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SINUS BRADYCARDIA AS AN EARLY MARKER OF HEART DAMAGE IN A GIRL WITH LYME DISEASE (CASE REPORT AND MINI REVIEW)

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Abstract. Objectives Early clinical markers of Lyme carditis and other life-threatening prognostic factors due to complications of borrelia infection are still the beginning of active research by scientists. The literature describes a small number of cases of Lyme carditis with arrhythmias or cardiac conduction disorders. Material and methods We reviewed the case of a 14-year-old girl. LC was diagnosed on the basis of history of disease, physical examination, laboratory examination data (positive anti-borrelia IgM in ELISA with confirmation in immunoblot. The presence of B. burgdorferi s.l. was detected by the method of immunoassay analysis using the Euroimmun AG test systems (Germany). The presence of B. burgdorferi s.l. was detected by the method of immunoassay analysis using the Euroimmun AG test systems (Germany). 2 instrumental methods: (ECG,Echo-cor) were used. Results We described a case of Lyme borreliosis in a 14-year-old girl, accompanied by sinus bradyarrhythmia and pericardial fluid -(hydropericardium) (passed after a course of antibacterial therapy). Analysis of this case indicates early detection and adequate treatment of Lyme carditis in young people with minimal deviations of the cardiogram. A case of Lyme carditis , in a child was confirmed.Echocardiography of the heart (at the time of admission to the hospital andat the time of discharge from the hospital was made.

Conclusions 1. Lyme carditis should be suspected in patients with a history of Lyme disease and minimal Electrocardiographic abnormalities , such as sinus bradycardia. 2. For differential diagnosis of Lyme carditis, it is necessary to make (carry out two-stage serological investigation of blood, especially using antibody screening tests by ELISA with subsequent confirmation by the Western blot assay.

Keywords ELISA, child, COVID-19, Lyme carditis

List of abbreviations:
ASL-O –antistreptolysin-O
WBC – white blood cells,
leukocytes APP- Acute-Phase Blood Protein
LC-Lyme carditis
LB-Lyme boreliosis
Borrelia burgdorfery-B.burgdorfery
Electrocardiography-ECG

Background.

Lyme disease is the most common disease transmitted by the ixodes ticks. It is endemic in Ukraine. Clinical manifestsation of the disease in the early stages often includes erythema migrans and early disseminated infection (multiple erythema migrans lesions, cranial nerve palsy--most commonly VII, aseptic meningitis or acute radiculopathy neurological (Bell's palsy, radiculoneuritis, meningitis) particularly with lymphadenopathy [1,2] or heart (atrioventricular block, myopericarditis, pancarditis) manifestation [3]. In Europe, only 0.3–4 % of all cases of borreliosis manifests itself in myocarditis [4]. Lyme carditis occurs when B. *burgdorfery* enters directly the heart tissue [5], which can cause conduction disorders. Lyme carditis can cause fainting, shortness of breath, heart palpitations, or chest pain. The most dangerous complication of conduction disorders is the full atrioventricular block of the heart. Its correction requires the use of pacemaker. Other reported serious and even fatal heart complications include heart arrhythmia, myocarditis, heart failure and sudden cardiac arrest [6]. Early LC, defined as signs and symptoms lasting less than 6 months after the tick bite, represent the vast majority of the cases (95%) [5]. The purpose of the work is to describe the peculiarities of the clinical manifestation of Lyme carditis on the basis of clinical, laboratory and instrumental methods of examination and identification of its early clinical markers.

Materials and methods. Our study was conducted, in Ternopil region (Western Ukraine) and consists of two parts: Study 1. During the first study we analysed case history, anamnesis morbi and in the course of the

second study we performed laboratory and instrumental examination of blood samples of person with lyme carditis. Study 2.Laboratory examination data (CBC, C-reactive protein, (ASL – O), positive anti-borrelia IgM, Ig G in ELISA with confirmation in immunoblot (Euroimmun AG test systems (Germany). According to the manufacturer's recommendations, the presence of specific IgM antibodies was considered positive, intermediate or negative, depending on the combinations of OspC antigens of the three species of Borrelia (B. afzelii, B. burgdorferi s.s. and B. garinii), p39 and VLsE Bb. At the same time, the presence of IgG was considered to be positive or negative, depending on the combinations of VLsE antigens of the three species of Borrelia (B. afzelii, B. burgdorferi s.s. and B. garinii) and other specific antigens: p18, p19, p20, p21, p58 , OspC (p25), p39, p83, Lipid Ba, Lipid Bb. Instrumental methods: (ECG, Echo-cor) were used.

«The authors declare that all the procedures and experiments of this study were conducted according to the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from the patient included in the study».

The authors received no funding for this study.

Results.

Case report. We reviewed the case of a 14-year-old girl. She was admitted to the regional hospital because of complaints of general weakness, rapid fatigue and discomfortin the left half of the chest, pain in the left ankle and metatarsal joint. Ankle pain syndrome was unbearable, and did not respond to the action of nonsteroidal anti-inflammatory drugs, flection was not in full volume. History of present illness : there was an evidence of a tick bite 2 months before, tick bite sign and redness in the area of the joint. At that time mother did not pay attention to symptoms. Thus, the girl was healthy did not receive preventive treatment, and did some in sports. Past medical history: None. A child from the first full-term uncomplicated pregnancy. Pertinent physical examination : Her mental and physical development corresponds to her age. The skin is pink. The general condition of the patient is moderate severity, body temperature 37,3 °C. Heart rate 56 bpm, the tones were preserved, some arrhythmia is presence . Respiratory rate: 20 breaths per 1 minute, auscultatory-vesicular. BP 110/70 mm Hg. SpO2=99 %

Tabl 1.

Parameter Lyme arthritis	Index
Hemoglobin, g/dl	11.5
Leukocytes, cells/μl	12,1
Platelets, cells/μl	
ESR, mm/h	40
CRP, mg/dl	40.7
Rheumatoid factor, IU/ml< 10	6.9
Antinuclear antibodies, titer < 1: 100	< 1:100
Joints ultrasound	bursitis and synovitis
ELISA (Borrelia burgdorfery RU/ml IgM	176.79-17.77
IgG-	72.2-77.6
Sialic acids	<2.0 mmol/L
total protein –	60 g/l

Taking into account , that the child before this case was healthy,engaged in sports.the history of tick bite, cardiac clinical symptoms and changes of ECG, the patient was scheduled for following examinations: serological tests for diagnosis of Lyme borreliosis, echocardiography, ECG in the dynamics. The peculiarity of this case was the deterioration of the patient's condition 2 months after tick bite. The treatment with non-steroidal anti-inflammatory drugs did not bring relief. Table I. Laboratory and ultrasound findings in the patient. The patient underwent serological testing for lyme borreliosis. Enzyme immunoassays for specific anti-Borrelia burgdorferi IgM and IgG were positive (176.8 U/ml and 72.2 U/ml respectively. Immunoblot assay identified the presence of several antibodies targeting the OspC Ba (B.afzelii), OspC Bb (B.burgdorferri), OspCBg (B.garinii), P39 Ig M, VlsE B.afzelii (Vls EBa) 34, VlsE B. burgdorferi (VlsE-Bb)33, VlsE B. garinii (VlsE-Bg)33, Lipid B.afzelii (LBa)8, Lipid (B. burgdorferri) (LBb)2, p83 (p83)12, Flagellum (p41)87, BmpA (p39)8, OspC (OspC)27, BB_A34 (p58)1, BB_K53 (p21)4, BB_Q03

(p20)3, BB_N38 (p19)4, BB_P38 (p18)29, Anti-human IgG (IgG) 63 were detected. ECG indicators at the time of admission (Fig. 1,2):

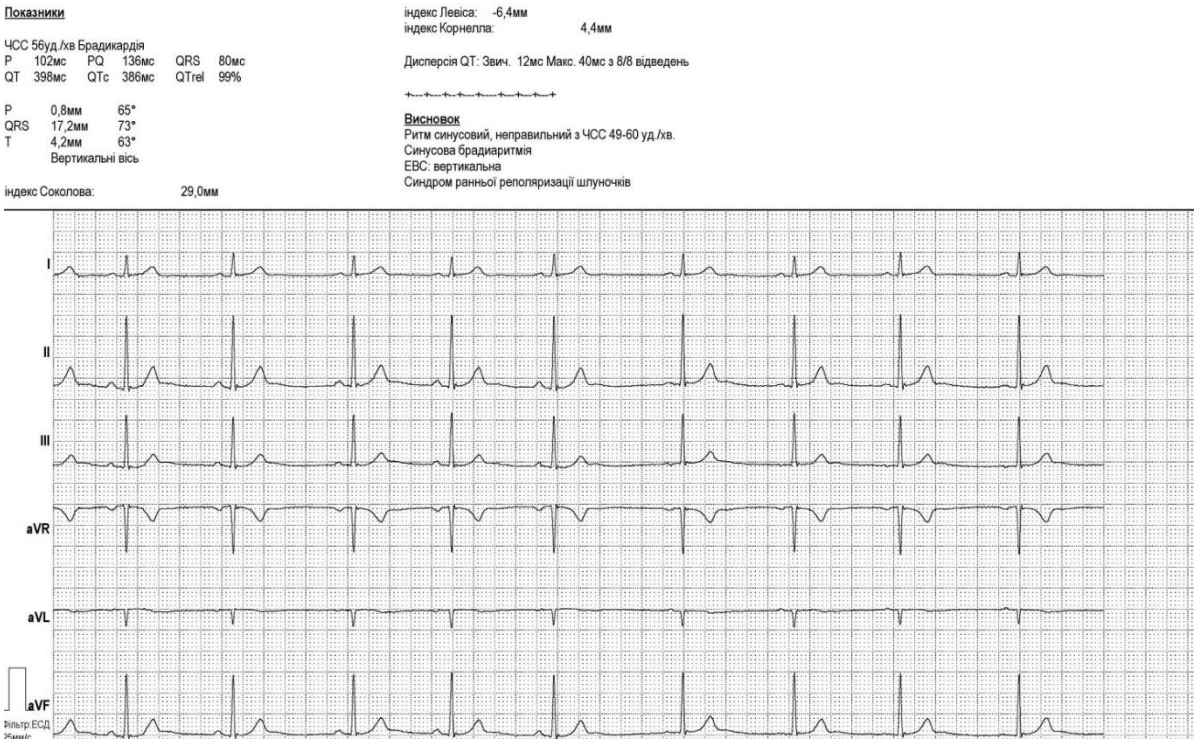

Fig 1.

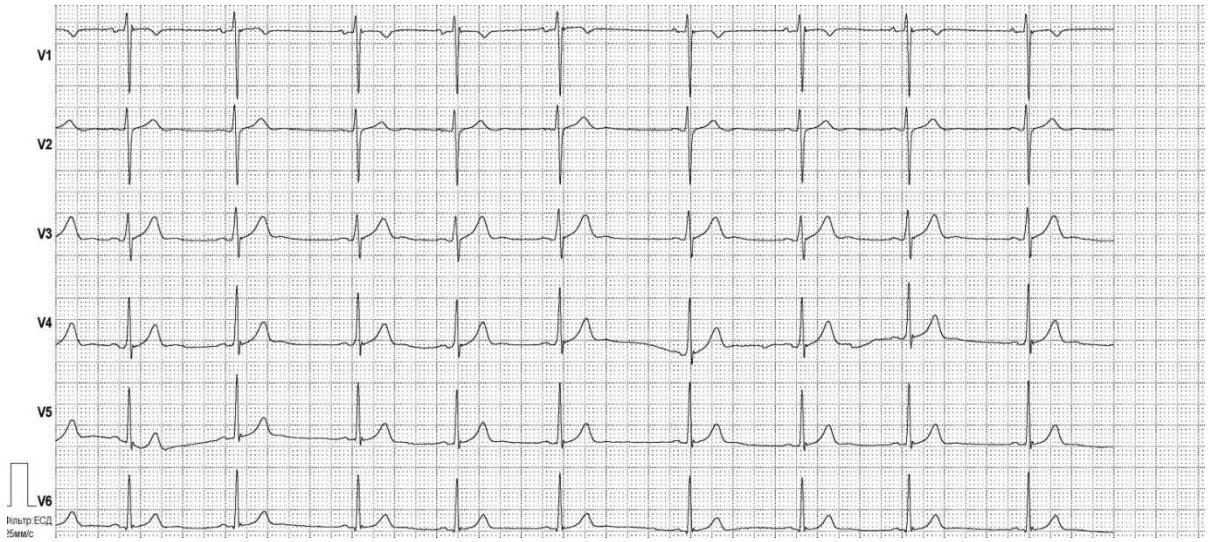

Fig 2.

Heart rate 56 bpn, bradycardia. P 102ms, PQ 136ms, QRS 80ms, QT 398ms, QTc 386ms,QTrel 99 %, P 0.8mm 65°, QRS 17.2mm 73°, T 4.2mm 63°. Vertical Axis, Sokolov Index:29.0mm, Levis Index: -6.4mm, Cornell Index: 4.4mm. Dispersion 12ms Max. 40ms with 8/8 leads. Conclusion: Sinus rhythm, with heart rate 49–60 bpm. Sinus bradyarrhytmia, vertical axis. Early ventricular repolarization syndrome. ECG at the time of discharge from the hospital (Fig. 3, 4):

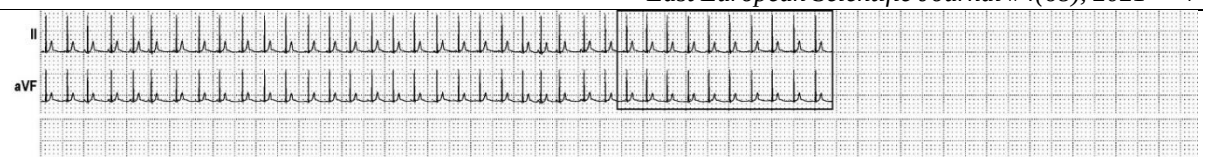

Fig3.

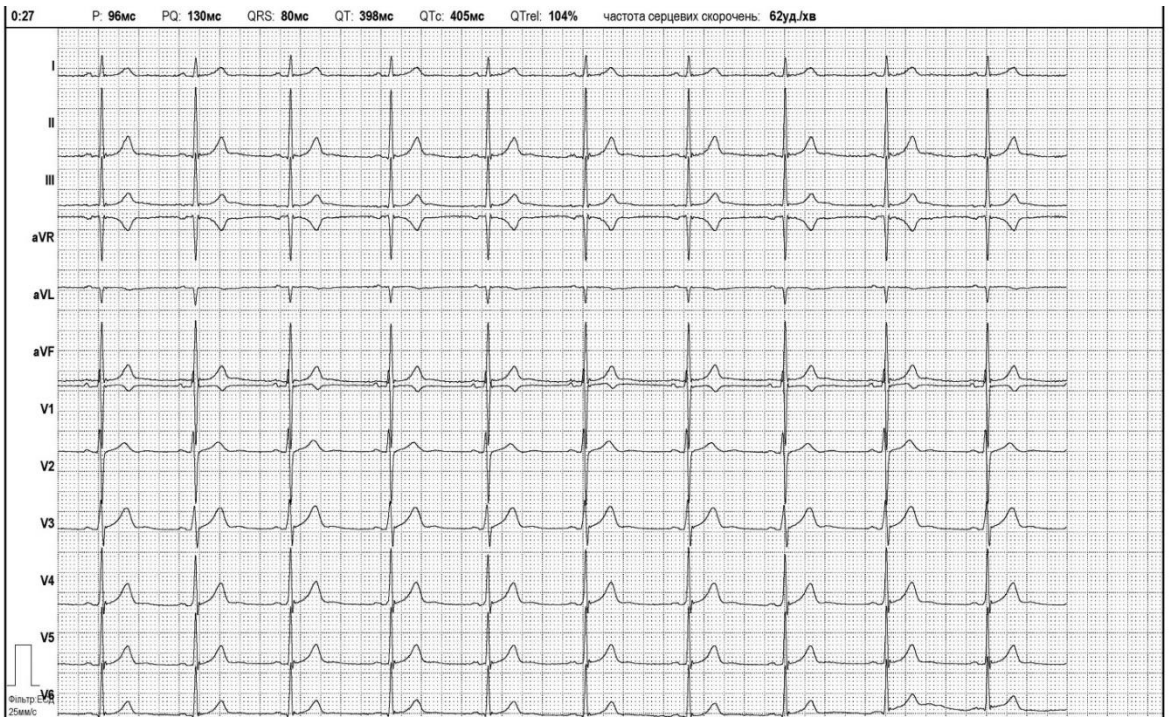

Fig 4.

Heart rate 62 bpm. P: 96ms PQ: 130ms QRS: 80ms QT: 398ms QTc: 405ms QT rel: 104 %.

Conclusion: Sinus correct rhythm 62 bpm. Vertical Axis. Early ventricular repolarization syndrome. Echocardiography of the heart (at the time of admission to the hospital). The chambers of the heart are not dilated. The structure and function of the valves are preserved. Left ventricular contractility is normal. A small echo-free space 4 mm thick (a small amount of fluid in the pericardium) is located behind the posterior wall of the left ventricle. Echocardiography of the heart (at the time of discharge from the hospital).

Table 2 .

Parameter	Before treatment	After treatment
Aorta, cm	2,1	2,1
LA, cm	2,2	2,2
IVS, cm	0,7	0,7
PW,cm	0,7	0,7
EDD, cm	3,7	3,6
EDV, ml	53	52
ESV, ml	21	20
RA, cm^2	9	9
RV,cm	1,7	1,7
EF,%	60	62

The chambers of the heart, the structure and function of the valves are normal. Left ventricular contractility is preserved. Fluid in the pericardiumis not determined. During this time, after treatment with antibiotics, the patient's condition became stable. The heart rate was normalized with 62 bpm, BP – 120/80 mmHg. Table 2. Echo-cor Options LA, left atrium; IVS, interventricular septum; PW, posterior wall; EDD, end diastolic distance; EDV, end diastolic volume; RA, right atrium; RV, right ventricle; EF, ejection fraction. Child's treatment. For antibiotic treatment Ceftriaxone 50 mg/kg/day intravenously once daily during 14 days was used. Nonsteroid therapy (ibuprophen) was prescribed. After treatment the patient's condition improved, signs of arthritis resolved and arthralgias decreased. Discussion. In our case we observed: history of tick bite and erythema migrans in child, signs and symptoms of carditis and arthritis, atrioventricular block or arrhythmia. Lyme disease at the beginning usually presents skin lesions accompanied by fever, flu-

like features – fatigue, low temperature, arthralgia, myalgia and nausea. In many cases, skin lesions can have partial central enlightenment, which looks like "bovine eye"[4,5]. If the infection is not localized, it can spread and affect the whole body [5]. Manifestations of LB. Miocardial presents in many different ways, ranging from mild symptoms to chest pain and palpitation, association with trancient ECG changes to life-threatening cardiogenic shock and ventricular arrhythmia [8,9]. In most cases, the clinical expression of myocarditis can be exemplified by 3 main patterns of presentation 1) recent-onset heart failure, 2) arryhytmias and 3) chest pain. Patients may experience bradycardia due to varying degrees of AV block, which often happens to be the only sign of Lyme carditis [10]. Heart symptoms (14 leukocytes/mm2 including up to 3) monocytes/mm2 with the presence of >7 CD3-positive T lymphocytes/mm2. According to some scientific research on autopsy, it was found that the cause of death was diffuse pancarditis with great lymphocytic infiltration and focal interstitial fibrosis [11]. Among the bacterial etiology of carditis: Brucella, Corynebacterium diphtheriae, gonococcus, Haemophilusninfluenzae, Actinomyces, Tropheryma whipplei, Vibrio cholerae, B.burgdorferi, leptospirosis, Rickettsia, Mycoplasma pneumoniae. Most cases of Lime carditis are clinically asymptomatic. If they are symptomatic, typical signs may be: complaints of fatigue, shortness of breath, heartbeats, fainting, syncope and chest pain [5.6.7.8]. Musculoskeletal manifestations include arthralgia and arthritis. Arthritis typically presents with recurrent brief attacks of joint swelling in 1 or more joints, typically involving the knees [12]. As clinical manifestations of the disseminated form of the disease, the patient may have multiple secondary EM, arthritis of large joints, neurological lesion [13] and heart lesions manifested by the atrioventricular unit [14]. How long after Borrelia infection can LC symptoms occur? The late-spread stage lasts for months or years after a tick bite and occurs in 60 % of patients who have not received treatment [2]. Such patients may experience periodic bouts of arthritis [9]. Borrelia disseminates from skin to other organs quickly. It replicates, kills host cells, and emerges through the membrane of that cell. Within days to weeks after infection, Borrelia has been recovered from blood, cerebrospinal fluid, myocardium [12,15]. The study was intended to identify changes in blood serum proteins in people infected with B. burgdorferi at the earliest stage [16]. In our study we detected signs of inflammatory activity (leukocytosis with neutrophilic shift to the left, increased ESR, increased C-reactive protein, sialic acids. Analysis of our study shows early LB-associated proteins revealed 1. Increase of acute phase proteins (C-reactive protein) assays for detecting host biomarkers associated with bacterial fevers, such as C-reactive protein (CRP) and are used in hospitals in Europe to differentiate between bacterial and non-bacterial infections [17] , (Tabl.1); Acute phase proteins (C-reactive protein, antistreptolysisin-O (ASL – O), as biomarkers of inflammation, can react within hours after borreliosis penetrate the skin [14]. 2. Borrelia antibody IFA as well as confirmatory IgG and IgM Western Blot were positive. Diagnosis of Lyme disease confirmed by laboratory tests prescribed according to the recommendations of the Centers for Disease Control and Prevention [14]. In response to infection, the host immune response to Borrelia integrates both cell-mediated and humoral mechanisms [15]. That's why its an importance of the 2 stage tests in the diagnostic of ethiology carditis of Lyme disease [14,17]. In Laboratory testing we found antibodies to B.burgdorferi in the serum [15,16]. Although serological testing can initially give false negative results, a second serological study should be conducted within two to six weeks especially in a patient with suspected Lyme carditis [14]. Immunoblot is a highly specific test used to diagnose Lyme disease. In our case Immunoblot assay identified the presence of several antibodies targeting the OspC Ba (B.afzelii), OspC Bb (B.burgdorferri), OspCBg (B.garinii), P39 Ig M, VlsE B.afzelii (Vls EBa) 34, VlsE B. burgdorferi (VlsE-Bb)33, VlsE B. garinii (VlsE-Bg)33. On the other hand, there is a suggestion the immunobOn the other hand, there is a suggestion the immunoblot assay tests for the presence of antibodies to specific B. burgdorferi antigens, including immunoglobulin (Ig) M antibodies to 3 spirochetal antigens (the 23/24, 39, and 41 kDa polypeptides) and IgG antibodies to 10 spirochetal antigens (the 18, 23/24, 28, 30, 39, 41,45, 60, 66, and 93 kDa polypeptides) [16]. Based on positive results of serological tests, Lyme borreliosis was confirmed. The diagnosis of Lyme carditis is challenging: it is usually made in the presence of other manifestations of Lyme disease—concomitant erythema migrans, arthritis, or neurologic disease, or such cardiac manifestations as ECG findings and congestive heart failure—together with positive serologic testing for B. burgdorferi infection. One should note that positive serologic testing is not by itself diagnostic, nor does negative serologic testing exclude the diagnosis [16]. In our study we estimated ECG Sinus bradyarrhytmia, vertical axis. Early ventricular repolarization syndrome. Echo-cor. A small echo-free space 4 mm thick (a small amount of fluid in the pericardium) is located behind the posterior wall of the left ventricle. Echocardiography can provide valuable information to assess the presence and stage of heart dysfunction, which is important for the management of these patients [16,17]. According to Nelson CA, SahaS, the 12-lead ECG and Holter can show depression of the ST segment, inversion of T-waves in the chest leads and conduction disorders [16].

The criteria for diagnosis of myocarditis are the following [12, 16]: 1. Standard ECG/Holter Daily Monitoring – AV-block degree 1–3 – Blockade of the bunch of Gis – ST/T changes (ST interval inversion), – Paroxysmal tachycardia, – Low voltage (if R in standard leads is less than 5 mm or less than 10 mm in the breast leads. - Frequent extrasystoles. In a previous study, Alida L.P.Caforio et al. [7] electrocardiogram (ECG) is usually abnormal in myocarditis though ECG signs are neither specific nor sensitive. Some ECG

changes are more suggestive of myocarditis than others. For example, ST-T segment elevation in myocarditis is typically concave (rather than convexin myocardial ischaemia) and diffuse with out reciprocal changes. A-V block in the presence of mild left ventricular dilata3tion can be due to various causes , but it may also be suggestive of Lyme disease, cardiac sarcoidosis, or giant cell myocarditis. In another recent publication [18], autor said that conduction system involvement is diverse, possibly including but not limited to bundle branch block, intraventricular conduction delay, prolonged QT interval, ventricular and fascicular tachycardias, and complete heart block.The most common LC electrocardiogram (ECG) findings in patients include atrioventricular (AV) conduction abnormalities (first, second, and third degree heart block) [19], atrial fibrillation and a full atrio-ventricular block [20] of a first-second grade type I . In our study echocardiography of the heart (at the time of admission to the hospital). A small echo-free space 4 mm thick (a small amount of fluid in the pericardium) is located behind the posterior wall of the left ventricle. Echocardiography helps to rule out non-inflammatory cardiac disease such as valve disease and to monitor changes in cardiac chamber size, wall thickness, ventricular function, and pericardial effusions [7]. According to scientific finding, in Echocardiography we found: enlargement of left or right ventricle, thickness of the wall of the left ventricle, pericardial effusion – for no other reason. [18]. The literature describes a number of cases of Lyme carditis with heart rhythm disorders or isolated block of nerve branches or other conduction disorders [21, 22, 23]. Differential diagnosis with other cardites was made. In viral myocarditis, the Coxsackie B family of the single-stranded RNA viruses, in particular the plus-strand RNA virus Coxsackievirus B3 and Coxsackievirus B5 are the most frequent cause [15]. Most studies have reported cases of the involving protozoa, fungi, parasites, allergy, autoimmune disorders are also causes of eosinophilic myocarditis [6]. Other causes of lyme carditis may be Chagas disease, Kawasaki disease [24]. Acute rheumatic cardiitis should also be excluded in diagnostic search in patients with violation of AB conduction due to acquired heart disease, especially in pediatric practice. Most cases are reversible AV-blocks of the first or second degree [19]. The mechanisms of development of infectious genesis carditis, are actively studied today, including a completely new infection SARSCoV-2 [16, 23, 26]. A recent incident reported by Kannangara et al. Diseases (2019) [25] describes manifestations of Lyme carditis (LC) vary from asymptomatic and symptomatic electrocardiographic changes and heart block (HB) reversible with treatment, to sudden death.tachycardia in the pediatric case of LC. The AV-block in Lyme carditis is usually reversible, and takes place after a course of antibiotics [20,22].

For diagnosis of carditis it is necessary to make CBC, CRP, ECG, Echo-cor, and which tests are needed for etiological diagnosis of carditis it necessary to make two-stage serological investigation tests of blood are needed. Oral antimicrobial agents are appropriate and effective for most manifestations of disseminated Lyme disease, including multiple erythema migrans and for patients with Lyme carditis treated as outpatients. For patients requiring hospitalization for Lyme carditis (high-grade atrioventricular block), initial therapy usually is parenteral but can be completed with oral therapy for a total course of 14 days (range: 14 to 21 days) [26]. Early clinical markers of LC and other predictors of life-threatening complications of borreliosis infection are still at the beginning of an active study by scientists. Therefore, the case described Lyme borreliosis, accompanied by sinus bradyarrhythmia and pericardial fluid (past after taking an antibacterial therapy course), may target early detection and adequate treatment of Lyme carditis in young people with minimal deviations in cardiogram indicators [27, 28, 29]. Clinicians, pathologists, immunologists, and molecular cardiologists must contribute to the new criteria, which should include clinical presentation, histopathology, immunohistochemistry, viral polymerase chain reaction, cardiac antibody assessment, and imaging results. Finally, it is possible that sinus bradycarditis or other minimal deviations from the normal ECG can be studied Manuscript body Download source file (60.08 kB) as potential early markers of heart lesions not only in Lyme borreliosis, but also in other infectious diseases, including COVID-19.

Conclusions

1. Lyme carditis should be suspected in people with a history of Lyme disease and minimal abnormalities in cardiograms, such as sinus bradycardia.

2. For diagnosis of Lyme carditis, it is necessary to make two-stage serological investigation of blood, especially use of antibody screening tests by ELISA with subsequent confirmation by the Western blot assay.

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ILIZAROV – FOUNDER OF DISTRACTION OSTEOGENESIS

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ИЛИЗАРОВ – ОСНОПОЛОЖНИК ДИСТРАКЦИОННОГО ОСТЕОГЕНЕЗА

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Summary. The article analyzes the unique personality of the XX century, the outstanding Soviet orthopedic surgeon-Gavriil Abramovich Ilizarov. The evolutionary process of the formation and development of the Ilizarov method in traumatology and orthopedics at the domestic and world level is shown. The undeniable advantages of the external fixation system developed by Ilizarov are revealed. The design of the Ilizarov compression-distraction apparatus is considered. Being familiar with G.A. Ilizarov, the author shares his memories about him, describes the biological and mechanical features of the influence on the bone and other tissues of the Ilizarov system, which has become a classic and internationally recognized in the treatment of patients with fractures, pathological conditions of the musculoskeletal system, congenital and post-traumatic orthopedic deformities of the limbs.

Аннотация. В статье осуществляется анализ уникальной личности XX столетия, выдающегося советского хирурга-ортопеда – Гавриила Абрамовича Илизарова. Показан эволюционный пусть становления и развития метода Илизарова в травматологии и ортопедии на отечественном и мировом уровне. Выявлены неоспоримые преимущества разработанной Илизаровым системой внешней фиксации. Рассмотрена конструкция компрессионно-дистракционного аппарата Илизарова. Будучи знакомым с Г.А. Илизаровым, автор делится своими воспоминаниями о нем, описывает биологические и механические особенности влияния на костную и другие ткани илизаровской системы, ставшей классической и признанной на мировом уровне при лечении пациентов с переломами, патологическими состояниями опорно-двигательного аппарата, врожденными и посттравматическими ортопедическими деформациями конечностей.

Keywords: Gavriil Ilizarov, osteogenesis, traumatology, orthopedics, distraction, Ilizarov apparatus, bone elongation.

Ключевые слова: Гавриил Илизаров, остеогенез, травматология, ортопедия, дистракция, аппарат Илизарова, удлинение костей.

Постановка проблемы. Метод удлинения, реконструкции и остеосинтеза костей по Илизарову получил огромное развитие с момента его внедрения Г.А. Илизаровым в Советском Союзе в 1960-х годах и западных странах в начале 1980-х годов. Метод Илизарова стал неотъемлемой частью арсенала, используемого ортопедическим сообществом во всем мире, эволюционное развитие которого и его нынешняя роль значительно улучшили качество жизни миллионов людей. Несмотря на большую универсальность его возможных применений при повреждениях и заболеваниях костей, метод Илизарова не может быть альтернативой ряду других методов, применяемых при некоторых специфических состояниях костей. Сам Гавриил Илизаров предостерегал не считать его систему унифицированной. Проблема исследования обуславливается отсутствием комплексного всестороннего анализа уникальной методологии советского врача Гавриила Абрамовича Илизарова в разрезе ретроспективного анализа его метода в ортопедии и травматологии с целью определения перспективных направлений дальнейших научных исследований в данной области с учетом личного знакомства автора статьи, ориентированных, в первую очередь, на внедрение и практическое использование в клинической работе.

Анализ последних исследований и публикаций. Вопросы, связанные с личностью великого доктора Илизарова, а также современные экспериментальные исследования касательно использования метода дистракционного остеогенеза (метода Илизарова) были освещены в трудах отечественных (А.Я. Адсмади, Э.И. Солод, М.А. Абдулхабиров, А.Н. Ивашкин, А.А. Артемьев, А.М. Кошуб [1], А.В. Губин, Д.Ю. Борзунов, Т.А. Малькова [3], Э.И. Илизарова-Абаева [4], Э.А. Меликов, А.Ю. Дробышев, И.А. Клипа, С.А. Снигерев, С.В. Шамрин [5],) и зарубежных исследователей (J.G. Birch, M.L. Samchukov [6], M. Bisaccia, C. Ibáñez [7], J.J. Jr. Gugenheim [11], D. Lowenberg, M. Githens [12], etc).

Так, по мнению J.G. Birch, M.L. Samchukov, внедрение на Западе в начале 1980-х годов циркулярного наружного фиксатора и метода Илизарова привело к быстрому прогрессу в удлинении конечностей, коррекции деформаций и реконструкции сегментарных длинных костных дефектов [6]. Механические особенности и

биологическая реакция на использование дистракционного остеогенеза с круговым наружным фиксатором являются уникальными аспектами вклада Г.А. Илизарова. В экспериментальных исследованиях, проводимыми авторами J.G. Birch, M. L.Samchukov, а также экпериментах D. Lowenberg, M. Githens наиболее распространенными показаниями для детей и подростков являются удлинение конечностей и коррекция угловой деформации [6;12]. Действительно, хирургическое применение и послеоперационное управление аппаратом Илизарова требуют кропотливого внимания к деталям как пациента, так и хирурга. Кроме того, от хирурга требуется тщательное понимание основных принципов работы аппарата Илизарова, механической осевой перестройки, потенциальных осложнений и биологической реакции на растяжение.

В трудах J.J. Jr. Gugenheim обосновывается, что метод Илизарова имеет широкое применение для формирования костной и мягкой ткани с помощью внешнего фиксатора, состоящего из небольших штифтов, натяжных проволок, колец, шарниров и дистракторов [12]. Успех методики, как справедливо утверждает автор, зависит от соблюдения принципов феномена «стресс напряжения» Илизарова: сохранение кровоснабжения тканей, частая дистракция с небольшими приращениями, полноценная функция конечности.

Выделение нерешенных ранее частей общей проблемы.

Несмотря на международное признание Илизарова, включая создание Ассоциации по изучению и применению методов Илизарова, приглашение Гавриила Абрамовича на международные симпозиумы, конференции, противостояние и скептицизм со стороны московского медицинского истеблишмента продолжалось до последних лет жизни Илизарова, ограничив великого врача во многих званиях и наградах, включая в себя возможность быть членом Академию медицинских наук СССР, удостоенным Нобелевской премии и др.

Цель статьи заключается в обзоре метода Илизарова и его неоспоримых преимуществах в современной хирургии при лечении различных патологических состояний опорно-двигательного аппарата.

Изложение основного материала.

Гавриил Абрамович Илизаров – советский врач, доктор медицинских наук, профессор, академик РАН, Герой Социалистического Труда, заслуженный изобретатель СССР, известный изобретением аппарата Илизарова для удлинения костей конечностей и метода хирургии, названного в его честь хирургией Илизарова.

Г.А. Илизаров родился 15 июня 1921 г. старшим из шести детей в бедной еврейской семье в Беловеже (Полесское воеводство, Польша). Вскоре после его рождения семья переехала к родителям его отца в г. Гусар (Азербайджан). Его отец, Абрам Илизаров, был горским евреем из Гусара, а мать, Голда Розенблюм, была еврейкой ашкеназского происхождения.

Г.А. Илизаров окончил Буйнакский медицинский Рабфак (учебное заведение, созданное для подготовки рабочих и крестьян к высшему образованию). В 1939 году поступил в Крымское медицинское училище г. Симферополь. После вспышки Великой Отечественной войны в 1941 году училище было эвакуировано в Кызылорде в Казахстане. После окончания школы в 1944 году Г.И. Илизаров был направлен в сельскую больницу в с. Долговка Курганской области в Сибири, расположенной 2000 км к востоку от Москвы. В 1950 году Илизаров получил место в отделении Курганской областной клинической больнице хирургом санитарной авиации. В 1955 году стал заведующим отделением травматологии и ортопедии Курганского областного госпиталя ветеранов войны.

Обучение в ординатуре и уникальные эксперименты Илизарова.

Ординатура Илизарова проходила в ортопедической хирургии, в ходе которой он разработал систему внешней фиксации (1951). Гавриил Абрамович обнаружил, что, осторожно отсекая кость, не разрывая надкостницу вокруг нее, можно слегка отделить две половинки кости и зафиксировать их на месте, и кость будет расти, заполняя образовавшийся зазор. Вместе с тем Г.А. Илизаров обнаружил, что кость отрастает с довольно равномерной скоростью у разных людей и обстоятельств.

Данные эксперименты привели к созданию так называемого аппарата Илизарова, который удерживает разорванную таким образом кость на месте благодаря каркасу и булавкам, проходящим через кость, и отделяет половинки кости на крошечную величину; повторяя это с течением времени со скоростью роста кости, можно удлинить кость на желаемую величину. Эта процедура была вдохновлена упряжью из лука на конной повозке. Первоначально для рамы использовались велосипедные детали.

Причиной создания системы внешней фиксации послужило пребывание в с. Долговка, где Илизаров столкнулся с огромным количеством патологий, однако имел минимальное количество доступных ему технологий. Открытые переломы часто приводили к септическому несращению. Хроническая боль, дренаж и разрушение костей с укорочением конечностей были обычным явлением. В свою очередь, Илизаров использовал - стерильные велосипедные спицы, прикрепленные к кускам металла в качестве внешней фиксации для этих септических несоединений. Благодаря клиническому опыту и лабораторным экспериментам на животных он обнаружил, что может устранить инфекцию и исцелить неединства путем постепенного, контролируемого манипулирования устройством. Самое значимое,

что ни антибиотиков, ни костной пластики не требовалось.

В дальнейшем Г.А. Илизаров попытался исправить неравенство длины конечностей. В ходе исследований он обнаружил, что может создать остеотомию в длинной кости, отвлечь концы фиксатором и сформировать новую костную ткань в медленно расширяющейся дистракционной щели. Создание правильной среды и техники для того, чтобы это происходило надежно и воспроизводимо, заняло годы критических экспериментальных и клинических исследований.

Проще говоря, если определенным образом растягивать ткани, они удлиняются или растут без необходимости пересадки. Этот процесс гистиогенеза дистракции зависит от адекватной васкуляризации и функционального использования конечности. Это явный отход от прежних представлений об удлинении и коррекции деформаций конечностей. Когда этот принцип используется в сочетании с циркулярным наружным фиксатором Илизарова в правильно спланированной и управляемой хирургии, возможности для управления проблемами костей и мягких тканей крайних конечностей огромны. В Советском Союзе метод Илизарова по существу является областью медицины, параллельной западной ортопедии, но отличной от нее.

Долгое время Илизаров сталкивался со скептицизмом, сопротивлением и политическими интригами со стороны медицинского истеблишмента Москвы, который пытался опорочить его, называя «шарлатаном». Однако неуклонно растущая статистика успешного лечения больных привела к росту известности Илизарова по всей стране. Он стал известен среди пациентов как «маг из Кургана». В 1968 году Г.А. Илизаров защитил докторскую диссертацию в г. Пермь и был удостоен звания доктора наук в обход степени кандидата наук, к которой первоначально готовилась диссертация.

Прорыв произошел в 1968 году, когда Илизаров успешно прооперировал Валерия Николаевича Брумеля, олимпийского чемпиона 1964 года и многолетнего рекордсмена мира в прыжках в высоту среди мужчин, который повредил правую ногу в результате аварии на мотоцикле. До прихода к Илизарову В.Н. Брумель около трех лет безуспешно лечился в различных клиниках и перенес семь инвазивных и 25 неинвазивных операций.

Противостояние московского медицинского истеблишмента продолжалось до последних лет жизни Илизарова. Еще в 1991 году, всего за год до своей смерти, Илизаров был избран действительным членом Российской академии наук. Несмотря на многочисленные награды и мировое признание, он не был избран в Академию медицинских наук СССР.

Международное признание Илизарова. Метод, разработанный в 1951 году профессором Советского Союза Гавриилом Илизаровым, представляет собой неожиданный прорыв в лечении большинства патологических состояний опорно-двигательного аппарата путем применения сложного внешнего фиксатора, создания остеотомии и постепенного и контролируемого манипулирования конструкцией с целью формирования новой ткани.

Наиболее заметное применение аппарата Илизарова – неравенство длины конечностей, также используется для лечения переломов, несращений, артритов и многоплоскостных деформаций конечностей. В западном мире управление этими состояниями традиционно опиралось на принципы и методы, которые во многих случаях сильно отличались от тех, которые отстаивал Илизаров [13].

Наружная фиксация для лечения переломов, осложненных значительной травмой мягких тканей, восходит к Гиппократу. В 1905 году A. Codivilla из Болоньи опубликовала первый отчет об хирургическом удлинении конечностей в английской литературе [8]. С тех пор, из-за ограниченных успехов и высокой частоты осложнений, было много попыток улучшить методы и устройства для удлинения конечностей. С начала 1970-х годов метод Вагнера стал самой популярной процедурой удлинения конечностей на Западе. В 1963 году профессор Хайнц Вагнер из Западной Германии разработал односторонний внешний фиксатор, состоящий из двух больших резьбовых штифтов (5 мм в диаметре), которые вставляются перпендикулярно как в проксимальный, так и в дистальный концы кости [17]. Штифты соединены с телескопическим прямоугольным стержнем. Открытая остеотомия выполняется осциллирующей силовой пилой или соединением нескольких сверл с остеотомом. Остеотомия сразу отделяется на 1 см и устройство фиксируется. На следующий день и каждый последующий день ручка в конце устройства поворачивается на один полный оборот, создавая 1 мм дистракции костных сегментов. Из-за недостаточной прочности и устойчивости консольной системы вес подшипника не допускается. Когда желаемая длина достигнута, для покрытия и костной пластики удлиняющегося промежутка требуется второй анестетик. Это делается через длинный разрез. Вес подшипника по-прежнему не допускается. Третий анестетик необходим, чтобы удалить аппаратуру, когда кость уже твердая.

К основным недостаткам метода Вагнера можно отнести необходимость трех анестетиков, несколько месяцев невесомости, сопутствующую остеопению всей конечности и длинные неприглядные шрамы [16]. В каждом клиническом обзоре удлинения конечностей Вагнера частота осложнений также была высокой, а способность достичь желаемого количества удлинения – низкой. Тем не менее, метод Вагнера был лучше своих предшественников Появившиеся в начале 1980-х годов сообщения о весьма успешном советском

методе удлинения конечностей без осложнений встретили на Западе некоторый скептицизм. Еще труднее было принять тот факт, что он был разработан в 1951 году, за 20 лет до метода Вагнера.

В западной прессе появились разрозненные сообщения об успешном лечении Брумеля Илизаровым. Первым иностранным медицинским посетителем был доктор Йоханнес Хеллингер из бывшей ГДР (Германская демократическая Республика), Медицинская академия Эрфурта в 1970 г. Он сделал первую публикацию в западном медицинском журнале о методе Илизарова. В 1980 году, в эпоху холодной войны, Карло Маури, итальянский альпинист, исследователь и фотожурналист, по настоянию своего российского коллеги Юрия Сенкевича, ездил в Курган, в Советский Союз. Он должен был лечиться у Илизарова от перелома большеберцовой кости, который неправильно зажил после несчастного случая на лыжах десять лет назад. Итальянские врачи давно оставили надежду на какое-либо хирургическое улучшение ноги. Илизаров отвлек застывшее несращение в большеберцовой кости на 2 см, излечив псевдартроз, исправил эквинусную деформацию дистракцией и удлинил ногу. В свою очередь, К. Маури окрестил Илизарова «Микеланджело ортопедии». По возвращении в Италию исцеление ноги Маури поразило хирургов-ортопедов. После этого Антонио Бьянки-Майокки и Роберто Каттанео пригласили Илизарова выступить в качестве приглашенного докладчика на конференции АО Италия в 1981 году в Белладжио. Так, Илизаров прочитал три лекции на конференциях более чем 200 участникам из Италии, Франции, Швейцарии, Австрии и Германии. В конце лекций Илизаров заслужил десятиминутную овацию. Это был первый раз, когда Илизаров выступил за пределами Железного занавеса.

В 1982 году в Италии была образована Ассоциация по изучению и применению методов Илизарова (АСАМИ). В 1983 году компания Medicalplastic, принадлежащая Bianchi-Maiocchi, лицензировала технику у советских властей, зарегистрировала товарный знак ILIZAROV и начала производить и продавать аппарат Илизарова. В последующие годы метод Илизарова быстро распространился по большей части Западной Европы. АСАМИ организовала курсы в Италии, Португалии, Швейцарии, Франции, Испании, Греции, Бразилии и Соединенных Штатах. Группы АСАМИ были сформированы во Франции, Испании, Бельгии, Португалии и Бразилии.

В 1986-1987 гг. метод был привезен в Северную Америку Виктором Френкелем, президентом Больницы болезней суставов, Дрором Пейли, Альфредом Д. Грантом и Стюартом Грином, которые в 1992 году отредактировали первый английский перевод книги Илизарова. Более 300 американских хирургов-ортопедов приняли участие в международном симпозиуме, организованном в 1987 году в Нью-Йорке Больницей болезней суставов и компанией Smith & Nephew д ля прослушивания лекций Илизарова. Компания Smith & Nephew начала распространение внешнего фиксатора Илизарова в США и по всему миру [2].

В 1989 году Дитмар Вольтер организовал в Гамбурге Илизаровскую конференцию. В 1990 году Илизаров приехал на вторую конференцию в Гамбург, где стал одним из основателей Немецкого общества Илизарова (Deutsche Ilisarow-Gesellschaft). Больница Немецкой ассоциации предотвращения несчастных случаев и страхования (Berufsgenossenschaftliches Unfallkrankenhaus, BGUK) в Боберге, Гамбург, стала крупным центром в Германии, применяющим и продвигающим методику Илизарова. Посещение Курганского центра изучения метода Илизарова стало обязательным для всех старших врачей больницы.

Конструкция аппарата Илизарова. Аппарат Илизарова (рис. 1), включает в себя круговой наружный фиксатор, состоит из тонких проволок (диаметром 1,5 мм и 1,8 мм), просверленных через кость чрескостный и закрепленных с обоих концов болтами и гайками под высоким натяжением (от 90 до 130 кг) к кольцам из нержавеющей стали. От двух до четырех проводов и от одного до двух колец требуются как проксимальные, так и дистальные к месту удлинения или деформации. Кольца соединены тремя или четырьмя расположенными по окружности резьбовыми стержнями. Поворачивая гайки на стержнях, кольца (и, следовательно, костные сегменты) отвлекаются. Использование колец и скрещенных проволок, а не одностороннего стержня и полуштифтов, дает устройству лучший контроль костных сегментов во всех плоскостях. Это важно для обеспечения возможности опоры на вес во время удлинения конечностей. Данная особенность позволяет корректировать другие деформации с одновременным удлинением конечностей или без него.

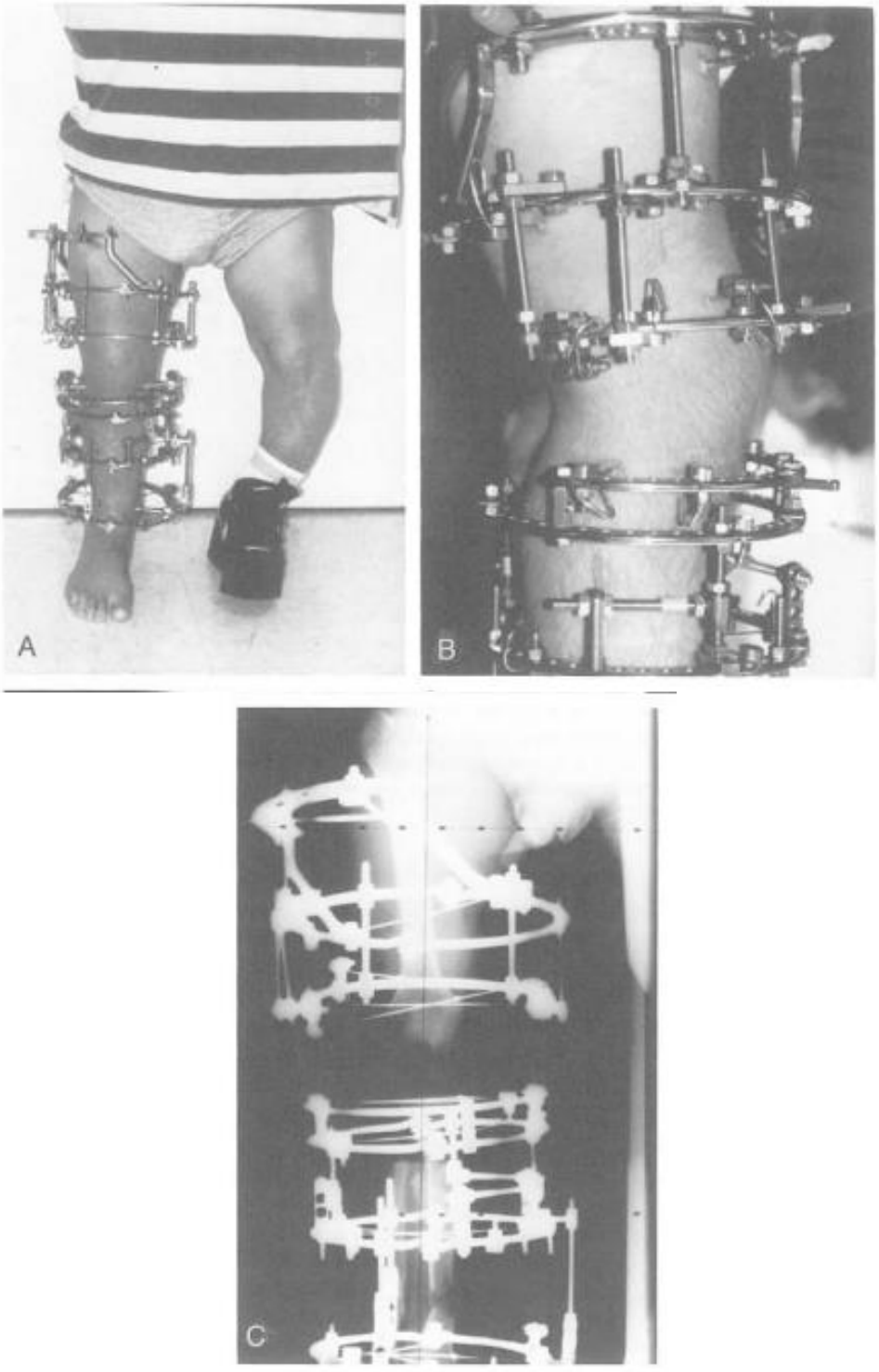

Рис. 1. Аппарат Илизарова
А) Феморальный и большеберцовый каркасы на месте при выполнении трехуровневой одновременной коррекции деформации;
Б) Видны небольшие разрезы для кортикотомии;
С) Рентгенографическое появление во время фазы нейтрализации [13]

Односторонние внешние фиксаторы могут просто удерживать кости на месте или выполнять одноплоскостное удлинение. Фактическое размещение проводов Илизарова и конструкция каркаса определяются индивидуальными потребностями пациента. Аппарат Илизарова – это, по сути, «хирургический эректор» с почти неограниченным количеством возможных вариантов расположения компонентов. Важной конструктивной особенностью аппарата Илизарова и причиной коррекции многоплоскостных деформаций является шарнир (рис. 2) – ось, вокруг которой в одной плоскости могут вращаться две подвижные части. Существуют также

универсальные шарниры, которые позволяют перемещаться между деталями более чем в одной плоскости. Шарниры просто сделаны из компонентов Илизарова и прикреплены к кольцам шатунами. Специальные провода, называемые оливковыми проводами, используются в определенных местах, когда используются петли. Металлический шарик на проволоке опирается на кору головного мозга, чтобы предотвратить неконтролируемое скольжение кости по проволоке.

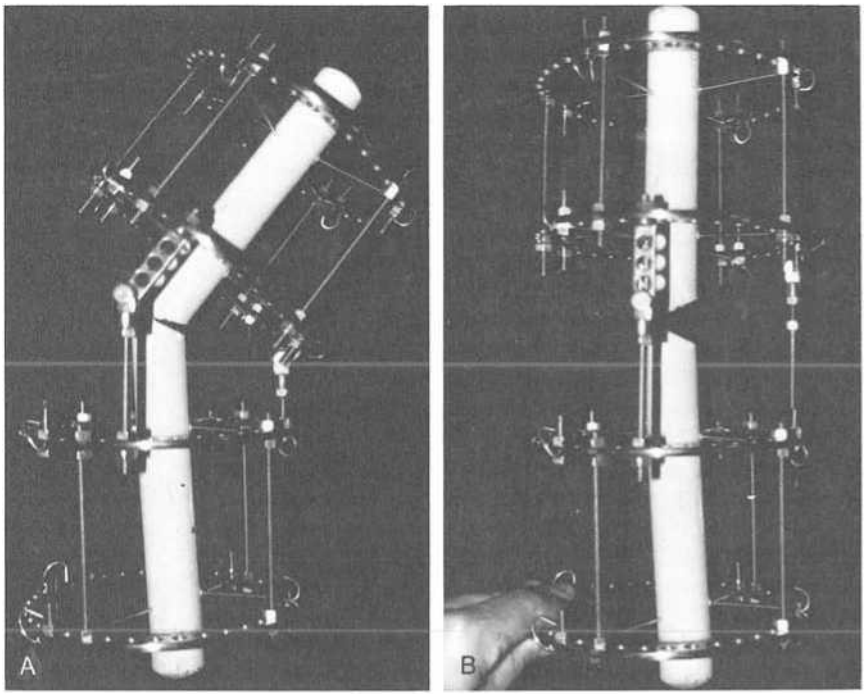

Рис. 2.
А) Простой шарнир, центрированный над выпуклой корой на уровне угловой деформации. Провода и кольца расположены перпендикулярно каждому сегменту кости.
В) После кортикотомии дистракция вогнутой коры происходит на 0,25 мм каждые 6 часов. Провода и кольца обоих сегментов параллельны при завершении коррекции деформации [13]

Во время коррекции деформации путем тщательного размещения шарниров и других компонентов можно одновременно с удлинением или последовательно исправить многоплоскостные деформации, не возвращаясь в рабочее помещение и не добавляя проволок. В Советском Союзе было проведено около 1 миллиона процедур. Эти цифры включают в себя удлинение конечностей, а также все другие приложения. Полученные результаты впечатляют. Увеличение длины конечностей достигается с меньшим количеством осложнений, чем при методе Вагнера, и большей универсальностью для коррекции других форм. Не стоит отрицать, что осложнения возникают и при методе Илизарова, но частота и тяжесть их возникновения значительно ниже.

Реабилитационные аспекты метода. Г.А. Илизаров давно осознал важность реабилитации для успеха своего метода. Его исследования показали, что переносимость веса и физиологическое использование конечности во время лечения имеют важное значение для качества и скорости образования новых костей, предотвращения остеопении во всей конечности и поддержания функциональной целостности мягких тканей.

Мягкие ткани, как и кость, подвергаются удлинению в результате дистракционного гистиогенеза. Только кость жестко фиксируется устройствами внешней фиксации. Мягкие ткани свободно пронизываются и при удлинениизависят от своих костных прикреплений для жесткого контроля. Из-за асимметричного расположения мышц вокруг и поперек суставов дистракция-приводит к контрактурам суставов и ограничению движения суставов, а также может привести к подвывиху или вывиху сустава [10]. К счастью, этих проблем можно избежать или свести к минимуму путем тщательного внимания к реабилитации. Хотя Илизаров сообщает о очень низкой частоте болей во время удлинения [18], опыт на Западе показывает, что пациенты часто имеют хроническую, тупую, ноющую боль во время дистракционной фазы удлинения, особенно при более длинных удлинениях [14]. Боль усиливается во время физиотерапии, при ходьбе и ночью. Илизаров считает, что боль возникает из-за неправильного применения прибора или из-за инфекции желудочно-кишечного тракта. Какое-то поведение боли является физиологией, а какое-то изучается. Даже сегодня в Кургане наркотики, как и аспирин, в дефиците. Жалоба на боль мало поможет ее разрешению [9; 13].

Открытие Центра Илизарова. В 1971 году был создан Курганский Научно-исследовательский институт экспериментальной и клинической

ортопедии и травматологии (КНИИЭКОТ). В центре применяется метод чрескостного остеосинтеза по Илизарову для удлинения или изменения формы костей конечностей. Илизаров возглавлял этот центр до 1991 года. Имея около 1000 коек, 24 операционных и 168 работающих врачей, Центр стал крупнейшей ортопедической клиникой в мире. После смерти великого хирурга-ортопеда центр был переименован в Российский научный центр восстановительной травматологии и ортопедии им. Илизарова (РИНЦ РТО).

За 40 лет работы Илизаров и его клинико-научные сотрудники опубликовали более 2000 статей по темам, начиная от биологии гистогенеза дистракции и заканчивая обзорами клинических результатов и техническими соображениями методики. Сегодня в штате центра работают десять профессоров, 34 доктора наук и 193 кандидата наук. Центр включает в себя амбулаторию, где ежедневно консультируются 250 пациентов, больницу на 800 коек, экспериментальное отделение и хирургию животных. Каждый год более 9000 человек проходят лечение в РИНЦ РТО.

Личное знакомство автора с Илизаровым. Будучи старшим научным сотрудником Центра бытового травматизма Центрального института травматологии и ортопедии имени Н.Н. Приорова в 1980 г, я был направлен на четырёхмесячную стажировку в Курганский научно-исследовательский институт клинической и экспериментальной травматологии и ортопедии, где были организованы курсы на кафедре усовершенствования врачей по илизаровской системе лечения переломов и деформаций. Данный период стал значимым этапом в моей жизни, поскольку позже меня пригласили в Турцию (Трабзон. Кафедра травматологии и ортопедии Медицинского факультета Черноморского технологического университета) для внедрения системы Илизарова. Дело в том, что Турция приобрела лицензию на изготовления аппарата Илизарова и продавала их во многие страны Африки. Дизайн аппарата был лучше, чем в СССР, а натягиватель спиц они сконструировали технологически удобным в эксплуатации. К занятиям и показательным операциям по Илизарову турецкие коллеги проявили большой интерес и илизаровские методики приобрели в Турции большую популярность, хотя их медицина давно ориентирована на немецкую.

Из личных воспоминаний: *В Кургане с утра проходили интенсивные теоретические и практические занятия. В первые же дни я попытался зайти к самому Г.А. Илизарову, но перед его кабинетом ежедневно было очень большое число пациентов из разных республик Советского Союза и даже других стран мира. Выстоять эту очередь не представлялась мне возможным, пропуская занятия. Поэтому однажды я передал записку секретарше Г.А. Илизарова о своем желании встретиться с ним. Вскоре я был приглашен на встречу в кабинет легендарного Гавриила Абрамовича.*

Более часа продолжалась встреча. Илизаров благодарно вспомнил мою статью о дистракции в хирургии кисти, сказав: «Я еще до кисти не добрался». Узнав, что я из Дагестана, Гавриил Абрамович заинтересовался всем, что происходит в Дагестане. Говорил, что «он сможет без единого рубля из бюджета Дагестана построить на берегу Каспия Центр травматологии и ортопедии для всего Северного Кавказа, если возжелает Дагестан, ибо к нему с очень большим почтением относятся председатель правительства Советского Союза Николай Иванович Рыжков». В тот же день Г.А. Илизаров отправил заказное письмо Магомедову М.М. – председателю Госсовета Дагестана, но я не удостоился ответа. Повторно из Москвы отправил такое же письмо и снова странное молчание…

В завершение той встречи я подарил Гавриилу Абрамовичу чернильный набор унцукульского производства, чему он очень обрадовался. А он подарил мне фотографию с надписью: «Родному Дагестану и замечательному многонациональному народу страны гор с любовью и с самыми добрыми пожеланиями». Позже, при встрече в Москве, Гавриил Абрамович спросил меня: «Мне выделили штаты. А не хочешь ли поехать со мной в Курган заведовать новым отделением?». Я благодарно обнял великого земляка и остался работать в ЦИТО, ибо должен был думать не только о себе, но и о семье: жене-профессоре мединститута и о дочке-студентке мединститута. Не легко привычное менять на неизвестное.

На мой взгляд, открытие «Биологический феномен (эффект) Илизарова» заслуживает Нобелевской премии, однако коммунистическая идеология ошибочно считала, что Ленинская премия (Илизаров был удостоен этой премии в 1978 г.), престижнее любой (в том числе и Нобелевской) «капиталистической» премии. Данный постулат тогдашнего времени помещал быть лауреатом Нобелевской премии и гениальному конструктору космических кораблей Сергею Павловичу Королеву, челюсть которого была сломана сапогом надзирателя во время репрессии, глумления и избиения его в застенках сталинского ГУЛАГа. Прискорбно, когда политики, диктаторы курируют науку и командуют над учеными.

Ассистировать на операции Г.А. Илизарову побаивались многие коллеги, ибо была у Гавриила Абрамовича привычка резко, громко и публично выражать свое недовольство. Но я сам навязался к нему ассистировать, и в предоперационной во время мытья рук, полушутя и полусерьёзно сказал ему: «Гавриил Абрамович! Мы же земляки и поэтому должны показать всем культуру и дружбу дагестанцев; поэтому не стоит нам друг на друга ругаться при других». Конечно, я рисковал, но он был человек с юмором, понял мою хитрость, улыбнулся и мы работали всегда очень коллегиально во время операции.

Стоит сказать, оперировал Илизаров виртуозно и чрезвычайно смело, но обдуманно. У него было фантастическое объемное мышление и представление о ходе и исходах операции. Особо запомнилась одна методика. Была у Гавриила Абрамовича наивысшая (навязчивая) идея: «Пока жив, – рассуждал он, – я должен определить возможности и пределы моей системы». И поэтому Илизаров брался лечить самых сложных пациентов с самыми невероятными деформациями. У него лечился даже гениальный композитор Дмитрий Шостакович, который написал письмо Генеральному секретарю ЦК КПСС Л.И. Брежневу о Гаврииле Илизарове. Затем последовал звонок министра здравоохранения СССР, академика Б.В. Петровского. После этого Гавриила Абрамовича пригласили в ЦИТО и уже сам М.В. Волков вынужден был публично предоставить слово Илизарову Г.А. для выступления. Большой актовый зал ЦИТО был переполнен и с восхищением слушали коллеги столицы в течение двух часов выступления Г.А. Илизарова с показом диапозитивов рентгеновских снимков пациентов до и после лечения. Это было его историческим выступлением.

Г.А. Илизаров не был «легким» человеком. Вообще, гениальные люди не бывают «удобными, сладкими и всепрощающими». Он уволил врача, который своей маме с переломами лодыжек по дежурству наложил гипсовую повязку и отпустил домой. Илизаров правомочно считал, что для использования традиционных способов лечения нужно работать не в научно-исследовательском институте, а в городской больнице.

Г.А. Илизаров начал разрабатывать лечение врожденного вывиха бедра с использованием аппарата своей конструкции. Это очень сложная патология. Со мной тогда в Кургане стажировался и руководитель детского и подросткового отделения Горьковского (ныне- Приволжский Федеральный Медицинский Исследовательский Центр (ПФМНЦ) в Нижнем Новгороде) научно-исследовательского института травматологии и ортопедии А.А. Абакаров (дагестанец). Он был признанным специалистом среди детских ортопедов Совестного Союза. Он поинтересовался методикой Илизарова по лечению пациентов с врождёнными вывихами бедра. Не стану в деталях описывать эту сложную методику, но подчеркну, что Г.А. Илизарову удавалось при этой патологии создавать новую опору для проксимального отдела бедра, удлинить так ногу так, что пациенты переставали хромать. Абакар Алиевич был в восторге от этой идеи и позже сам успешно и первым в стране использовал многие идеи Гавриила Илизарова.

Однажды, когда Г.А. Илизаров проводил спицу в проксимальный отдел бедра, она (спица) застряла. Обратившись ко мне, он спросил: «Магомед, где наша спица?». Недолго думая, прозвучал мой ответ: «Гавриил Абрамович! Кажется, она чуточку запуталась по дороге и ушла кзади головки бедра; переправить бы её кпереди». Илизаров так и поступил. Операция завершилась благополучно. Надо знать, что никто и никогда не смел ему возразить, но тут он спокойно выслушал меня и даже поблагодарил после операции.

Теплые воспоминания об Илизарове со стороны друзей. Великому Илизарову тоже пришлось преодолеть огромные препятствия, сложности, трудности и даже коварства прежде, чем он получил Всесоюзное и Всемирное признания. Нейрофизиологи считают, что «Зависть болезнью мозга», а в христианстве зависть считают «одним из семи смертных грехов». К сожалению, наука и медицина тоже не свободны от зависти, подлости, лжи и даже коварства.

Расул Гамзатович и Гавриил Абрамович были в большой дружбе между собой. Стихотворение Расула Гамзатова, посвященные Гавриилу Илизарову, было опубликовано в главной газете Советского Союза- «Правда».

Гавриил Илизаров, искусный лукман,
Я приеду в Курган, но не в гости,
А затем, чтоб любви, пострадавшей от ран,
Ты срастил перебитые кости.
Кто удачи тебе подарил талисман,
Мне гадать лишь даётся свобода:
Может, горный Урал, может, наш Дагестан,
Где приписан ты к небу от рода?
Как в бою отступать заставляя недуг,
На печаль заработал ты право,
Ведь излечивать вывих душевный, мой друг,
Тяжелее, чем вывих сустава.
Знай, в студенты твои перешёл бы сам Бог,
Если б ты, не жалея усилий,
Связь времён, Гавриил, восстанавливать мог,
Словно связки людских сухожилий.
А в Курган я приеду, зови не зови,
И скажу: «Моё сердце утешь ты,
Человек, превеликою силой любви
Возвращающий людям надежды» (1984 г.).

Писатель и академик Б.Ш. Нувахов в своей книге об Илизарове пишет: «Я был дружен с этим замечательным врачом и ученым. Такие личности рождаются раз в столетие. Бывал у него в Кургане и вместе с ним во многих странах мира и в родном для нас Дербенте. Гавриил Илизаров бывал и у меня в гостях вместе с Расулом Гамзатовым и его супругой Патимат Саидовой. Гавриил Абрамович был замечательным собеседником, человеком с большим достоинством и внутренней свободой. Его влюбленность во врачебную профессию была фантастической». В те же дни в газете «Московская правда» (главный редактор Полторанин М.Р.) была опубликована статья Б.Ш.Нувахова (он тоже дагестанец) с разоблачением коварства М.В. Волкова.

Директор ЦИТО (Центрального института травматологии и ортопедии имени Н.Н. Приорова), академик АМН СССР М.В. Волков выразил мне (дагестанцу) не удовольствие этими публикациями, что стало для меня серьезным тормозом в научной и служебной карьере. Талантливый ученый,

главный травматолог-ортопед Минздрава СССР М.В. Волков, к сожалению, стал тогда главным оппонентом Г.А. Илизарова, что негативно отразилось и на судьбе самого Мстислава Васильевича. Жаль, ибо вместе они могли бы поднять советскую травматологию и ортопедию на невиданные высоты в планетарном масштабе. Тут роковую роль сыграли и «друзья» директора ЦИТО тоже. Лучше иметь одного друга с правдой, нежели сотни с лестью.

Вместе с тем нельзя не отметить теплое отношение А.А. Каплунова к Илизарову, посвятившего ему книгу «неизвестный Илизаров: штрихи к портрету» (Записки очевидца), где А.А. Каплунов повествует о годах совместной работы автора с известным российским ученым хирургом-новатором академиком Г. А. Илизаровым. В ней раскрыты черты личности Илизарова как человека, наставника и руководителя на этапе становления разработанного им метода лечения и обретения первого признания в отечественной медицинской науке. А.А. Каплунов так высказался об Илизарове: «Это крупная личность, самобытный гений и великий врач XX века».

Дмитрий Дмитриевич Шостакович, великий композитор и музыкант, пациент Илизарова следующим обращением характеризует отношение к Г.А. Илизарову: «Мне дорог Гавриил Абрамович Илизаров, и я с большим уважением отношусь к его талантливым сотрудникам. Гавриил Абрамович обладает удивительным даром: возвращать людям здоровье, работоспособность, радость. Он не просто врачует болезнь, он исцеляет человека. Было бы отрадно, если бы у нас в стране побольше работало таких преданных медицине и людям одарённых врачей, как приверженцы учения Илизарова».

Разделяю восторг профессора Л.Д. Воронцова, утверждавшего, что «кость, доселе считавшаяся мало податливым органом, в руках умельцев при применении компрессионно-дистракционного остеосинтеза превращается чуть ли не в глину, какую-то пластическую массу, легко поддающуюся изменению. Я в полном восторге от успехов, достигнутых школой Илизарова!».

Г.А. Илизаров был фанатично влюблен в свою систему, работал без устали, много ездил по многим странам мира с лекциями и для проведения показательных операций. «Гений – это высшая способность концентрировать внимание на изучаемом предмете», – считал Иван Павлов, лауреат Нобелевской премии, физиолог.

По мнению Э.И. Илизаровой-Абаевой, «Илизаров – реформатор, изменивший представление о костной хирургии второй половины XX столетия». Приведу цитату из выступления Заслуженного деятеля науки РФ, заведующего кафедрой травматологии, ортопедии и медицины катастроф Московского государственного медико-стоматологического университета имени А.И. Евдокимова, профессора Василия Иосифовича Зоря в Москве, проходившее 4 ноября 2017 года на Международной конференции «TRAUMA-2017»: «Гавриил Илизаров – апостол травматологии и ортопедии. Такого гения не было, нет и не предвидеться в травматологии и ортопедии».

Из цитат самого Илизарова: «С годами испытываю не только ничуть не затухающий, но постоянно растущий интерес к своей профессии, своем делу. И, конечно, свойственное настоящему врачу чувство ответственности за результаты своего труда. Стремление видеть, как можно больше людей счастливыми».

Выводы и предложения. Несмотря на то, что метод Илизарова, очевидно, очень мощный инструмент, необходимо понять причины его широкого спектра показаний и его текущей полезности в Советском Союзе и других странах Восточной Европы. Большую роль в развитии метода Илизарова сыграли финансовые и технологические ограничения Советов в обеспечении пациентов достаточным количеством соответствующих антибиотиков. Отсутствие антибиотиков, с одной стороны, создавало значительные заболевания костей и суставов для лечения и, с другой стороны, ограничивало определенные варианты лечения. Риск глубокой инфекции, осложняющей внутреннюю фиксацию даже при закрытых переломах или чистой, плановой операции, был и остается высоким.

Существует острая нехватка товаров и услуг. У Советов не было ни технологии, ни финансовой поддержки протезных устройств, которые сделали бы ампутацию разумной альтернативой для некоторых проблем с мышечным скелетом. Хирургически спасти сильно деформированную конечность дешевле, чем ампутировать, даже если это означает месяцы госпитализации и реабилитации за тысячи миль от дома [14].

В 1970-х гг. во всей Европе и Соединенных Штатах произошло возрождение использования внешней фиксации для лечения переломов и деформаций конечностей. Достижения в области материалов и методов уменьшили осложнения мягких тканей, ранее исключавшие использование этого метода. Одновременно в Кургане, в тогдашнем Советском Союзе, Г.А. Илизаров разработал свою методику дистракционного остеогенеза. Это важное достижение способствовало удлинению конечностей, устранению многих осложнений и уменьшению объема хирургического вмешательства. Эта техника сохраняет остеогенные элементы в конечности. Он усовершенствовал высокочастотный, мелкошаговый ритм дистракции, который позволял хорошей кости регенерироваться и уменьшал осложнения мягких тканей, такие как повреждение нервов и сосудов. Этот метод дает хорошее качество костеобразования, сводя к минимуму распространенность неравномерности (требующей дальнейшей костной пластики) или преждевременной консолидации удлиненного

сегмента (требующей повторной остеотомии и остеоклаза). Удлинение сегмента конечности до 140% теперь не только возможно, но и является обычным делом. Своими клиническими наблюдениями и экспериментальными (в КНИИКЭТО мощное экспериментальное отделение с современным оборудованием) опытами Г.А. Илизаров и его коллеги доказали, что срастание переломов при использовании компрессионно-дистракционной системы происходит в два, а то и в три раза ускорено, нежели при лечении переломов с использованием накостных пластин и внутрикостных штифтов. Гавриил Илизаров подчеркивал, что он разработал не только аппарат, но главное- систему компрессионно-дистракционного остеосинтеза.

По мере того, как методы Илизарова осваивались в Европе и США, достижения в области материалов и биомеханики внешних фиксаторов быстро модифицировали методику. Это расширило показания к лечению врожденных и приобретенных пороков развития конечностей. Различные конфигурации внешней фиксации, модифицирующие кольцевой фиксатор на унипланарные и бипланарные рамы и добавляющие трансфиксационные штифты и полуштифты к методам фиксации проволоки, теперь являются стандартными [15].

Осложнения все еще мешают успешному лечению недостатков конечностей. Эти осложнения достаточно предсказуемы, чтобы изменить номенклатуру в литературе по удлинению конечностей. Только те осложнения, которые изменяют прогнозируемый результат, являются действительно «осложнениями». Будущие тенденции к совершенствованию метода Илизарова позволят снизить частоту осложнений.

Метод Илизарова является важным дополнением к арсеналу методов лечения хирурга-ортопеда. Сочетая стабильную внешнюю фиксацию с точной и контролируемой кортикотомией и используя новое для западной медицины понимание биологии удлинения тканей, сложные деформации и значительное неравенство длины конечностей могут быть успешно устранены с помощью малоинвазивной хирургии.

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ПИРОГОВ Н.И. СПАС МЕНДЕЛЕЕВА Д.И. И ПОМОГ МЕЧНИКОВУ И.И.

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PIROGOV N.I. SAVED MENDELEEV D.I. AND HELPED MECHNIKOV I.I.

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Аннотация. Не известно о знакомстве Дмитрия Менделеева с Илья Мечниковым, но судьбе было угодно спасти Дмитрия Ивановича от серьезного недуга и помочь Илье Ильичу продолжить учебу в Европе. Этим третьим гением был великий анатом, хирург и педагог Николай Иванович Пирогов. Кратко о кратких встречах двух гениев с Николаем Пироговым настоящая статья.

Annotation. It is not known about Dmitry Mendeleev's acquaintance with Ilya Mechnikov, but fate wanted to save Dmitry Ivanovich from a serious illness and help Ilya Ilyich continue his studies in Europe. This third genius was the great anatomist, surgeon and teacher Nikolai Ivanovich Pirogov. Briefly about the brief meetings of the two geniuses with Nikolai Pirogov, this article.

Ключевые слова. Мечников Илья Ильич, Пирогов Николай Иванович, Дмитрий Иванович Менделеев, Киев, Германия, Крымская война, туберкулёз, Нобелевская премия, педагогика, химия, хирургия.

Keywords. Mechnikov Ilya Ilyich, Pirogov Nikolai Ivanovich, Dmitry Ivanovich Mendeleev, Odessa, Germany, Crimean war, tuberculosis, Nobel Prize, pedagogy, chemistry, surgery.

Предисловие. **Дмитрий Иванович Менделеев** (1834-1907 гг.) –химик, физик, экономист, педагог, воздухоплаватель, натуралист инициатор создания Русского химического общества, почетный член более 70 Академий наук и научных обществ мира, директор Главной палаты мер и весов; он заложил основы теории растворов, изобрел промышленный способ фракционного разделения нефти и бездымный порох, создал физическую теорию весов, предложил приемы точного взвешивания, восстановил прототипы древнерусских мер- фунта и аршина; интересовался демографией, таможенной и промышленной политикой России, проектировал ледокол для проведения научных исследований в высоких широтах.

Дмитрий Менделеев - величайший ученый в истории человечества, открывший один из фундаментальных законов Естествознания - Периодический закон химических элементов, которому подчинено все Мироздание. 1984 год, по решению ЮНЕСКО, был Годом Менделеева, а журнал “Researche” назвал его «самым великим ученым всех времен”.

Мечников Илья Ильич (1845- 1916 гг.) выдающийся русский и французский биолог, лауреат Нобелевской премии в области физиологии и медицины, первооткрыватель фагоцитоза и внутриклеточного пищеварения, один из основоположников иммунологии, эмбриологии и геронтологии.

Николай Иванович Пирогов- (1810-1881 гг.) - гениальный хирург, анатом, педагог создатель первого атласа топографической («ледяной») анатомии- предтеча компьютерной томографии, основоположник русской военно-полевой хирургии и основатель русской школы анестезии,

О Д.И. Менделееве. Дмитрий Менделеев был последним, семнадцатым ребёнком в семье директора Тобольской гимназии Ивана Павловича Дмитриева. С детства страдал слабым здоровьем. Когда Дмитрию было 10 лет, умер отец и осиротевшей семье помогла Василий Дмитриевич Корнилов- брат матери- Марии Дмитриевны. В. Д. Корнильев переехал в Москву, а небольшой стекольный завод в селе Верхние Аремзяны оставил сестре, но завод сгорел, это и послужило толчком для переезда семьи сначала в Москву, куда семья добралась на лошадях. затем в Петербург, но, ни в Московский, ни в Петербургский университет Дмитрию поступить не удалось. Поэтому он направил документы в Медико-хирургическую академию, но не выдержал пребывания в анатомическом театре, ибо при присутствии на перо же вскрытии трупа ему стало дурно. И получилось по поговорке: «Нет дороги - иди в педагоги». Дмитрий Иванович в 1850-м году поступил в Главный педагогический институт.

В первый год обучения И. Менделеев среди 28 воспитанников оказался на 25-м месте и единственным из однокурсников согласился на повторное прохождение обучения. Через год он оказался уже седьмым, а вскоре, завоевал репутацию одного из самых способных студентов. Ему часто приходилось проводить время в институтском лазарете, и сырой климат Петербурга плохо сказался на здоровье студента. У него открылось кровохарканье. Это был зловещий признак страшной и дольно распространённой в те годы болезни (кавернозный туберкулёз легких- «чахотка»).

*«Лишь только я огляделся по поступлении в институт, я сблизился с Дмитрием Ивановичем. Меня поражало его пристрастие к высшей математике несмотря на то, что он ясно обнаруживал себя физико-химиком. К биологическим наукам он выражал также большое расположение… Однако он не ограничивался науками этого факультета и интересовался науками, проходимыми на историко-филологическом факультете, так как он успевал выбрать время, чтобы быть на лекциях профессоров и того факультета. Кроме того, он посещал мастерскую гальванопластических работ, устроенную в здании Академии наук… От такого широкого и горячего интереса к наукам страдал его физический организм, выражаясь кровохарканьем и расстройством нервов» (*М.А. Папков о Д.И. Менделееве).

Узнав, что у И. Менделеева от туберкулеза умерли отец и три сёстры, институтский врач Кребель не сомневался в диагнозе и прогноза своего не скрывал. Когда в палату, где лежал Менделеев, заглянул директор института, бледный и худощавый студент спал. И сказал врач директору: «Ну, этот уже не поднимется». Придворный медик Н. Ф. Здекауер усомнился в диагнозе и на всякий случай дал пациенту рекомендательное письмо к "находящемуся где-то в Крыму» Николаю Ивановичу Пирогову. Николай Фёдорович Здекауер-ближайший друг Н.И. Пирогова, заслуженный профессор Императорской медико-хирургической академии, лейб-медик, действительный тайный советник.

В Крыму шла война, всюду были раненные. С поездкой в Симферополь связан курьезный случай: по ошибке Гирса - директора департамента Министерства образования Дмитрий Иванович был направлен в Симферополь, находившийся на тот момент в зоне боевых действий. Менделеев после этого наговорил чиновнику дерзостей и дело дошло до того, что мирил их сам министр образования. «Пошел в министерство, да и наговорил дерзостей директору департамента Гирсу. На другой день вызывает меня к себе И. И. Давыдов: «Что ты там в департаменте наделал. Министр требует тебя для объяснений». В назначенный день, к 11 часам утра, я отправился на прием к министру. В приемной было много народу и, между прочим, директор департамента. Я сел в одном углу комнаты, директор в другом. Начался прием. Жду час, другой, третий, ни меня, ни директора к министру не зовут. Наконец, в четвертом часу, когда прием кончился и все ушли, отворяется дверь и из кабинета, опираясь на палку и стуча своей деревяшкой, выходит (он был хромой, после ампутации одна нога у него была на деревяшке) министр Авраам Сергеевич Норов. Он был добрым, но грубоватым министром. Остановившись среди комнаты, посмотрел на меня, на директора и говорит: «Вы что это в разных углах сидите, идите сюда». Мы подошли. Он обратился к директору: «Это что у тебя там писаря делают? Теперь в пустяках напутали, а потом в важном деле напортят. Смотри, чтобы этого больше не было». А потом ко мне: «А ты, щенок. Не успел со школьной скамейки соскочить и начинаешь старшим грубить. Смотри, я этого вперед не потерплю… Ну, а теперь поцелуйтесь». Мы не двигались. «Целуйтесь, говорю вам!» Пришлось поцеловаться, и министр нас отпустил».» (Д. И. Менделеев).

Н.И. Пирогов спас Д.И. Менделеева. Добравшись до Симферополя (октябрь 1855 г.), Менделеев не сразу пошел к Пирогову, а долго выбирал время для визита, потому что Пирогов дни и ночи не отходил от операционного стола. По городу ходили слухи о том, что Пирогов буквально жертвует своим здоровьем ради спасения раненых и что те, равно как и сестры милосердия, его просто боготворят. Говорили, что даже отказывают себе в сне и пище.

Наконец Менделеев собрался с духом и отправился в госпиталь. Визит этот едва не закончился так же, как и давнишнее посещение анатомического театра, обмороком. По свидетельству современников, зал, в котором стояли операционные столы, был буквально залит кровью, не говоря уже о передниках врачей и сестер. Воздух был наполнен стонами и воем, а по сравнению с вонью, смрад симферопольской улицы, казался райским ароматом. По углам комнаты стояли переполненные бочки с ампутированными конечностями. Пирогов оперировал безостановочно: сделав самое важное, оставлял помощников завершать операцию и быстро переходил к другому столу…

Три дня подряд являлся Менделеев в этот зал и каждый раз не осмеливался обратиться к Николаю Ивановичу. Наконец, его заметили сестры, подошли и, узнав, в чем дело, доложили хирургу. Тот ничуть не удивился, попросил подождать и вскоре каким-то чудом нашел возможность обстоятельно расспросить и осмотреть неожиданного пациента. Несмотря на разницу в возрасте (Пирогову тогда было 46 лет, а Менделееву –всего 21 год), они были птицами одного полета — естествоиспытателями до мозга костей, к тому же их характеры и судьбы были на удивление похожи. Оба происходили из простых многодетных семейств (Пирогов был тринадцатым ребенком), оба потеряли в детстве отцов и познали связанную с этим нужду и оба почти мальчиками стали казеннокоштными студентами. Оба, волею судеб, оказались вхожими в сообщество близких А.С. Пушкину людей.

Обоим суждено было испытать несчастную первую любовь. Оба были трудоголиками, оба плохо ладили с людьми, особенно с женщинами, и страдали от непонимания, оба в расцвете сил и таланта будут оторваны от любимого дела.

И. Тихий. **Н. И. Пирогов осматривает больного Д. И. Менделеева**

Диагноз «туберкулез» Пирогов отверг сразу, обругал Здекауэра немчурой и подарил его письмо переставшему от счастья дышать пациенту: мол, вы, батенька, еще нас со Здекауэром переживете. «А что же тогда кашель, слабость, кровохарканье? Отчего они? — А когда это с вами первый раз случилось?» «-В начале учебы был с товарищами в театре. Певицу- итальянку бисировали; сильно кричал…». «-От восторга, значит, тоже проявляется…».

Пирогов предположил наличие у посетителя неопасной сердечной болезни, в целом же счел недомогание следствием многолетнего душевного смятения, переживаний, которые впечатлительный Менделеев перенес, глядя на смерть близких, а главное — мучительной неопределенности последних лет, прошедших под знаком скоротечной болезни. Доктор дал пациенту советы, которыми, наверное, с радостью воспользовался бы сам: «работать всласть, но не переутомляться; побольше гулять и путешествовать и, главное, никогда, ни в чем не перечить своей натуре». «Нате-ка вам, батенька, письмо вашего Здекауера. Сберегите его, да когда-нибудь ему и верните. И от меня передайте, что вы нас обоих переживете». Так оно и вышло.

Дмитрий Менделеев с благодарностью вспоминал ту встречу с Н. И. Пироговым: "**Вот это был врач! Насквозь человека видел".** Менделеев через два года вернулся в Петербург, защитил кандидатскую (1856 г.), а затем и докторскую (1865 г.). А исполнилось тогда Дмитрию Ивановичу всего 31 лет.

Менделеев и Нобелевская премия. В 1905 году Нобелевским комитетом единодушно было принято решение о выдвижении Менделеева на присуждении ему премии. Его кандидатура сразу была включена в «малый список» претендентов, где, помимо Дмитрия Ивановича, были немецкий профессор из Мюнхена Адольф фон Байер и парижский Анри Муассан. В итоге, комитет выбрал кандидатуру фон Байера, объясняя тем, что Байер выдвигался на премию на протяжении 5-ти лет, а Менделеев впервые, и, также, открытая незадолго до этого нулевая группа (инертных газов), дополняющая периодическую систему, не успела получить такой поддержки в комитете и за его пределами коей в течении ряда лет пользовался фон Байер.

В 1906 году Нобелевский комитет присудил премию Менделееву. Решения комитетов обычно не оспариваются, но бывают исключения… В тот

год Шведская королевская академия наук отказалась утвердить это решение, в чём сыграло решающую роль влияние С. Аррениуса, лауреата 1903 года за теорию электролитической диссоциации. Менделеев категорически не принимал гипотезу шведского ученого об самопроизвольном распаде молекул в растворе на ионы. Он, как и многие ведущие ученые того времени, считал предположение Аррениуса абсурдным. В итоге, премию присудили Ф. Муассану за открытие фтора.

Ещё одна причина противостояния Нобелевского комитета против Д.И. Менделеева. Борясь с хищническим потреблением углеводородов, Дм. Менделеев вступает в конфликт с Людвигом Нобелем- старшим братом знаменитого Альфреда. Пользуясь нефтяным кризисом и стремясь к монополии на добычу и перегонку бакинской нефти, Нобели спекулировали слухами об ее истощении. Менделеев доказал необоснованность подобных слухов к неудовольствию Нобеля. Между прочим, именно Д.И. Менделеев еще в 1860-е годы предложил строительство нефтепроводов и доставку с их помощью сырой нефти в Центральную Россию. Однако Нобели, хорошо сознавая выгоду в этом для государства Российского, отнеслись к его предложению крайне отрицательно, поскольку увидели в этом ущерб собственному монополизму. Однако ровно через 20 лет Нобели с успехом внедрили предложение Менделеева как собственное.

Н.И. Пирогов консультировал Д.И. Менделеева. Имеется картина заслуженного художника Украинской ССР Ивана Антоновича Тихого (1927-1982 гг.), на которой профессор осматривает Дмитрия Ивановича. И.А.Тихий долго изучал исторические материалы, письма, фотографии и книги тех лет прежде, чем приступить к работе над картиной «**Н. И. Пирогов осматривает больного Д. И. Менделеева**". В картине Дмитрий Менделеев изображен красивым, голубоглазым, не больным юношей. Картина была выполнена по заказу Министерства здравоохранения СССР в 1964 году и хранится в постоянной экспозиции в Национальном мемориальном комплексном музее Н. И. Пирогова в Виннице.

Если бы эти двое великие не встретились, то может развитие химии в мире сильно затормозилось бы. Для врачей тема встречи Д.И. Менделеева и Н.И. Пирогова имеет вечное измерение, ибо вопрос правильной диагностики стоит остро во все времена.

Помощь Н.И. Пирогова и И.И. Мечникову. И это произошло по стечению следующих обстоятельств, подтверждая народную мудрость «не было бы счастье, да несчастье помогло». Известно, что чиновникам не нравились самостоятельность и независимость Н.И. Пирогова, действовавшего по принципу: «Я люблю Россию, люблю честь родины, а не чины». 13 марта 1961 г. император России Александр 11 издал постыдный указ об увольнения Н.И. Пирогова от должности Попечителя Киевского учебного округа, которому тогда исполнилось всего 50 лет. *Отставку Пирогова была воспринята в России «как большую потерю для народного образования». «Отставка Н.И.Пирогва* мерзейших дел дураков против Руси развивающейся» (А.И.Герцен в журнале «Колокол».

Однако, по настоянию нового министра народного просвещения А.В. Головина (в 1962 г.) Н.И. Пирогову поручили курировать молодых российских ученых стажировавшихся за рубежом». В те годы в Европе находились более 100 русских стажеров. Среди них были И.М. Сеченов, Д.И. Менделеев, А.Г. Столетов, К.А. Тимирязев, С.М. Соловьев, П.В. Киреевский, Л.Н. Модзалевский и другие, ставшие позже выдающимися учеными в разных сферах науки. Как всегда, Николай Иванович взялся и за эту работу тоже творчески, страстно и с огромной пользой для России.

А по субботам Пирогов собирал у себя дома студентов для обсуждения волнующие их вопросы. Н.А.Модзалевский вспоминал «Часто также собираемся у Пирогова. Это наш патриарх. Я ещё не видывал человека столь человечного: так он прост и месте с тем глубок. Удивительно всего, как этот человек таких лет и чинов мог сохраниться во всей чистоте, и притом же у нас на Руси, переживший целое николаевское царствование».

Пирогов сам выезжал в 25 университеты Швейцарии, Италии, Франции и Англии, где учились студенты из России с целью изучения программы и опыта обучения в ведущих университетах Европы. Ходатайство Пирогова сыграло судьбоносную роль в оставлении Дм.Мечникова в Европе для продолжения научного исследования, что завершилось присуждением в 1908 году ему Нобелевской премии за фагоцитарную теорию иммунитета.

После знакомства с выдающимся зоологом того времени Рудольфом Лейкартом у И. Мечникова возникло пламенное желание остаться в Германии для продолжения научной работы. На помощь родителей он не мог рассчитывать. Р. Лейкарт посоветовал И. Мечникову лично обратиться к Н.И. Пирогову, который отнесся к желанию 19-летнего стажера с большим вниманием и выхлопотал ему стипендию Министерства народного просвещения на два года (по 1600 в год). Через год по предварительной договорённости он приехал в Неаполь к Н.И. Пирогову для отчета. Вот как сам И. Мечников описывает эту встречу: «Пирогов принял меня очень любезно, расспрашивал о моих занятиях, о неаполитанской фауне, о моих планах на дальнейшее будущее и при этом выказал себя не начальником, а добрейшим руководителем, симпатичный характер которого запечатлелся у меня на всю жизнь».

В заключении отмечу, что счастье в том, что она на всех драматических серпантинах и трагических поворотах своей истории не

оставалась в одиночестве без гениальных просветителей и отважных пророков! Их всегда было мало, но они были! Гении просвещения и совести нисходят к нам не часто, но Гении всегда, понимая людскую не обустроенность и, освещая сумраки надеждой, взывая к уставшим россиянам с мольбой: не сломаться, встать, идти, продолжать жить и созидать вопреки всему. Россия не всегда будет обездоленной, униженной, отвергнутая, коррумпированной и без устали обворовываемой, ибо она обладает генетикой рождать гениев, героев, и бунтарей, каким был и Николай Иванович Пирогов тоже. Россия, её богатства, перспективы и управления, как никогда ранее, остро нуждаются сегодня и всегда в таких просвещенных, творческих, героических, патриотичных и беспокойных личностях, как Николай ПИРОГОВ!

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THE EFFECT OF MELAXEN ON THE FUNCTIONAL STATE OF THE KIDNEYS, LIPID PEROXIDATION AND METAL ACCUMULATION IN RATS UNDER CONDITIONS OF COMBINED USE OF CADMIUM, LEAD AND ZINC SALTS

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ВЛИЯНИЕ МЕЛАКСЕНА НА ФУНКЦИОНАЛЬНОЕ СОСТОЯНИЕ ПОЧЕК, ПЕРЕКИСНОЕ ОКИСЛЕНИЕ ЛИПИДОВ И НАКОПЛЕНИЕ МЕТАЛЛОВ У КРЫС В УСЛОВИЯХ СОЧЕТАННОГО ПРИМЕНЕНИЯ СОЛЕЙ КАДМИЯ, СВИНЦА И ЦИНКА.

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Abstract. The research presents the effect of melatonin at a dose of 10 mg / kg on the renal water-electrolyte excretory function, on the degree of heavy metals' accumulation in the tubular bones of rats, and the activity of lipid peroxidation and antioxidant cell defense in isolated and combined use of zinc chloride at a dose of 1 and 20 mg/kg, lead acetate at a dose of 20 mg/kg, and cadmium sulfate at a dose of 0.5 mg/kg. It was found that in the intragastric administration of heavy metals, the level of diuresis against the background of melaxen introduction was lower than in the isolated use of metals. The renal electrolyte-excretory function of rats using melatonin in conditions of cadmium, lead and zinc intoxication was characterized by a decrease in the excretion of potassium, sodium and calcium compared to the indicators of the animals with the only metal salts introduction. Proteinuria was observed in all experimental groups, with the exception of the group with combined introduction of zinc chloride at a dose of 1 mg / kg and melatonin. Application of melatonin under conditions of intoxication produced by heavy metals helped to reduce the severity of LPO reactions; decalcification of bones and a decrease in the accumulation of metals in the animals' bone tissue were noted.

Аннотация. В работе представлено влияние мелатонина в дозе 10 мг/кг на водо-электролитовыделительную функцию почек, на степень накопления тяжелых металлов в трубчатых костях крыс и активность перекисного окисления липидов (ПОЛ) и антиоксидантной защиты клеток (АОЗ) в условиях изолированного и сочетанного введения хлорида цинка в дозе 1 и 20 мг/кг, ацетата свинца в дозе 20 мг/кг и сульфата кадмия в дозе 0,5 мг/кг. Выявлено, что при внутрижелудочном введении тяжелых металлов уровень диуреза на фоне введения мелатонина оказался ниже, чем при изолированном использовании металлов. Электролитовыделительная функция почек крыс с введением мелатонина в условиях кадмиевой, свинцовой и цинковой интоксикации характеризовалась снижением экскреции калия, кальция и натрия с мочой по сравнению с показателями в группах животных с введением только солей металлов. Во всех опытных группах наблюдалась протеиниурия, за исключением группы с введением хлорида цинка в дозе 1 мг/кг и мелатонина. Применение мелатонина в условиях интоксикации

тяжелыми металлами способствует уменьшению выраженности реакций ПОЛ, отмечается декальцинация костей и снижение накопления металлов в костной ткани животных.

Key words: heavy metals, cadmium sulfate, zinc chloride, lead acetate, melatonin, lipid peroxidation.

Ключевые слова: тяжелые металлы, сульфат кадмия, хлорид цинка, ацетат свинца, мелатонин, перекисное окисление липидов.

Введение. Известным свойствам тяжелых металлов влиять на функции многих систем и органов посвящено множество научных работ. Избыточное поступление этих экотоксикантов приводит к повреждению печени, почек [1,2,3,4,5], системы крови [6] и кровообращения, дыхательных путей. Однако методов эффективной профилактики интоксикации тяжелыми металлами немного. Одним из них является использование мелатонина, как вещества, оказывающего через множество рецепторов регулирующее влияние на функции почек [8]. Кроме того мелатонин обладает антиоксидантной, противовоспалительной, антиапоптозной и иммуномодуляторной формой активности [7,9,10]. В нашей работе исследованы эффекты мелатонина на функции почек и АОЗ при хронической интоксикации солями кадмия, свинца и цинка в различных дозах и сочетаниях.

Методы исследования. Опыты проведены на крысах самцах линии Вистар массой 200-300г., поделенных на несколько вариантов (n=90): 1) фоновые (интактные) крысы; 2) животные с внутрижелудочным использованием ацетата свинца в дозе 20 мг/кг и мелатонина (препарат «Мелаксен»); 3) крысы с введением хлорида цинка (1мг/кг) + мелатонин; 4) крысы с интрагастральным введением соли цинка в дозе 20 мг/кг + мелатонин; 5) крысы с совместным введением ацетата свинца 20 мг/кг и хлорида цинка 1 мг/кг + мелатонин; 6) животные с введением соли свинца 20 мг/кг и хлорида цинка 20 мг/кг + мелатонин; 7) крысы с внутрижелудочным введением сульфата кадмия в дозе 0,5 мг/кг + мелатонин 8) крысы с сочетанным введением сульфата кадмия 0,5 мг/кг и хлорида цинка 1 мг/кг+мелатонин; 9) крысы с использованием сульфата кадмия 0,5 мг/кг и хлорида цинка 20 мг/кг + мелатонин.

Животные во время эксперимента находились на обычном пищевом рационе и имели доступ к пище и воде. Световой режим - естественный. Эксперименты осуществляли в соответствии с «Международные рекомендации по проведению медико-биологических исследований с использованием лабораторных животных» (1985), 11-ой статьёй Хельсинской декларации Всемирной медицинской ассоциации и правилами лабораторной практики в РФ (приказ МЗ РФ от 01.04.2016 г. № 199). Раствор сульфата кадмия в дозировке 0,5 мг/кг массы (в пересчете на металл), цинка хлорида в дозе 0,1 мг/кг и 20 мг/кг, а также ацетата свинца в дозе 20 мг/кг вводили интрагастрально, ежедневно в течение 30 дней. В качестве препарата мелатонина использовался синтезированный аналог гормона шишковидной железы препарат «Мелаксен» в дозе 10мг/кг, который вводили также с помощью зонда в желудок, каждый день в течение месяца. Для изучения функции почек в условиях спонтанного диуреза животные помещались в обменные клетки, где в течение шести часов у них собиралась моча. По истечении времени эксперимента животные забивались под наркозом с тиопенталом для исследования тканей и плазмы. В плазме крови и моче определялась концентрация креатинина, общего белка, общего кальция на спектрофотометре PV1251C-26 с помощью наборов «АгатМед» (Россия), содержание натрия и калия с помощью пламенной фотометрии на автоматизированном пламенном фотометре ФАП-2. Расчёт показателей водо - и электролитовыделительной функции почек производили по формулам Наточина Ю.В. (1974). В подготовленных минерализованных пробах (по ГОСТ26929 и 30178-96) определялось содержание кальция с помощью спектрофотометра, а кадмия, свинца и цинка - на атомно-абсорбционном спектрофотометре («Квант-АФА»). Содержание белка определяли спектрофотометрически (аппарат СФ-26). Содержание общего кальция плазмы крови измеряли спектрофотометрически с помощью аппарата *PV1251C-26.* Обработка результатов исследования, исходя из количества выборок и нормального распределения рядов сравнения, установленного по критерию Шапиро- Уилка (Wф>> Wm), проводилась с применением критерия «t» Стьюдента с использованием программы *GraphPad Prizm* 6.1. О наличии факторных влияний судили при критическом уровне достоверности (p) меньшем 0,05. Линейный коэффициент корреляции Пирсона (r-Pearson) вычисляли, применяя пакет программ Microsoft (EXEL). Для расчетов и построения графиков использовались программы MICROSOFT EXEL.

Результаты и их обсуждение. Исследования показали, что совместное с мелаксеном использование солей кадмия и свинца способствовало некоторому понижению повышенного (относительно фоновых значений) при изолированном введении металлов, уровня спонтанного диуреза, что было обусловлено замедлением скорости клубочковой фильтрации (СКФ) и повышением канальцевой реабсорбции воды (КРВ) (рис.1).

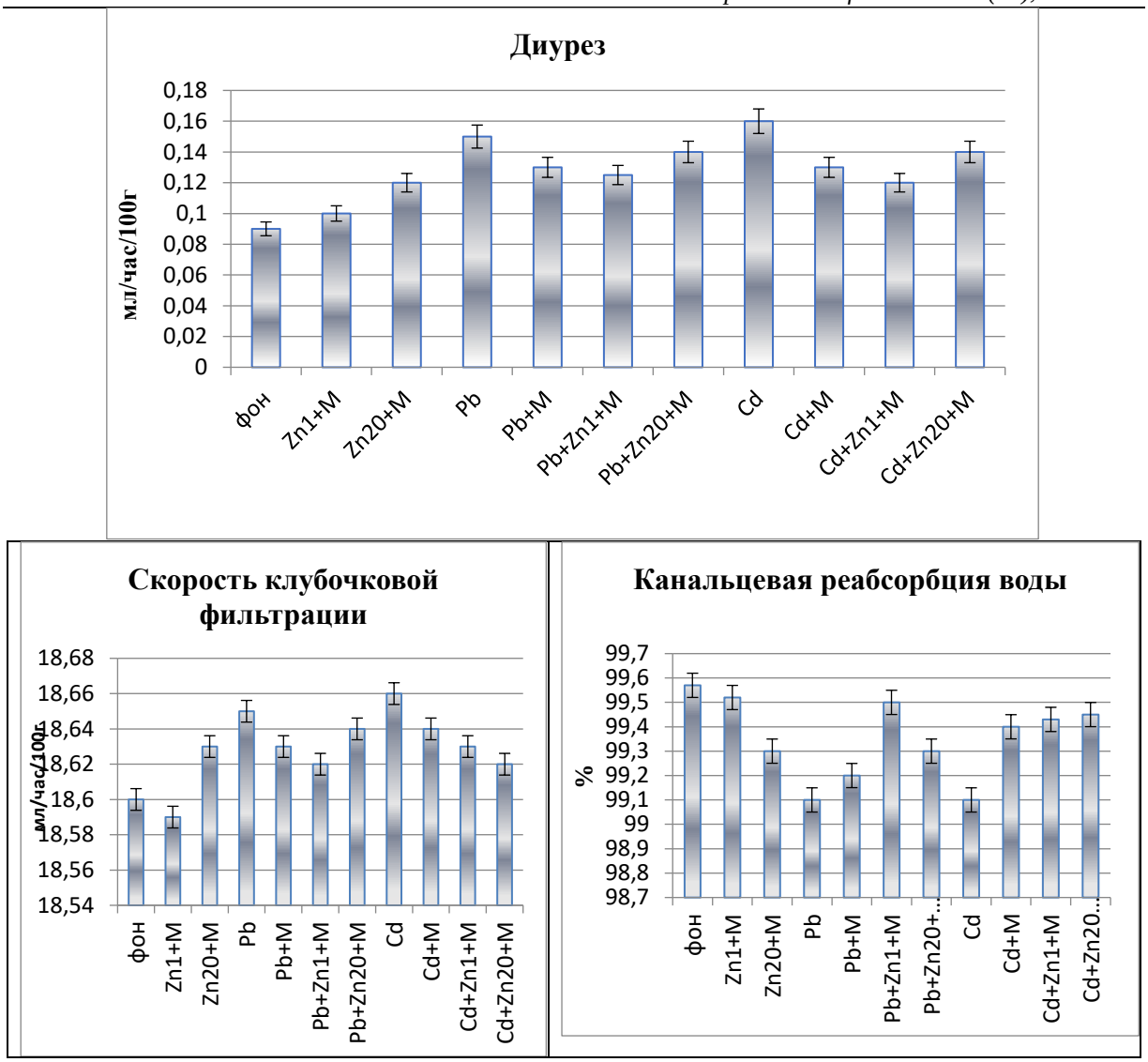

Рис.1. Влияние мелатонина на диурез и водовыделительную функцию почек в условиях хронической интоксикации солями металлов.

В группах с сочетанным введением солей металлов, мелаксена и хлорида цинка в дозе 1 мг/кг и 20 мг/кг также наблюдалась полиурическая реакция, однако менее выраженная, чем при изолированном использовании сульфата кадмия и ацетата свинца.

Электролитовыделительная функция почек у крыс с введением мелаксена на фоне кадмиевой и свинцовой интоксикации характеризовалась понижением ($p<0,001$), относительно значений в опытах с изолированным использованием металлов, экскреции кальция, натрия и калия с мочой, однако, эти показатели оставались выше фоновых значений (табл.1). Использование мелаксена на фоне введения хлорида цинка в дозе 1 мг/кг не вызывало изменений в выведении электролитов, а в дозе 20 мг/кг приводило к увеличению ($p<0,05$), относительно фонового уровня, натрий- калий и кальциуреза.

Табл.1.

Влияние мелатонина на экскрецию электролитов и белка при внутрижелудочном введении сульфата кадмия, ацетата свинца и хлорида цинка у крыс.

	Стат. показатель	Э Ca	ЭNa	ЭK	Э белка
		мкмоль/час/100г			мг/час/100г
Фон	M±m	0,22±0,003	12,4±0,015	6,1±0,14	1,20±0,01
Zn1+M	M±m	0,23±0,06	12,5±0,09	6,19±0,13	1,22±0,02
	P				
Zn20+M	M±m	0,26±0,009	12,6±0,08	7,24±0,07	2,1±0,001
	P	*)	*)	*)	*)
Pb	M±m	0,33±0,005	14,22±0,09	8,12±0,08	2,35±0,04

	P	*)	*)	*)	*)
Pb+M	M±m	0,25±0,003	13,5±0,12	7,93±0,07	2,53±0,05
	P	**)	*)**)	*)	*)**)
Pb+Zn1+M	M±m	0,24±0,011	13,1±0,1	7,34±0,15	2,4±0,005
	P	**)	*)**)***)	*)**)	*)
Pb+Zn20+M	M±m	0,34±0,012	13,67±0,08	7,44±0,014	2,55±0,03
	P	*)	*)**)***)	*)**)***)	*)**)***)
Cd	M±m	0,34±0,001	14,12±0,12	7,92±0,07	2,35±0,41
	P	*)	*)	*)	*)
Cd+M	M±m	0,26±0,009	13,2±0,05	7,35±0,11	2,3±0,04
	P	*)	*)**)	*)**)	*)
Cd+Zn1+M	M±m	0,27±0,007	12,56±0,04	7,22±0,14	2,23±0,05
	P	*)	*)**)	*)**)	*)
Cd+Zn20+M	M±m	0,28±0,01	13,82±0,12	7,45±0,08	2,31±0,03
	P	*)	*)**)***)	*)**)***)	*)

*)–*по отношению к фоновым значениям;*

***) -по отношению к значениям в группах с изолированным введением металлов;*

****) - по отношению к значениям в группах с сочетанным введением солей цинка и мелатонина.*

В группах с кадмиевой и свинцовой интоксикацией на фоне введения мелаксена при дополнительном использовании хлорида цинка в дозе 20 мг/кг также отмечалось повышение (p<0,001) выведения с мочой кальция, натрия и калия относительно фона. Внутрижелудочное введение хлорида цинка в дозе 1 мг/кг в аналогичных условиях способствовало некоторому понижению (p<0,05) экскреции кальция, натрия и калия относительно значений с изолированным использованием солей металлов. Во всех опытных группах отмечалась выраженная протеинурия, за исключением группы с введением мелатонина на фоне хлорида цинка 1 мг/кг (табл.1).

Изучение активности системы ПОЛ у животных почти всех опытных групп выявило увеличение (p<0,001) относительно фоновых значений, содержания малонового диальдегида (МДА) в эритроцитах и накопление гидроперекисей (ГП) в плазме крови (табл.2). Угнетение АОЗ в этих опытах проявилось снижением активности супероксиддисмутазы (СОД) и каталазы. Интактными оказались эти показатели у крыс с применением хлорида цинка в дозе 1 мг/кг на фоне введения мелатонина. Следует отметить, что в группе с введением хлорида цинка в дозе 1 мг/кг на фоне использования мелатонина при кадмиевой и свинцовой интоксикации содержание ГП и МДА повышалось, но было ниже, чем при изолированном применении сульфата кадмия и ацетата свинца соответственно. Уровень СОД и каталазы в группе с сочетанным применением мелатонина и хлорида цинка в дозе 1 мг/кг на фоне введения соли кадмия несколько повышался (p<0,05), относительно пониженных изолированным введением металла значений (табл.2.).

Табл.2

Влияние мелатонина на активность ПОЛ и состояние АОЗ при интрагастральном введении сульфата кадмия, ацетата свинца и хлорида цинка у крыс.

	Стат. показатель	ГП	МДА	СОД	Каталаза
		мкмоль/л		(ед.инг.)	(Me/гHb)
фон	M±m	4,1±0,09	27,2±0,35	65,7±0,15	6,1±0,28
Zn1+M	M±m	4,11±0,08	27,4±0,9	65,8±0,18	5,9±0,83
	P				
Zn20+M	M±m	4,7±0,07	28,2±0,085	62,4±0,22	5,52±0,06
	P	*)	*)	*)	*)
Pb	M±m	5,2±0,12	28,42±0,07	60,14±0,8	5,3±0,09
	P	*)	*)	*)	*)
Pb+M	M±m	4,95±0,012	28,24±0,05	61,13±0,18	5,5±0,07
	P	*)	*)	*)	*)
Pb+Zn1+M	M±m	4,8±0,042	27,23±0,14	61,4±0,85	5,32±0,13
	P	*)**)***)	*)**)***)	*)	*)
Pb+Zn20+M	M±m	5,42±0,04	29,8±0,7	61,3±0,72	5,1±0,81
	P	*)***)	*)**)***)	*)	*)
Cd	M±m	5,3±0,04	28,12±0,08	62,13±0,9	5,5±0,1
	P	*)	*)	*)	*)
Cd+M	M±m	4,93±0,2	28,3±0,1	63,5±0,3	5,6±0,123
	p	*)	*)	*)	*)
Cd+Zn1+M	M±m	4,79±0,03	27,35±0,09	64,7±0,3	5,13±0,04

	P	*)**)***)	*)	*)**)***)	*)**)
Cd+Zn20+M	M±m	5,5±0,12	30,9±0,05	61,5±0,83	5,21±0,09
	P	*)**)***)	*)**)***)	*)	*)**)***)

*)**)***) - значения как в табл.1.*

Содержание цинка в костях животных в группах с сочетанным применением используемых солей металлов на фоне введения мелатонина значительно увеличивалось (p<0,001) и сопровождалось декальцинацией костной ткани (табл.3).

Табл.3.

Влияние мелатонина на содержание кальция, цинка, свинца и кадмия в костях при внутрижелудочном введении сульфата кадмия, ацетата свинца и хлорида цинка у крыс.

	Стат. показатель	Zn мг/100г	Cd мг/100г	Pb мг/100г	Ca г/100г
фон	M±m	67,7±1,2	0,03±0,001	19,1±0,03	250±3,2
Zn1+M	M±m	87,3±0,4	-	-	248±1,3
	P	*)			
Zn20+M	M±m	102,1±1,2	-	-	210±1,2
	P	*)			*)
Cd	M±m	-	0,1±0,004	-	149±1,12
	p		*)		*)
Cd+M	M±m	-	0,09±0,001	-	151,5±0,85
	P		*)		*)
Cd+Zn1+M	M±m	85,4±0,9	0,12±0,002	-	155,3±1,21
	P	*)	*)**)		*)**)***)
Cd+Zn20+M	M±m	103,2±0,83	0,17±0,04	-	144,8±0,95
	P	*)	*)**)		*)**)***)
Pb	M±m	-	-	51,3±0,8	153,5±0,9
	P			*)	*)
Pb+M	M±m	-	-	50,9±0,48	155,2±1,1
	P			*)	*)
Pb+Zn1+M	M±m	88,5±0,5	-	98,5±0,5	160,3±1,2
	P	*)		*)**)***)	*)**)***)
Pb+Zn20+M	M±m	99,75±0,35	-	99,75±0,35	159,5±0,95
	p	*)		*)**)***)	*)**)***)

*)**)***) - значения как в табл.1.*

Содержание кадмия в костях крыс с использованием сульфата кадмия и хлорида цинка в разных дозировках на фоне применения мелатонина значительно повышается (p<0,001) и относительно фона и относительно значений в группе с применением только соли кадмия (табл.3). В этих же группах отмечается понижение (p<0,001) кальция в костном матриксе, наиболее выраженное при введении соли кадмия и хлорида цинка 20 мг/кг и мелатонина. Значительное увеличение (p<0,001) по сравнению с фоном, содержания свинца в костях характерно и для животных, получавших ацетат свинца на фоне введения мелатонина и еще более выраженное при использовании комбинации хлорида цинка в дозе 20 мг/кг с мелаксеном и солью свинца (табл.3). Также характерна выраженная потеря кальция костной тканью у животных этих групп по сравнению с фоновыми значениями и относительно показателей с введением ацетата свинца на фоне только мелатонина.

Таким образом, влияние мелатонина на функции почек у крыс в условиях интоксикации солями цинка, свинца и кадмия проявляется в некотором уменьшении диуреза и экскреции кальция, натрия и калия по сравнению с показателями в группах с изолированным введением металлов, а также снижении выраженности реакций ПОЛ и степени накопления тяжелых металлов в костях.

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EPIDERMAL GROWTH FACTOR RECEPTOR, HUMAN TELOMERASE SUBUNIT - HTERT, PROTEIN KINASE D1 AND P16^{INK4A} IN NORMAL KERATINOCYTES AND PREMALIGNANT LESIONS OF SKIN AND ORAL CAVITY.

Abstract. Identification of *mutations* as markers for early malignant transformation could be more appropriate not only for early diagnosis of cancer but could potentially influence treatment strategies in HNC, since mutations in EGFR and Ras genes are closely associated with resistance to cancer treatment. Mutations in Ras, p53 (early), the gene of **EGFR** (in BCCs not detected), and **HTERT** promoter could be used as markers of cancer transformation. Mutations in p53 are detected early in PMDs, associated with high risk for early transformation into Oral SCC. Mutations in Ras are not detected in Potentially Malignant Disorders (PMDs), with exception of Actinic keratoses (AK), Keratoacanthomas (KA) and papillomas. Since increased expression of hTERT is an early event in the pathogenesis of hyperproliferative skin deseases, overexpressed hTERT is considered as a proproliferative (proinflammatory) marker, rather than cancer marker, in contrary to its mutations. Mutations in *HTERT* are detected in both Spinocellular carcinoma (SCC) and Basocellular carcinoma (BCC), UV-signature.

Increased expression mainly of **MMP-9** and **MT1-MMP (MMP-2),** are now considered as markers for aggressive cancer phenotype in both cancers. Using EMT markers (vimentin, fibronectin, N-cadherin, vs, E-cadherin; and transcriptional factors - Snail, Slug, Twist; HIF-1α, α-SMA), we could not differentiate late PMDs of early cancer lesions. These markers are useful for detection of aggressive alteration in tumour pathogenesis, which is of importance when a surgical procedure is planned. **COX-2** stain was highest in SCCs and aggressive BCCs. Increased expression of PKD1 was detected in BCCs in contrary to SCC. There is no currently data for the expression of **PKD1** in PMDs, leading to SCC, nor for detected mutations in PKD1 gene in SCC and BCCs. PKD1 is upregulated and down-regulated in BCC and SCC, respectively. We speculate here that the molecular mechanism - increased NFκB-hTert-PKD1-NFκB-hTert, resulting in p16^{INK4a} mutations and turn of PKD1 function, is connected with the progression of chronic inflammation in cancer development.

Keywords: PKD1, Protein Kinase D1, hTert, EGFR, p16^{INK4a}, mutations, MMPs, Metalloproteinases, PMDs, Potentially Malignant Disorders, Leukoplakia, Erythroplakia, Actinic keratose (AK), Keratoacanthoma (KA), SCC, Spinocellular carcinoma, BCC, Basocellular carcinoma;

Abbreviations:

1. PMDs - Potentially Malignant Disorders
2. SCC - Squamous cell carcinoma (Spinocellular carcinoma)
3. oSCC – oral SCC
4. cSCC – cutaneous SCC
5. NHSCC – Head and Neck SCC
6. HNC – Head and Neck Cancer
7. BCC - Basal cell carcinoma (Basocellular carcinoma)
8. EMT - Epithelial to Mesenchymal Transition
9. MET - Mesenchymal to Epithelial Transition
10. EGFR (HER1, ErbB1) - Epidermal Growth Factor Receptor
11. EGF - Epidermal Growth Factor
12. HB-EGF - Heparin-binding EGF
13. AR - Amphiregulin
14. TGF-α - Tumor (Transforming) Growth Factor-alpha
15. MAPK - Mitogen-Activated Protein Kinase
16. ERK1/2 - Extracellular signal Regulated Kinase ½
17. PI3K – phosphatidylinositol 3-kinase
18. mTOR - mammalian Target of Rapamycin
19. STAT - Signal Transducer and Activator of Transcription
20. HH - Hedgehog
21. PTCH - Patched
22. SMO - Smoothened
23. GLI - Glioma Associated
24. HIF1 - Hypoxia Inducible Factor-1
25. PKC – Protein Kinase C
26. PKD1 – Protein Kinase D1
27. *PRKD1* - PKD1 gene
28. NF-κB - Nuclear Factor kappa B
29. MMPs – Matrix Metalloproteinases
30. COX-2 - Cyclooxygenase-2 (PGHS-2 - Prostaglandin H Synthase)
31. hTERT - Human telomerase catalytic protein subunit

Introduction:

Oral PMDs (potentially malignant disorders) are known as leukoplakia, erythroplakia, lichen planus, submucous fibrosis, actinic cheilitis (cheratosis) and palatal keratosis associated with inverted smoking, discoid lupus erythematosus, Marjolin ulcer, immunodeficiency in relation to cancer predisposition and some inherited cancer syndromes.[1,2] The most common are leukoplakia, erythroplakia, lichen planus and submucous fibrosis. Leukoplakia is a clinical presentation that is defined by the WHO (World Health Organozation) as a "white patch or plaque that cannot be characterized clinically or pathologically as any other disease" and is, by far, the most common precancer, accounting for over 80% of potentially malignant oral disorders. In addition, leukoplakia is also a relatively common oral lesion, ranging from 1 to 5% in the general population. However, it does not have the highest malignant transformation risk among precancerous lesions. Oral erythroplakia, a relatively rare (reported incidence: 0.02–0.80%) red lesion of the oral cavity that cannot be removed, has a much higher malignant transformation risk than leukoplakia; up to 50% of these lesions are invasive OSCC and 40% are carcinoma *in situ.*[3]

Evaluation of an asymptomatic patient for early-stage cancer, based on its physical features alone, is frequently compromised because malignant and benign lesions may not be clinically distinguishable. Consequently, approximately 60% of oral cancers are advanced by the time they are detected, and approximately 15% of patients have another cancer in a nearby area such as the larynx, esophagus or lungs. Early diagnosis of oral cancer plays a key role in disease progression, treatment response, and ultimately, quality of life and patient survival. Therefore, there is a need to identify and use molecular biomarkers to evaluate individuals with potentially malignant disorders who are at a high risk of developing OSCC and those with early-stage malignant lesions.[3]

Alterations in genes and pathways that regulate cellular signaling, cell cycle, proliferation, differentiation, apoptosis, genomic stability, motility, angiogenesis and metastasis are significantly associated with development and progression of a potentially malignant disorder to OSCC. Aberrant expression and function of molecules involved in these signaling networks have been considered as biomarkers for risk assessment of malignant transformation. These biomarkers, includes increased expression of hTERT (Human telomerase catalytic protein subunit), EGF-R (Epidermal Growth Factor Receptor) (and its ligands - EGF (Epidermal Growth Factor), TGF-α (Tumor Growth Factor-alpha), HB-EGF (Heparin-binding EGF) and AR (Amphiregulin) (see below))), upregulated NF-*k*B closely connected with increase in COX-2 (Cyclooxygenase-2; PGHS-2 - Prostaglandin G/H Synthase) expression.[4] COX-2 is a target gene of NF-*k*B and COX-2 stain was found increasing from hyperplasia to dysplasia and was highest in squamous cell carcinoma.[5] Its expression was significantly higher in the infiltrating pattern of BCC compared with the nodular and superficial subtypes in the primary BCC group. Furthurmore expression was significantly higher in the recurrent BCC group than in the primary BCC group.[6]

Although there is some but not significant advance in the understanding of molecular mechanism connected with the development of PMDs and their progression to cancer (in oral cavity mostly to SCCs), there is almost no advanced in their diagnostic markers or in their treatment strategies, consequence of which is often their malignisation or cancer development.

Squamous cell carcinoma (SCC) is the second most common cancer arising in the head and neck with

devastating effects on communication, swallowing, and, most importantly, survival. This tumor is occurring most commonly in the oral cavity, oropharynx, hypopharynx, and larynx, arising from the spinous layer of the skin and epitelium. Sun exposure (UV- light), ionizing radiation, papilloma viruses (PPV) infection, immune suppression, and chronic inflammation (or chronic trauma-mucosa) may lead to development of SCC.[7] Men are at greater risk than women with the two greatest risk factors consistently being tobacco and alcohol use.[8] In 2011, there were over 11,460 deaths from head and neck squamous cell carcinoma cancers (HNSCC) in the United States and over 300,000 deaths worldwide. Most HNSCC patients present with stage III/IV disease have a 5-year survival rate below 40%. HNSCC patients with metastatic disease have extremely poor prognosis and a survival rate of less than 10%.[9]

Oral and Cutaneous squamous cell carcinoma is a malignant tumor that can occur in normal skin and oral mucosa, but commonly evolves from precursor lesions pre-cancer or PMD (potentially malignant disorders) (see above). Oral epithelium differs from the skin only by the lack of stratum corneum.

Basal cell carcinoma (BCC) are slow-growing, locally invasive, rarely metastatic epidermal skin tumours which mainly affect white skinned people. This type of cancer is often located on the face and neck and is difficult to limit clinically. It can significant local destruction and disfugurment if treated inadequatly. It is consider to arise from multipotential (stem) cells within the basal layer of the epidermis or follicular structures and can develop without a premalignant lesion in both hereditary and sporadic fashion. It can significantly more rarely (contrary to SCC) develop on the basis of precancerous lesions, including: post-radiation dermatitis (60% of cases transforms into BCC); nevus sebaceous (transformation into BCC is 15%); actinic keratosis (transformation into BCC is 10%); chemical keratosis, xeroderma pigmentosum; inflammatory changes with scars and hypertrophied scars after burn injuries.[10]

Except UVB, ionizing radiation, chemical carcinogen (e.g. arsenic) and possibly infectiones with human papillomaviruses, have been associated with BCCs development. Some common deseases as post-organ transplant patients (most frequently after heart and renal transplantation), pharmacological immunosuppression combined with UVB radiation, leukaemic patients are also risk factors. Global statistics unanimously indicate that BCC is one of the most common neoplasms in Europe, Australia and the USA, and the number of new cases is increasing every year. Only in the USA, more than one million cases of skin cancers are detected every year (American Cancer Society, 2008).[10,11]

Epidermal Growth Factor Receptor (EGFR) in skin and oral pathology

EGFR is mapped to chromosome 7 short arm q22, spanning 110 kb of DNA divided into 28 exons. In normal cells, the expression of EGFR is estimated to be from 40,000–100,000 receptors per cell, whereas overexpression of more than 10^6 receptors per cell is observed in cancer cells. EGF regulates its own receptor, as it increases EGFR RNA expression by stimulating the expression of ETF (EGFR-specific transcription factor). Other proteins that modulate the EGFR promoter include E1A, Sp1, and AP2. The interaction between DNA topoisomerase I and c-JUN has also been shown to regulate EGFR gene expression.[12]

Activation of the epidermal growth factor receptor (EGF-R) a receptor tyrosine kinase, has been shown to stimulate cell cycle progression of normal epidermal keratinocytes. In normal skin, the EGF-R (also known as HER1 or ErbB1) is most strongly, although not exclusively, expressed in the basal layer of the epidermis, consistent with the involvement of the EGF-R in epidermal growth control. A lot of observations indicate that abnormalities in expression of the EGF-R and/or its ligands EGF (Epidermal Growth Factor), TGF-α (Tumor Growth Factor-alpha), HB-EGF (Heparin-binding EGF) and AR (Amphiregulin) are common features of hyperproliferative (PMDs) and neoplastic epithelia. For example, in psoriatic epidermis, the EGF-R is overexpressed not just in the basal layer, but in all nucleated strata of the epidermis, consistent with the suprabasal proliferation that occurs in this disease. Furthermore, both TGFα and AR are found at elevated levels throughout the nucleated layers of psoriatic epidermis. In squamous carcinomas, overexpression of the EGFR is commonly observed consistent with the view that EGFR signaling is upregulated and constitutive in such tumors. Furthermore, epithelial neoplasms frequently coexpress high levels not only of the EGF-R but also of its ligands, EGF, TGFα or AR thereby creating constitutive autocrine loops dependent on the EGF-R. Direct support for a role of EGFR activation in the development of skin tumors comes from studies in transgenic mice, in which overexpression of TGF-α targeted to the epidermis elicits hyperplasia, hyperkeratosis, papillomas, and squamous cell carcinomas.[13,14]

EFGR is involved in multiple downstream signaling pathways influencing cell growth, angiogenesis, and invasion. Downstream EGFR signaling activates the mitogen-activated protein kinase (MAPK) pathway (Ras/Raf/MEK/ERK1/2), p38 MAPK, JNK as well as the phosphatidylinositol 3-kinase (PI3-K)/protein kinase B (Akt) pathway. Activation of the MAPK pathway leads to increased expression of antiapoptotic proteins like Bcl-x2 and inhibition of proapoptotic proteins like BAD. Signaling through the PI3-K/Akt pathway ultimately leads to inhibition of the tumor suppressor gene p53.[14] All these results pointed the role of EGFR in a proliferative state and inhibition of tumor suppressor function.[15] Other pathways which are activated from EGFR are phospholipase PLCγ – PKC/PKD1, Src and STAT. Amplified EGFR signaling induces uncontrolled cell growth and a malignant phenotype.[16]

The enhanced EGFR expression on the keratinocytes in OLP (Oral Lichen Planus) lesions and

the up-regulation of EGF-like ligands in keratinocytes and infiltrating mononuclear cells could contribute to the carcinogenesis and pathogenesis of OLP. Of the receptors, only EGFR mRNA and protein were more highly expressed in OLP compared with NOM (normal oral mucosa) tissues. Regarding the ligands, the mRNAs of Amphiregulin (AREG), Epiregulin (EREG), and Heparin-Binding EGF-like growth factor (HB-EGF) were more highly expressed in OLP compared with NOM tissues. These ligands were strongly expressed by infiltrating lamina propria lymphocytes as well as epithelial keratinocytes in OLP lesions, as shown by immunohistochemistry.[17]

Acording another work, genes involved in human OLP (Oral Lichen Planus) pathogenesis are identified and ranked according to their number of interactions, in order to obtain a broader view of its molecular mechanisms and to plan targeted experimentations. 132 genes were identified and five of them (namely, JUN, EGFR, FOS, IL2, ITGB4) were classified as leaders. Interestingly, all of them but EGFR were up-regulated and were widely distributed in the network (in term of topological parameters such as stress, eccentricity and radiality) and showed higher topological coefficients than the other genes.[18]

Zhao M *et al.* studied the role of EGFR in the genesis of squamous cell by means of observation on its expression in oral lichen planus (OLP), squamous cell papilloma (SCP) and squamous cell carcinoma (SCC). The expression of EGFR was weak in OLP without erosive and ulcerative lesion. The strongly positive rates of EGFR in OLP with erosive and ulcerative lesion group, SCP group and SCC group were 20%, 25% and 60%, respectively. There were significant differences between the OLP with erosive and ulcerative lesion and OLP without erosive and ulcerative lesion. The expression of EGFR increased significantly from OLP, SCP to SCC.[19]

In another study Ribeiro DC *et al.* investigate the immunoexpression of epidermal growth factor receptor (EGFR) in a sample of oral leukoplakias (OL) and to determine the receptor's association with dysplasia, tobacco consumption, lesion site, and proliferation rate. EGFR is expressed in leukoplakia regardless of dysplasia, but EGFR positivity should be more frequent in lesions sited in areas of high cancer risk. According authors the association between EGFR and p27 may represent an important mechanism in the control of cellular proliferation and malignant progression of oral epithelium and therefore warrants further investigation. Although EGFR should be overexpressed in some oral leukoplakias, the factors that may interfere with this expression and the influence of this receptor on epithelial proliferation have yet to be investigated.[20]

Yamada T. examined the relationship between the oral leukoplakia and cancer, an immunohistochemical study of the EGF-receptor had been performed by the avidin-biotin peroxidase-complex method and silver enhancement with anti-human EGF-receptor antibody (Bio-yeda). Totally 61 cases of leukoplakias without relation to cancer, 20 cases of leukoplakias with relation to cancer and 31 cases of squamous cell carcinomas were examined. Seventy percent of leukoplakias and 55% of cancer cases were positive for the EGF-receptor. The proportion of the EGF-receptor-positive cells in the leukoplakia cases was slightly decreased in proportion to the degree of the epithelial dysplasia. The proportion of the EGF-receptor-positive cells in the poorly differentiated type cancer cases (3%) was fewer than that in the differentiated type cancer cases (31%). In the leukoplakia related to cancer, the leukoplakia and cancer of the same patient did not always show the same pattern of EGF-receptor.[21]

Bagan JV *et al.* studied compared the epidermal growth factor receptor (EGFR) copy number in patients with potentially malignant oral disorders (PMODs) and oral squamous cell carcinoma (OSCC). They estimated the EGFR copy number in both groups using real-time reverse-transcription polymerase chain reaction assays. They used laser microdissection (LMD) for EGFR amplification, and overexpression was performed. Group 1 comprised 20 patients with oral leukoplakia and group 2 comprised 19 cases of OSCC. The EGFR copy number was higher in group 2 (9.1 ± 6.2) than in group 1 (3.8 ± 1.5). The greatest copy number was found in the non-homogeneous leukoplakias, but the difference in homogeneous cases was not significant (Mann-Whitney test, $P>0.05$). In group 2, the EGFR copy number was higher in advanced stages than in early stages, but again lacked statistical significance. The EGFR copy number may be a useful biomolecular marker to differentiate PMODs from OSCC. The EGFR was higher in non-homogeneous leukoplakias and in the advanced stages of OSCC.[22]

One of the most well-known biomarkers in HNSCC (head and neck SCC) is the Epidermal Growth Factor Receptor (EGFR). Overexpression of EGFR in HNSCC has been associated with poorer overall survival and recurrence, and up to 90% of HNSCC patients express high EGFR.[8]

Several lines of evidence have shown that stimulation with EGF, as well as H2O2, UV, therapeutic agents, or ionizing radiation cause the EGFR to translocate to the nucleus, with nuclear EGFR signaling playing roles in cell proliferation, tumor progression, DNA repair. High levels of nuclear EGFR have been found in various types of cancers, and nuclear EGFR signaling has been reported to mediate radio-resistance and chemo-resistance, such as to ionizing radiation and cisplatin. Nuclear EGFR appears to be the full-length receptor. The mechanism by which the EGFR translocates to the nucleus has been studied, but is still far from clear. However, there is no established mechanism for the translocation of endosomal EGFR to nucleus.In the nucleus, the EGFR supports gene regulation by acting as a transcriptional co-activator. It has been shown that nuclear EGFR aids in the transcription of important cell cycle progression mediators, including CYCLIN D1 and c-MYC, among other proto-oncogenes . The interaction of nuclear EGFR to the CYCLIN D1 gene promoter has been better studied, and has been shown to require EGFR interaction with two proteins, Mucin-1 (MUC-1) and RNA helicase A (RHA). In this way, nuclear EGFR

signaling represents another way by which the EGFR promotes cell cycle progression, highlighting the breadth and redundancy of the EGFR signal transduction network in cancer progression.[12]

Epidermal growth factor receptor (EGFR) and p16 (a surrogate marker of human papillomavirus [HPV] infection) expression are strong prognostic factors in patients with head and neck squamous cell carcinoma (HNSCC). Husain H et al. examined an HPV-negative (SQ20B) and an HPV-positive (UMSCC47) HNSCC cell line for EGFR and γH2AX expression. A tissue microarray containing 123 cores obtained from 101 HNSCC tumors was analyzed for EGFR expression by automated quantitative analysis and p16 expression by immunohistochemical staining, and these results were correlated with available clinical data. SQ20B had higher EGFR expression than UMSCC47. Nuclear localization of EGFR on activation with transforming growth factor-α was observed in SQ20B, but not in UMSCC47. SQ20B also had increased γH2AX foci compared to UMSCC47, suggesting that SQ20B has more DNA damage compared to UMSCC47. Total and nuclear EGFR was reliably obtained from 80 of 101 patients. p16 levels were determined in 87 of 101 patients. p16 levels were strongly associated with the oropharyngeal subsite and poorly differentiated histology. Expression of total and nuclear EGFR was higher in p16-negative tumors compared to p16-positive tumors.[90]

According Yamakoshi *et al.*, the level of $p16^{INK4a}$ expression in early papillomas was slightly but consistently higher than those seen in normal skin. This level of $p16^{INK4a}$ expression does not appear to be high enough to induce senescence cell cycle arrest. Thus, it is tempting to speculate that $p16^{INK4a}$ may play a more important role or roles in late papillomas, presumably preventing malignant conversion of benign tumors. Indeed, by 30 wk after DMBA/TPA treatment, 33% of $p16^{INK4a}$ knockout mice (C57BL/6 background) had at least one carcinoma compared with 5% of the wild-type mice (unpublished data), indicating that $p16^{INK4a}$ plays an important role or roles in preventing malignant conversion of benign tumors. These results are somewhat consistent with a previous study showing that the tumor-free survival of DMBA treated mice was substantially reduced in $p16^{INK4a}$ knockout mice.[190] However, according Xu J *et al.*, the low-risk HPV–positive oral papillary lesions in this study showed a lower proliferative index by MIB-1 staining and patchy p16 or no p16 staining.[197]

Expression of p16 and c-Myc could not be used for differentiation of AK from SCC, because of the simillar staining, although increased expression of both p16 and c-Myc during the progression of skin from actinic keratosis to in situ squamous cell carcinoma to invasive squamous cell carcinoma was observed.[198,199,44]

Ultraviolet light plays a fundamental role as an initiator and promoter of carcinogenesis of SCC, allowing the accumulation of genetic alterations that allows a selective growth advantage. The TP53 (p53) gene often mutates and Ras is frequently activated, but with low frequency of mutations. Recently, frequent mutations in the epidermal growth factor receptor (EGFR)[23] have been detected in lung cancer, mainly deletions in exon 19 and L858R mutation in exon 21. These are located at the EGFR tyrosine kinase domain (TK). EGFR TK mutations produce activation of the signaling pathways downstream and preferentially activated antiapoptotic pathways (PI3K/AKT, JAK-STAT and ERK/MAPK). These mutations are correlated with the clinical response of patients to tyrosine kinase inhibitors (poor response to Gefinitib, Erlotinib). Glioblastoma shows another EGFR mutation (EGFRvIII)[23], corresponding to a deletion of the extracellular domain, and it is present in 24-67% of these tumors. This variant has been found in 42% of HNSCC, related to the poor response to monoclonal antibody cetuximab (competitively inhibits EGFR). Interestingly, EGFRvIII displays ligand-independent signaling, but has low constitutive activity. The low constitutive activity is enough to impart cancer cells with increased signaling, however its growth advantage is due to the fact that these receptors are not downregulated by endocytosis.[12] Many observations show that there are abnormalities in the expression of epidermal growth factor receptor (EGFR) and/or its ligands in HNSCC with frequent activation of multiple pathways downstream EGFR, and unrelated to RAS mutation.[24] EGFR gene is often amplified (30% of OSCCs[2]) and/or with activating mutations in cancer cells.[16] Other mutations detected in SCC are activating mutations or gains of Ras genes (amplification in 30% of OSCCs, mutation in 35% of OSCCs, mutation in 5% of oral cancers, but in 55% of lip cancers). Mutations in *TP53* (UV-signature) (mutation in 79% of HNCs), loses of *CDKN2A* ($p16^{INK4A}$) (hypermethylation in 44% oral leukoplakia lesions, in 76% of OSCCs), and inactivating mutations of NOTCH.[25,2]

SCC typically exhibits a broad spectrum of progressively advanced malignancies, ranging from premalignant actinic keratosis (AK) (precursor lesions) to squamous cell carcinoma *in situ* (SCCIS), invasive cSCC and finally metastatic cSCC. The primary risk factor for AK is chronic UV exposure. Genetically, AKs and cSCCs are associated with amplifications and activating mutations of the Ras oncogene indicate that 11% of cSCCs harbor activating Ras mutations (6% HRAS, 3% NRAS, 2% KRAS; n = 371 cases). In cSCCs Ras is frequently activated, but with low frequency of mutations. *Ras* mutations are infrequent in Western patients and detected in fewer than 5% of oral cancers. In contrast, 55% of lip cancers have *H-ras* mutation, which is also present in 35% of oral cancers in Asian populations in association with betel nut chewing. Whereas only benign tumors were observed after *KRasG12D* expression alone, combined p53 deletion and oncogenic *KRas* expression initiated invasive cSCCs.[26,27] There is no data for detected Ras mutations in other PMDs with exception of Keratoacanthomas (KA) (28.6%)[196] and papillomas.[44] KRAS gene exon 2 G12C presented mutation in the Oncocytic Schneiderian (sinonasal) papillomas (OSP) - associated adenocarcinoma.[188]

Up to 30% of all human tumors harbor mutations in canonical *RAS* genes (*KRAS*, *HRAS*, *NRAS*).[28,29] K-ras is a downstream mediator of EGFR-induced cell signaling, and ras mutations confer constitutive activation of the signal pathways without EGFR activation.[2] *KRAS* mutations have been associated with primary resistance to Gefitinib or Erlotinib (EGFR kinase inhibitors).[30,14,31]

In organotypic 3D culture of human esophageal cells (keratinocytes) EGFR overexpression and mutant p53 resulted in transformation and invasive growth.[32] Overexpression of inactivated or mutated forms of p53 in oral epithelial dysplasia has been associated with high risk for transformation to early stage OSCC.[33]

P53, a tumor suppressor gene, has been implicated in the early pathogenesis of HNSCC, as it controls cell growth through regulation of the cell-cycle and apoptosis. P53 acts as transcription factor of cell cycle inhibitors such as $p21^{Waf1/Cip1/Sdi1}$ and prevents the cell from going beyond phase G1 of the cell cycle, permitting DNA repair. If this is not possible, p53 induces apoptosis of these cells to avoid the transmission of potentially carcinogenic information.[2] In a study analyzing HNSCC patients with a history of tobacco and alcohol use, Brennan *et al*. found a significantly higher proportion of patients with mutations of p53 and other distinct sites when compared to nonsmokers and nondrinkers. p53 inactivation or mutations have been found in up to 50% of HNSCC patients and have been shown to be associated with decreased survival. Kuo *et al.* detected mutations in 35% of OSCCs, Balz *et al*. in 79% of HNCs (head and neck carcinomas).[34,2] In addition to p53, mutations in the retinoblastoma (Rb) gene are involved in the pathogenesis of HNSCC.

Park HR *et al.* evaluated the expression profiles of p63, p53, survivin, and hTERT in usual skin cancers, including squamous cell carcinoma (SCC) and basal cell carcinoma (BCC) and putative preneoplastic epidermal lesions, including actinic keratosis (AK), Bowen's disease, and porokeratosis. BCCs showed diffuse p63 expression and SCCs heterogeneous p63 expression with negativity in terminally differentiated squamous cells. All preneoplastic epidermal lesions showed p63 expression in all cell layers. p53 was found in seven of 10 cases of BCCs, all 10 cases of SCCs, and nine of 10 cases of Bowen's disease. AK and porokeratosis revealed focal to moderate p53 expression. Survivin was found in eight of 10 cases of SCCs and eight of 10 cases of Bowen's disease. Six of 10 cases of BCCs revealed weak survivin positivity. AK and porokeratosis showed survivin expression confined to the basal layer. hTERT expression was found in most cases of skin cancers and preneoplastic lesions. p63 expression may be a marker of basal/progenitor cells and a diagnostic marker in skin tumors. p63 expression is not related to p53 expression in these tumors. This study points to a putative role of survivin and hTERT in the development of certain skin cancers. In addition, authors' data support the concept of porokeratosis being a premalignant condition.[35]

Although BCC are with basal origin, EGFR is expressed at a significantly higher level in SCC than in BCC.[36] In general, BCCs seem to have relatively stable genomes – the few published study suggest that they have lower levels of genomic instability than do many extracutaneus cancers.[14] Methylation commences in UV exposed skin at a relatively early age and occurs in skin prior to the onset of recognizable preneoplastic changes in BCCs.[37] There is no any data concerning detected mutations in EGFR in BCC, although PKD1 is lining down from the receptor and its increase expression could be explain by positive mutations (amplification) or gain in the PKD1 or EGFR genes. However, in BCCs are detected activation mutations mostly in HH (Hedgehog) pathway[14], inactivating mutations in the tumor suppressor PTCH1 (90% of sporadic BCC), and activating mutations in other Hedgehog pathway genes, such as constitutively activate SMO15, SHH, or GLI[40] are found less frequenly (~10% of tumors)[25,14,169] in TP53 (UV-signature) (56% of all types of BCCs[2], Ras dysregulation in 100% and mutations in 10% to 30% (50%) of BCCs[38,39] and hTert mutations in 56% (78%) of BCCs.[25,11] None of the 61 non-melanoma skin cancer (30 SCC and 31 BCC) samples revealed any PIK3CA and AKT1 hotspot mutations at the investigated loci. The authors conclude that PIK3CA and AKT1 hotspot mutations do not contribute to the activation of the PI3K/AKT signalling pathway in non-melanoma skin cancer.[41] Deletion of 10q23, where *PTEN* is located, is infrequent event in human BCC[11], PKCs and STAT3 mutations in BCC are also rare.[42,43,44]

Tsao AS *et al.* summarized that patients with head and neck premalignant (and malignant) changes consist of a diverse population and should be treated differently depending on their molecular genotype. Patients with minimal genetic changes may be treated with single agent retinoids, $v.D_3$ or other agents. Those with more accumulated genetic changes will require combination chemoprevention therapies. Lesions that have advanced genetic changes with mutant p53 may benefit from targeted p53 therapy, and those lesions that express EGFR and COX-2 may require inhibitors of EFGR and COX-2. Other strategies include the oncolytic adenovirus dl1520 (ONYX-015), which selectively targets p53-deficient cells. Ongoing trials and future strategies include studying EGFR inhibitors, vascular endothelial growth factor receptor (VEGF-R) inhibitors, demethylating agents, farnesyltransferase inhibitors, celecoxib, vitamin E, and Bowman–Birk inhibitors.[45]

hTERT in skin and oral pathology

Telomeres are specialized chromatin structures at the ends of eukaryotic chromosomes and are crucial for genome (chromosome) stability, cell growth control and carcinogenesis. Normally, they protect chromosomes from end to end fusion, degradation and recombination. At each DNA replication cycle, 30-150 base pairs of telomeric DNA are lost, driving cells into metabolic state of irreversible growth arrest and replecative senescence.[46,47,30,48] Telomeres are repetitive DNA (TTAGGG) elements at the ends of the

chromosome. Telomerase is a multimeric ribonucleoprotein containing an RNA component that includes in its sequence the template for telomere synthesis and a catalytic protein subunit that is a reverstranscriptase (hTERT). The RNA component of the enzyme is expressed constitutivly, but catalytic subunit-hTERT is thought to be activity-limiting component of the telomerase holoenzyme. Telomerase is active in germ cells, stem cells and approximately 90% of cancers (including BCC and SCC), but not in most normal somatic cells (the proliferative basal layer of epidermis expresses telomerase, but cell cycle arrest and differentiation of these cells correlates with inhibition of telomerase activity).[30,49] Human telomerase catalytic protein subunit hTERT is crucial for telomerase activity. Although increased hTERT expression is sufficient to immortalize normal human cells in culture, spontaneous immortalization is extremely rare which suggests that hTERT expression is under strong negative control. Characterization of the hTERT promoter has allowed for the analysis of potential mechanisms of hTERT expression and regulation. The hTERT promoter is very complex and contains a great number of canonical and non-canonical sequences that bind or potentially bind a variety of transcription factors.[46] It is known that NF-κB transactivates c-Myc to stimulate hTERT promoter activity in intimal smooth muscle cell, human T lymphocytes, HTLV-I–associated adult T-cell leukemia and irradiated HER2-positive tumour-derived breast cancer cells.[50,51,52,53] A feed-forward regulation between *TERT* and NF-κB was suggested as telomerase directly regulates NF-κB dependent gene expression by binding to the NF-κB p65 subunit. This leads to the recruitment of a subset of NF-κB promoters such as Interleukin 6 (IL6) and TNFα. These cytokines, which are critical for inflammation and cancer progression, together with NF-κB, can transcriptionally upregulate telomerase levels.[54]

Telomerase reactivation in telomerase-negative cells can be achieved by chromatin remodeling, such that the promoter region of *hTERT* is more accessible. Myc:Max complexes activate transcription by binding to E-boxes, but these sites are often being competed for by the Mad:Max repressor complex. Mad represses the *hTERT* promoter through the interaction of HDACs. This complex can be repressed, though, by chromatin condensation through HDAC inhibitors.[55]

Also present within the core *hTERT* promoter are GC-boxes, which are binding sites for Sp1 transcription factor. Sp1can interact with *c-Myc* and stimulate telomerase expression through the transcriptional abilityof MBD1-containing chromatin-associated factor1(MCAF1). Furthe rinvolvement of Sp1 and *hTERT* expression are explored. Mutations in any of the five GC-boxes reduce core promoter activity. Other key binding sites found in the *hTERT* promoter include AP1,which binds the Jun/Fos dimer as a transcriptional repressor, AP-2, which shows tumor- specific *hTERT* upregulation, and HIF-1,which upregulates *hTERT* expression in hypoxic events. Mutations that generate an ETS binding site play a role in increasing *hTERT* promoter activity.[55]

Canonically DNA methylation is associated with gene silencing. This hypermethylation is associated with gene silencing of tumor suppressors such as *p16* and *hMLH1* (a part of DNA mismatch repair). *hTERT* is an exception to this rule, though, considering that the majority of the *hTERT* promoter region contains hypermethylated CpG islands in most cancer cells where it is expressed. Methylation status can vary among cell lines. Hypermethylation decreases the affinity of transcriptionl activators for the *hTERT* promoter region, while hypomethylation allows for binding of transcriptional repressors.[55]

5-aza-20-deoxycytidine (5-azadC) is acommon DNA demethylating agent involved in the reexpression of *hTERT* in *hTERT*-negative cells. Demethylationby5-azadC restores the binding capability of CTCF to the first exon of *hTERT* and E2F-1 to the promoter. Therefore,one of the main roles of *hTERT* methylation is probably to prevent binding of the CTCF and E2F-1 repressors and permit transcription. Hypermethylation of the *hTERT* promoter during senescence is linked to diminished telomerase activity, as well as *hTERT* mRNA expression. Exposing these cells to 5-azadC restores *hTERT* expression.[55] Regulation of hTert is reviewed in details in:[54,55].

In a study Crowe DL *et al.* examined the regulation of telomerase expression in anchorage-deprived normal human epidermal keratinocytes and squamous cell carcinoma lines. Anchorage-deprived cells underwent rapid loss of telomerase activity. Attachment loss was associated with increased ERK1 activity, G1 to S phase progression, and subsequent G2 arrest. Adhesion to collagen via specific integrin subunits inhibited ERK1 activity and telomerase repression. Loss of telomerase expression was associated with recruitment of an Rb/HDAC1 (transcriptional co-repressors, coregulator of Snail) repressor complex to the -98 E2F site of the hTERT promoter. The authors propose a mechanism by which anchorage deprivation inhibits telomerase activity in stratified squamous epithelial cells and squamous cell carcinoma lines.[49]

hTERT expression is used as a surrogate for telomerase activity. It has been suggested that telomerase activity is a biomarker of cell proliferation, not of malignant transformation (see below). Telomerase activity is detected in the proliferative basal layer of epidermis, but anchorage-deprived cells underwent rapid loss of telomerase activity. In the studies of Liu H *et al.*, staining for hTERT was found in the nuclei of epidermal cells, and more intensely in the nucleoli of cells in psoriasis, primarily in some proliferating keratinocytes. This indicates that telomerase activity does not always correlate with the malignant phenotype. The finding, that staining for hTERT was observed in keratinocytes in the upper to middle layers of the epidermis in psoriasis vulgaris, supports the concept that proliferating and nonproliferating cells can express human telomerase RNA (hTER). It may be speculated that one possible

mechanism of epidermis hyperplasia in psoriasis might be the increased telomerase activity in psoriatic epidermis.[56]

hTEP1 (telomerase associated protein 1) and hTR (human telomerase RNA component) but also hTERT (human telomerase reverse transcriptase) expression were detected in the basal cells of normal oral mucosa, and the cells expressing these mRNAs were also seen in the upper layer of leukoplakia of gingiva, and a heterogeneous pattern of expression was observed in the oral SCC tissues. These results indicate that there are at least two steps in the increase of telomerase activity during carcinogenesis in oral squamous cells; a change in distribution of cells expressing these telomerase components and the over-expression of hTERT gene in individual cells.[57]

Oral carcinogenesis is a multi-step process. One possible step is the development of potentially malignant disorders known as leukoplakia and erythroplakia[33]. The objective of the study of Cabral *et al*. was to use immunohistochemistry to analyze the patterns of expression of the cell-cycle regulatory proteins p53 and $p16^{INK4a}$ in potentially malignant disorders (PMD) of the oral mucosa (with varying degrees of dysplasia) and in oral squamous cell carcinomas (OSCC) to correlate them with the expression of telomerase (hTERT). Fifteen PMD and 30 OSCC tissue samples were analyzed. Additionally, 5 cases of oral epithelial hyperplasia (OEH) were added to analyze clinically altered mucosa presenting as histological hyperplasia without dysplasia. p53 positivity was observed in 93.3% of PMD, in 63.3% of OSCC and in 80% of OEH. Although there was no correlation between p53 expression and the grade of dysplasia, all cases with severe dysplasia presented p53 suprabasal immunoexpression. $p16^{INK4a}$ expression was observed in 26.7% of PMD, in 43.3% of OSCC and in 2 cases of OEH. The $p16^{INK4a}$ expression in OEH, PMD and OSCC was unable to differentiate non-dysplastic from dysplastic oral epithelium. hTERT positivity was observed in all samples of OEH and PMD and in 90% of OSCC. The high hTERT immunoexpression in all three lesions indicates that telomerase is present in clinically altered oral mucosa but does not differentiate hyperplastic from dysplastic oral epithelium. In PMD of the oral mucosa, the p53 immunoexpression changes according to the degree of dysplasia by mechanisms independent of $p16^{INK4a}$ and hTERT.[33]

Shimamoto H examined the status of telomerase activities in oral squamous cell carcinomas (OSCCs), precancerous lesions, and also cell lines established from OSCCs, by using a non-radioactive PCR-based TRAP (telomeric repeat amplification protocol) assay. Telomerase activities in 23 of 30 OSCCs, 8 of 17 leukoplakias, 0 of 5 normal tissues, and in 8 of 8 OSCC cell lines and 0 of 5 normal human keratinocyte cultures. According the authors the received results indicated that telomerase activity might have some association with carcinogenesis and might be used as a tumor marker in OSCC.[48]

In the of Palani J *et al.* study, immunohistochemistry (IHC) was used to detect the expression of hTERT protein in oral squamous cell carcinoma (OSCC) (n=30), leukoplakia (n=15), oral submucous fibrosis OSF (n=15) and normal oral mucosa (n=10). The cellular localization of immunostain, intensity of stain, mean nuclear labeling index (LI) and mean nuclear labeling score (LS) of hTERT protein were studied. There was increased expression of hTERT protein in OSCC and leukoplakia samples when compared to normal oral mucosa. The cellular localization, LI and LS in OSF were significantly different from OSCC and leukoplakia.[58]

Bettendorf O *et al.* analyzed telomerase activity in capsule tissues in a rat model with chronic inflammation and in tumor. Significant elevated telomerase activity was found in tumor tissue compared with nonneoplastic tissue ($p = 0.047$). Cases with a strong inflammation in capsule tissue showed a specific telomerase activity. In these cases, there were no significant differences in telomerase activities compared with malignant tumor tissue. The authors demonstrated elevated telomerase activity and its diagnostic limits around model implants in a rat model, and visualize its expression not only in malignant tissue but also in inflammatory cells. So the quantitative measurement of telomerase activity should not be applied in general as a marker for malignancy in capsule tissue.[59]

To investigate the effects of human telomerase reverse transcriptase (hTERT) on the growth of Capan-2 human pancreatic cancer cell and apoptosis. mRNA and protein expressions of hTERT, Bcl-2 and cyclooxygenase (COX)-2 were assessed by real time PCR and Western blot. Knockdown of hTERT by siRNA can inhibit the growth of Capan-2 cell. The inhibitory effect is associated with the downregulation of Bcl-2 (anti-apoptosis protein) and pro-inflammatory protein COX-2.[60]

Additionally, Li J *et al.* used short hairpin RNAs (shRNAs) specifically targeting hTERT were constructed and expressed in Hep-2 cells. Cell proliferation was measured by CCK-8 assay. Expression of hTERT, cyclin D1, cyclin E, c-myc, and GAPDH was detected by RT-PCR and Western blot; cyclin D1 and hTERT proteins in laryngeal squamous carcinoma tissue microarray were analyzed by quantum dots immunofluorescence. hTERT silence by shRNAs decreased the proliferation of Hep-2 cells by 76.8% at day 4 (96 h). Furthermore, transfection with hTERT shRNA for 48 h also significantly reduced expression of hTERT, cyclin D1, and c-Myc, but not cyclin E. Quantum dots immunofluorescence analysis of 36 laryngeal squamous carcinoma tissue samples found that hTERT expression was highly correlated with cyclin D1 expression. Down-regulating human telomerase reverse transcriptase (hTERT) expression will significantly suppress the cell viability of laryngeal squamous cell carcinoma Hep-2, which was mainly due to the inhibition of cyclin D1 and thus G1/S phase transition.[61]

Combination of P53 mutation and telomerase overexpression may induce tumourigenesis in NSCLC (Non-small-cell lung cancer). There is multiple

evidence of elevated COX-2 levels in NSCLC and their importance in lung carcinogenesis. Overexpression of COX-2 has been proposed as a biomarker for biologically aggressive types of NSCLC and poorer survival. The authors showed that P53 mutations were identified in 34.4% of tumours, the majority of them occurring in SCC (squamous cell carcinoma, 55.6%). K-RAS was mutated in 12of NSCLC tumours, the majority of the mutations being found in ADC (adenocarcinoma, 27.0%). Mutational screening detected three different COX-2 mutations and five different P53 mutations, published for the first time. With RT-PCR the authors observed that the expression of the tested genes, hTERT and COX-2, was highly significant for ADC and SCC. Statistical analysis of the combined results revealed significant correlation between expression of COX-2 and hTERT, hTERT expression and staging and survival. A positive correlation between COX-2 expression and K-RAS mutation was also observed.[62]

In another experiment, using GM847 cell line that presents alternative lengthening of telomeres (ALT) phenotype, the authors showed that expression of oncogenic *RAS* failed to fully transform the cells. Nonetheless, with ectopic expression of *TERT*, the cells acquired a tumourigenic phenotype, suggesting that *TERT* had an additional function required for cellular transformation and not depending on its ability to maintain telomeres. Following this line of reasoning, several studies confirmed the capacity of *TERT* to cooperate with other factors to promote tumourigenesis, for instance, when reduced viability and increased cancer incidence was noted in K5-*TERT* mice with a p53 (+/−) genetic background, indicating that telomerase could cooperate with loss of p53 function in inducing tumourogenesis in ageing organisms.[63]

Recent studies found TERT promoter mutations in a wide array of other human cancers, including hepatocellular cancer, bladder cancer, thyroid cancer, gliomas. However, activating mutations in the TERT promoter were recently identified in up to 71% of cutaneus melanoma, in atypical fibroxanthomas and pleomorphic dermal sarcomas, tumors arising in heavily UV-damaged skin. Griewank K. *et al.* investigated the presence of TERT promoter mutations in 32 BCCs and 34 cutaneous SCCs using conventional Sanger sequencing. TERT promoter mutations were identified in 18 (56%) BCCs and in 17 (50%) cutaneous SCCs. The recurrent mutations identified in their cohort were identical to those previously described in cutaneus melanoma, and showed a UV-signature.[25] In another study authors found TERT promoter mutations in 78% of BCC and 50% of SCC.[25] Additionally, significant correlation was found between telomerase activity and mRNA expression of EGFR in 15 cases, including non-neoplastic salivary glands and human salivary gland carcinomas.[37]

Fabricius EM *et al.* explored demonstrated telomerase activity in frozen tissues from BCC and their tumor-free margins by the PCR ELISA. In their next study the authors examined in the same frozen sections immunohistochemical presence of hTERT in the nucleus. The hTERT expression in the BCC was distributed heterogeneously. The score values established by the anti-hTERT antibodies used were variably or significantly increased. In the stroma they tended to be negative, so the authors disregarded stroma hTERT. Proof of hTERT did not differ uniformly from telomerase activity. They compared the high with the lower median hTERT values in the Kaplan-Meier curve. Patients with lower hTERT scores in the center or tumor margin as shown by some of the antibodies suffered relapse earlier. Finally, they compared the hTERT expression in BCC tissues with the hTERT scores in HNSCC tissues from their previous study. Only one anti-hTERT antibody (their Ab 7) yielded significantly higher scores in BCC than in HNSCC.[64]

In a study using 23 urothelial cancer cell lines, Borah and coworkers confirmed that *TERT* promoter mutations correlate with higher levels of *TERT* mRNA, TERT protein, telomerase enzymatic activity and telomere length. Furthermore, *TERT* promoter mutations represent a possible mechanism for telomerase reactivation and also correlate with worse patient outcome and reduced disease-specific survival in two independent patient cohorts.[54] For instance, in tissues that are constantly self-renewing, such as the gastrointestinal tract or the bone marrow, telomerase is already epigenetically activated and during transformation cells do not need to acquire mutations to activate telomerase. In contrast, tumor entities originating from cells with a low turnover rate, including glial cells and hepatocytes, more frequently harbor mutations that activate telomerase, mostly point mutations in the promoter region of hTERT as mentioned above. Promoter mutation being the most common point mutations in hepatocellular carcinoma, glioblastoma (GBM), bladder cancer and melanoma, where they can even constitute potential biomarkers.[65]

However, hTERT expression is also up-regulated in tumors via multiple genetic and epigenetic mechanisms including hTERT amplifications (3%), hTERT structural variants (3%), hTERT promoter mutations (31%) and epigenetic modifications through hTERT promoter methylation (53%). Specifically, hTERT gene amplification can result from telomere dysfunction in addition to breakage at fragile sites and formation of chromosomal fusions. In a large cohort made of 31 different types of cancer, it was demonstrated that 3% out of 95% of hTERT expressing tumours presented hTERT amplifications. Therefore, hTERT might be a target for amplification during tumorigenesis, which contributes to the dysregulation of telomerase activity that usually occurs in human tumors. Increased hTERT gene copy number is associated with upregulation of hTERT expression, related to acquired drug resistance, and correlated with worse clinical outcomes in breast, skin and thyroid cancer. However, in bladder cancer, no correlation was observed between increased hTERT gene copy number and hTERT mRNA, telomerase activity, or telomere length, suggesting that hTERT gene amplification may

require another companion alteration for telomerase reactivation.[66]

Bladder, thyroid, cutaneous melanoma, basal cell and squamous carcinoma and oligodendrogliomas are examples of cancers where TERTpMut are widespread through different stages and grades of the disease, suggesting their role as an early tumorigenic event. Additionally, not all TERTpMut tumors display telomerase activation and some premalignant lesions also displayed these genetic alterations at the hTERT promoter region. Together, these results support the fact that TERTpMut may act as early events in the oncogenic process.[66]

In 2013, two pivotal studies described two recurrent non-coding mutations within the hTERT promoter region in both familial and sporadic melanomas. These two mutations were located at -124 and -146 bp upstream from ATG (chr5:1,295,228 G>A and 1,295,250 G>A, C>T on opposite strand). In fact the authors demonstrated that TERTpMut acquired at the transition from benign nevus to malignant melanoma do not support telomere maintenance suggesting that TERTpMut contribute to tumorigenesis in two distinct ways. Initially, TERTpMut do not prevent telomere shortening but act "healing" the shortest telomeres and later telomeres are critically short leading to genomic instability and telomerase reactivation. Whether coincidental or reasonable, recurrent hTERT mutations seem to occur in the unmethylated region, which supports the hypothesis stating ETS family factors binding to these sites activate hTERT expression.[66]

However, there are other cancers that do not harbor TERTpMut (testicular germ cell tumors; breast cancer, colorectal carcinoma, prostate cancer) but have telomerase activation. These observations suggest that in hTERT dependent tumors without TERTpMut, other mechanisms responsible for telomerase activation might be at play, as a hypermethylation of promoter repressive region.[66]

Also, TERTpMut are not only prognostic factors for poor clinical outcomes, but also predictors of radiotherapy resistance. Furthermore, BRAF/NRAS mutations are associated with decreased disease-free and melanoma-specific survival. In liver disease, TERTpMut are present in pre-malignant nodules and predict high risk for advanced disease and reduced disease-free and overall survival in hepatocellular carcinoma patients. Thyroid cancer patients with TERTpMut are associated with clinically aggressive and recurrent disease, with lower disease-free and overall survival when combined with BRAF mutations. TERTpMut are a moderately prevalent genetic event in non-small cell lung cancer (NSCLC) associated with patient age, gender and distant metastasis. These studies emphasize the hypothetical existence of a companion mechanism, necessary not only for telomerase activation but also to maintain the selfrenewal capacity allowing cancer disease progression in TERTpMut patients.[66]

Additionally, *TERT* has been described to have influence in several other molecules and pathways, which modify responses to inflammation, cell death, apoptosis and DNA damage responses, EMT and oncogenesis. *TERT* binds to c-MYC and recruits the complex to heparanase promoter to upregulate heparanase expression promoting invasion and metastasis of gastric cancer cells; furthermore, *TERT*-activated Wnt/β-Catenin signalling promotes c-MYC expression, which could in turn activate *TERT* transcription and expression in a positive feedback loop; (iii) finally, it has been shown that *TERT* regulation of ITGB1 in the MDM2-FOXO3a- ITGB pathway is able to promote gastric cancer invasion.[55]

Young and coworkers suggested two epigenetics mechanisms for the maintenance of a young phenotype in normal human fibroblasts with *TERT* re-expression. The first was the freezing of the epigenomic state of young proliferating cells by the stabilisation of DNA methylation; the second, the maintenance of low levels of the cell cycle inhibitor p21, mediated at least partially by DNMT1's transcriptional repressor activity. In human cervical cancer cell lines, p27/kip1, a tumour suppressor protein, can inhibit *TERT* mRNA expression and telomerase activity through post-transcriptional upregulation by interferon-c (IFNc)/IRF1 signalling. This suggests that p27 may have the function of tumour suppressor by inhibiting *TERT* expression. It was also described that *TERT* overexpression upregulated the expression and transcriptional activity of a key cell cycle regulator, cyclin D1, in human prostate epithelial cell lines. This means that *TERT* could have a role in the modulation of cyclin D1 expression. *TERT* is capable of activating the transcription of vascular endothelial growth factor (*VEGF*) in WI-38 and HeLa cells, this activation being independent of telomerase activity and telomere maintenance. Suppression of *TERT* expression abrogates the cellular response to DNA double-strand breaks. Loss of *TERT* does not alter short-term telomere integrity but instead affects the overall configuration of chromatin. Cells lacking *TERT* exhibit increased radiosensitivity, diminished capacity for DNA repair, fragmented chromosomes, demonstrating that loss of *TERT* impairs the DNA damage response.[55]

HIF-1, upregulates *hTERT* expression in hypoxic events. Ahmed and coworkers described that in a mild oxidative stress background, telomerase did not prevent telomere shortening under hyperoxia as it translocated gradually from the nucleus to mitochondria. TERT mitochondrial localisation reduced mtDNA damage levels under oxidative stress and improved mitochondrial function, with lower levels of ROS and with enhanced mitochondrial membrane potential. Haendeler and coworkers also reported TERT's protective role in mitochondria in an oxidative stress background. TERT binds to *ND1* and *ND2* genes, protecting against mtDNA damage, reducing mitochondrial ROS, and increasing energy metabolism and conferring higher protection from apoptosis.[55]

TERT can be targeted to the mitochondria by an N-terminal leader sequence, and mitochondrial extracts from seven different human cell lines show telomerase activity. The cellular effects of mitochondrial

telomerase are controversial. Some authors argue that mitochondrial telomerase increases hydrogen peroxide (H_2O_2)-mediated mitochondrial DNA (mtDNA) damage. Ectopic *TERT* expression in human cells correlates with increase in mtDNA damage after H_2O_2 treatment. The same group later demonstrated that this increase in mtDNA damage after H_2O_2 exposure is dependent on the presence of *TERT* itself. Further experiments using a dominant negative *TERT* mutant show that telomerase must be catalytically active to mediate the increase in mtDNA damage. Mutations in the N-terminal mitochondrial leader sequence of *TERT* cause a complete loss of mitochondrial targeting without affecting catalytic activity. Cells carrying this mutated *TERT* not only have significantly reduced levels of mtDNA damage after H_2O_2 treatment, but strikingly also do not show any loss of viability or cell growth. Thus, the authors proposed that localisation of *TERT* to the mitochondria renders cells more susceptible to oxidative stress-induced mtDNA damage and subsequent cell death. Nuclear targeted *TERT*, in the absence of mitochondrial localisation, is associated with diminished mtDNA damage, increased cell survival and protection against cellular senescence.[55]

The expression of stem cell markers CD117, Oct4, and Sox2 was significantly higher in TEL^{pos} cells than in TEL^{neg} cells, the latter were not able to form spheres and had a much lower tumorigenic potential as only one in eight injected mice showed a tumor in contrast to seven out of eight mice which received TEL^{pos} cells. In addition, the TEL^{pos} cells displayed multipotency, as cells isolated from the tumors of mice injected with TEL^{pos} cells acquired both phenotypes, TEL^{pos} and TEL^{neg}. But most interestingly, it was shown that telomerase inhibition seems to be a promising strategy for stem cell targeted therapy in osteosarcoma: hTERT can modulate classical cancer pathways including NF-κB, TGF-β/Smad, and Wnt signaling that all contribute to the metastatic potential and stem cell phenotype of cancer cells.[65]

PKD1 (Protein Kinase D1) in skin and oral pathology

Protein kinase D1 (PKD1), a ubiquitous serine/threonine kinase, was originally described as a novel μ isoform of the protein kinase C (PKC) family, as it shares two cysteine-rich domains (C1a and C1b) that bind phorbol esters and diacylglycerol as in the PKC family. Unlike other members of the PKC family, PKD1 also has a unique pleckstrin homology (PH) domain, differentiating them from other members of the PKC family, and the catalytic domain of PKD1 is most closely related to calcium calmodulin–dependent kinases (CaMK).[67,68,69]

Protein Kinase D1 (PKD1), has been implicated in numerous cellular functions, including cell survival, proliferation, differentiation, migration, cell-cell adhesion and epithelial mesenchymal transition (EMT). PKD1 has been reported to be downregulated in advanced prostate, breast and gastric cancers, shown to play a role in tumorigenesis and metastasis, and upregulated in BCCs (basocellular carcinoma) and pancreatic cancer. Embryonic deletion of PKD1 in mice is lethal, suggesting PKD1 plays a crucial role in development, which can not be replaced by other PKD family members, PKD2 and PKD3.[67,68,69]

Analysis of BCC (basal cell carcinoma) lesions of Ristich *et al.* showed increased expression of PKD1 when compared with normal epidermis, but not in SCC lesions (squamous cell carcinoma). So as the authors wrote, the question remain: are the enhanced PKD1 levels in BCCs are simply a marker of their basal origin or does this elevated PKD1 contributes to the pathogenesis of BCCs.[70] Thus, another question is curently adequate, lack of PKD1 expression in SCCs despite increase expression of EGFR, is a consequence of its spinous layer origin, or is a consequense of PKD1 gene (PRKD1) mutation(s) (silencing, methylation).[11] An analysis of 530 HNSCC tumors from the TCGA via cBioPortal demonstrated low levels of DNA methylation on PRKD1 gene. Further analysis indicated 13% cases (67 out of 530 cases) of PKD1 had loss of heterozygosity (LOH), while only three cases (< 1%) of PKD1 showed homozygous deletion. Thus, a combination of genetic and epigenetic alterations contributed to the downregulation of PKD1 expression in HNSCC.[71]

Using only immunohistochemical analysis authors showed that expression of PKD1 in normal epidermis was primarily restricted to the stratum basalis, the proliferative compartment of the epidermis, supporting the concept that PKD1 promotes proliferation of normal keratinocytes and that this kinase is probably connected with hyperproliferative disorders of the skin (increased expression of PKD1 was detected also in involved psoriatic lesions).[70] According Ryvkin V *et al.* contrary to mouse keratinocytes (KCs), PKD1 is undetectable in human keratinocytes.[72] There is no data for the expression of the kinase in other skin or oral (head and neck) PMDs.

Our study, using normal human cultured keratinocytes, which resembles basal epidermal keratinocytes, showed that PKD1 is presented in these cells in very low protein and mRNA levels, detectable only using p-PKD1 Ser744/748 (Ser738/Ser742 in human) antibody after short term PMA treatment (known activator of classical and novel PKCs and PKDs) and Quantitative Real-Time PCR. The antibody of Cell Signaling is with species reactivity for human, mouse, rat, monkey (from the datasheet of producer). Knockdown of PKD1 in normal human keratinocytes, using siRNA for PKD1, resulted in altered cell phenotype-enlarged cells, inhibition of keratinocytes proliferation (decreased expression of PCNA-proliferative marker) and promotion of cells differentiation (increased expression of K10 and involucrin).[73] Furthermore, using antisense oligonucleatide for PKD1 we show that keratinocytes proliferation was inhibited with more than 70%, which was connected with increased phosphorylation/activity of ERK1/2 (Extracellular signal Regulated Kinase 1/2) (PKD1 inhibited ERK1/2 phosphorylation/activity)(our unpublished results). Similar results were obtained using antisense oligonucleotides for PKCα and PKCε - inhibited

proliferation mesuread by decrease of [^{3}H] Thymidine incorporation and increased ERK1/2 phosphorylation/activity. The two PKC isoforms were proved to activate PKD1 in plethora of cell cultures, which suppose that EGF stimulates proliferation in NHEK (normal human epidermal keratinocytes) in a PKC-PKD1-ERK1/2 dependent mode.[74] EGF is the main growth factor secreted from keratinocytes in the model of human autonomous proliferated keratinocytes. Using the ERK1/2 inhibitor PD98059 and the two PKC inhibitors Go6976 and Go6983, with different substrate specificity, Praskova *et al.* showed that EGF stimulates keratinocytes proliferation in PKC-ERK1/2 dependent mode. The obtained results are similar to those received with antisense oligonucleotides for PKC isoforms in normal keratinocytes.[75] PKD1 (PKCμ), PKCα and PKCε failed to influence the phosphorylation and binding activity of c-Myc (our unpublished results). The phosphorylation and binding activity of the transcriptional factor was diminished by knockdown of CaMKIIδ, using also antisence oligonucleotide, which inhibited keratinocytes proliferation and opposingly decrease ERK1/2 phosphorylation/activity and c-Myc binding activity.[76] Similar resuts were obtained from Praskova *et al.* using also unspecific CaMKinase inhibitor KN-62.[77] The degree of inhibition of proliferation, ERK1/2 phosphorylation/activity and c-Myc binding activity were higher in KN-62 treated cells, which suppose expression of other CaMKinase isoforms in NHEK.[76,77]

In contrast in hTERT keratinocytes, obtained from normal human epidermal keratinocytes infected with amphotropic retroviral vectors encoding hTERT (catalytic subunit of telomerase), also called N/Tert-1 or N-hTERT keratinocytes, the expression of PKD1 was increased near 9-fold (mRNA)[73] and knockdown of the kinase, using siRNA, resulted in effective decrease of protein kinase levels, inhibition of ERK1/2 expression and activity, as well as EGFR expression and activity 48 h after transfection, and according our previous results decreased mRNA expression of K10 and involucrin 72 hours after transfection (inhibition of their differentiation).[78,79] Therefore, increased expression of PKD1 in hTert keratinocytes, opposed to normal human keratinocytes, possess prodifferentiation function, which can beat least partly result of 9 fold increased PKD1 expression (our result), leading to different substrates affinity and binding, hence different function or this turn of function could be a result of $p16^{INK4a}$ mutations.[73,78,79] In postconfluent normal keratinocytes ERK1/2 expression is increased, which is most probably connected with initiation of differentiation ot inhibition of hTert expression.[74,49] Most of the authors supposed that ERK1/2 functions to mediate pro-proliferative and pro-survival pathways from many different stimulus, inscluding growth factors and cell adhesion molecules. Second, differentiation-positive signals are associated with redused ERK1/2 activity. Agents which promoted differentiation and apooptosis signals through p38 MAPK-dependent mechanisms. p38α and p38δ are the main p38 MAPK isoforms, mediating activation of gene expression, although p38α, β, and - δ (but not -γ) are expressed in keratinocytes.[80]

Sustain ERK1/2 activity simultaneously with increased expression of K10 and involucrin are characteristics of differentiating keratinocytes[49], but it remains quationary increased expression of EGFR. If we consider increased expression of telomerase and loss of $p16^{INK4a}$ function, this cell line could be considered as a model of a premalignant (PMD) cell line, which are characterisised with up-regulated EGFR and/or its ligands and ERK1/2. PKD1 regulate in opposing manner differentiation in normal keratinocytes and hTert keratinocytes, in both of which increased proliferative cell potential as a result of over expression od EGFR and ERK1/2 (chronical inflammation, increased hert expression), are limited at least in part by PKD1 possesing prodifferentiation activity – expression of early markers of differentiation – K10 and involoucrin. It is good to mention according us ERK1/2 in human keratinocytes participates not only in the regulation of proliferation, but also in the control of their differentiation, similarly to PC12 cells, although only several papers report differentiation connected ERK1/2 activity in keratinocytes.[49,81,74] Recent findings suggested that the inhibition of the p38 MAPK pathway, ERK1/2 pathway and JNK pathway down-regulated filaggrin (differentiation marker) in NHEKs.[82] PKD1 is with near nine fold higher levels of mRNA expression in hTert keratinocytes in comparision with normal keratinocytes, which could altered the affinity to substrates, which are not targeted by the kinase in their normal extremely low levels in normal human keratinocytes, undetectable by Rykin *et al.*[73,70,72] or overactivation of its ordinary substrates (ERK1/2). Bertrand-Vallery V *et al.* reported that repeated exposures to sublethal doses of UVB induce an alternative differentiation state rather than premature senescence in N-hTERT (hTERT) and in cultivated human keratinocytes.[83] For the first time we detected that ERK1/2, which are thought to be constitutive expressed kinases in cells are inducible enzymes upregulated, together with EGFR, from PKD1 in hTert keratinocytes.[79]

hTERT keratinocytes have been obtained from normal human epidermal keratinocytes, which were infected with amphotropic retroviral vectors encoding hTERT, catalytic subunit of telomerase, catalytic subunit-hTERT is thought to be activity-limiting component of the telomerase holoenzyme. hTERT is expressed only in germ cells, stem cells of renewal tissues and in cancer cells (including BCC and SCC). Telomerase activity is not detected in most somatic tissues (the proliferative basal layer of epidermis expresses telomerase, but anchorage-deprived cells underwent rapid loss of telomerase activity). In NHEK and in SCC4 cell line attachment loss was associated with decrease hTERT and increased ERK1 activity, G1 to S phase progression, subsequent G2 cell cycle arrest and differentiation. Adhesion to collagen via specific integrin subunits inhibited ERK1 activity and telomerase repression.[49] Erk1/2 knockdown in

keratinocytes was not associated with decreased cyclin D1 expression, in contrast to fibroblasts.[84]

Dickson *et al.* showed that expression of hTERT alone permits keratinocytes to escape complete growth and to enter a phase of slow growth of variable length from which rapidly dividing immortal variants emerge.[47] The hTert(+) cells have a normal karyotype and the cells have undergone more than 80 population doublings (PDs) after hTert retroviral transduction while control cells exhibit senescence-associated proliferation arrest after 8 PDs.[47,85] Such immortalized cell typically have identifiable defects in $p16^{INK4a}$ expression but retain functional p53. The mechanism that triggers $p16^{INK4a}$ accumulation appears to sense the senescence state of keratinocytes, but preventing telomerase erosion does not avoid its activation. Loss of this mechanism, whether by $p16^{INK4a}$ gene deletion, mutation, or altered regulation of expression, together with telomere stabilization effected by hTERT expression is necessary to enable keratinocytes to become immortalized. It was supposed that immortalization of the keratinocytes by forced expression of telomerase and subsequent spontaneous events leading to loss of this $p16^{INK4a}$-dependent mechanism (first mutation-see below) generally does not disrupt either other normal growth control mechanisms or affects the ability of the cells to form a differentiated epithelium. hTERT keratinocytes continued to express the differentiation-related markers involucrin and K10 in suprabasal cells of stratified colonies and form differentiating epidermis in organotypic culture. They form more robust epithelium with a denser and more columnar basal cell layer and more layers of spinous cells than that formed by mild-life-span normal keratinocytes.[47] This epithelium resembles pathohistological changes observed in psoriatic lesions. In psoriatic epidermis is detected mutations of CARD14, encoding a nuclear factor of kappa light chain enhancer in B cells (NF-kB) (epidermal regulator of NF-kB), within skin epidermis and increased expression and activity of hTERT, EGFR, STAT3, PKD1, NF-kB, and other kinases.[86,56,13,56,70,86]

Previously we supposed a model in which transient activation followed by a sustained low basal activity of ERK1/2 is connected with keratinocytes proliferation and sustained high activity (expression) of ERK1/2 is connected with promotion of their differentiation.[79,74,49] Corelation between the duration and strenght of ERK1/2 phosphorylation/activation and the consequent physiological cells events was previously detected in rat PC12 pheochromocytome cells, fibroblasts, macrophages and T-limphocytes.[87,88,89] Factors such as cell-surface receptor density, expression of scaffolding proteins, the surrounding extracellular matrix, and the interplay between kinases and phosphatases modulate the strength and duration of ERK signaling. Furthermore, the spatial distribution and temporal qualities of ERK can markedly alter the qualitative and quantitative features of downstream signaling to immediate early genes (IEG) and the expression of IEG-encoded protein products. As a result, IEG products provide a molecular interpretation of ERK dynamics, enabling the cell to program an appropriate biological response.[89,184]

Thus, lower levels of ERK ½ expression in hTERT keratinocytes knockdowned for PKD1 together with lack of expression of K10 and involucrin is a phenotype characteristic for basal proliferating keratinocytes, suggesting that PKD1 in hTERT keratinocytes possess prodifferentiation role.[78,79] Additional study have to prove or reject the hypotesis, that PKD1 in hTERT keratinocytes will inhibit their proliferation. The results suppose different function of PKD1 in normal human and hTERT keratinocytes, which suppose that the kinase (and/or other kinases) could possess different role in PMDs (see below). The alteration in the function of the kinase is a consequence only of the forced expression of hTERT, followed by spontaneous events leading to loss of this $p16^{INK4a}$-dependent mechanism, and increase in PKD1 expression.[78,73,47] Other mutations are not detected. p16 is a cyclin-dependent kinase inhibitor that inhibits pRb phosphorylation and blocks cell cycle progression at the G1 to S checkpoint (see below). Loss of p16 expression by deletion, mutation, or hypermethylation is common in HNSCC.[90] Previously it was detected that expression of total and nuclear EGFR was higher in p16-negative tumors compared to p16-positive tumors (HPV positive tumours mostly).[91] Doorslaer and Burk showed that oncogenic types papilloma virus (HPV) specifically activate the hTERT promoter, while non-oncogenic types do not.[92] However, as it was mentioned above increased forced expression of hTERT leads to spontaneous loss of $p16^{INK4a}$-dependent mechanism.[47] Significant correlation was found between telomerase activity and mRNA expression of EGFR in 15 cases, including non-neoplastic salivary glands and human salivary gland carcinomas.[37]

Surprisingly for us, the expression of EGFR in hTret keratinocytes was also down-regulated from PKD1 silencing.[79] In normal skin, the EGF-R is most strongly, although not exclusively, expressed in the basal layer of the epidermis, consistent with the involvement of the EGF-R in epidermal growth control. EGFR is known to be an important regulator of multiple epidermal functions, including cell cycle progression, differentiation, cell movement and cellular survival.[13] Using microarray analysis of a confluent cell density-induced model of keratinocyte differentiation, the authors identified 2,676 genes that are regulated by epidermal growth factor (EGF), a ligand of the EGFR. The authors further discovered, and separately confirmed by functional assays, that EGFR activation abrogates all of the known essential processes of keratinocyte differentiation by *1*) decreasing the expression of lipid matrix biosynthetic enzymes, *2*) regulating numerous genes forming the cornified envelope, and *3*) suppressing the expression of tight junction proteins.[93] Several observations indicate that abnormalities (increase) in the expression of the EGF-R and/or its ligands EGF, TGF-α and AR are common

features of hyperproliferative premalignant deases and neoplastic epithelia.[13]

Additionally, *TERT* has been described to have influence in several other molecules and pathways, which modify responses to inflammation, cell death, apoptosis and DNA damage responses, EMT, invasiveness (metastasis formation) and stemness. For instance, in a comparison between normal and *TERT* immortalised fibroblasts using a cDNA microarray, Lindvall and coworkers verified that *TERT* immortalised cells had 172 differentially expressed genes; one of them is epiregulin, a potent growth factor associated with cancer. The results suggest that both activation of telomerase and subsequent induction of epiregulin are required for a sustained cell proliferation.[54,65]

Additiionally, ectopic expression of telomerase in HMECs leads to an increased ability to proliferate. This expression results in a diminished requirement for exogenous mitogens and correlates with telomerase-dependent induction of genes that promote cell growth. Inhibition of one of these genes, for instance of the epidermal growth factor receptor (*EGFR*), reverses the enhanced proliferation caused by telomerase.[54] However in hTtert keratinocytes PKD1 up-regulates EGFR expression, probably as consequence of $p16^{INK4a}$ mutations detected in these cells.

In oral squamous cell carcinoma (OSCC) an hTERT knockdown leads to reduction in MMP2 and MMP9 expression levels, thereby inhibiting invasiveness. Ding *et al.* demonstrated that hTERT, independent from its catalytic activity, is able to increase the cells' invasive potential by inducing MMP9 expression in an NFκB-dependent manner in several non-telomerase expressing cell lines such as osteosarcoma U2OS and the cervical carcinoma cell line HeLa. In cell lines with endogenous telomerase expression (293T and MCF7), a genetic knockdown of hTERT resulted in decreased MMP9 levels and a lower invasive potential. Interestingly, Ponnala *et al.* reported that silencing of MMP9 leads to a reduction in hTERT expression and telomerase activity via β1 integrin-dependent FAK signaling and deregulation of Myc/MAX/MAD ratios in GBM cells. From these findings, it was concluded that loss of telomerase activity due to MMP9 silencing leads to increased DNA damage that causes cells into a senescence stage or, after further replication, into apoptosis. Since it is established that MMP9 is overexpressed in tumor cells of several cancers compared to resident cells and normal tissue, a therapeutic reduction in MMP9 could be a potential approach for selective targeting of hTERT in cancer cells but not in resident cells. hTERT directly affects the levels of p65, the subunit of the NF-κB transcription factor, in the cytoplasm and the nucleus of tumor cells. This example demonstrates that hTERT functions in tumor cells are beyond the effect of replicative immortality.[65]

Although BCC are with basal origin, EGFR is expressed at a significantly higher level in SCC than in BCC.[36] Basal cell carcinoma (BCC) of the skin is a highly compact, non-metastatic epithelial tumour type that may arise from the aberrant propagation of epidermal or progenitor stem cell (SC) populations. Increased expression of GLI1 is a common feature of BCC and is linked to the induction of epidermal SC markers in immortalized N/Tert-1 keratinocytes. GLI1 over-expression is linked to additional SC characteristics in N/Tert-1 cells including reduced epidermal growth factor receptor (EGFR) expression and compact colony formation that is associated with repressed extracellular signal-regulated kinase (ERK) activity. ERK activity was predominantly negative in 13/14 BCCs (superficial/nodular).[40] However, N/Tert-1 keratinocytes possess $p^{16INK4a}$ mutation, which is not observed in BCC[11], hence this cell line is not the most suitable for studing molecular alterations in BCC. Additionally, transgenic mice overexpressing Sonic hedgehog (Shh) or a mutated variant of Smoothened (SMOH) show epidermal proliferations in late embryonic skin that partially resembles BCC. To test the hypothesis that GLI-1 is the downstream effector that drives tumorigenesis, transgenic mice were generated specifically overexpressing GLI-1 in the basal layer of epidermis and outer root sheet of hair follicles. These mice develop several types of spontaneous skin tumors, including BCC, TEs, cylindromas, and trichoblastomas. Furthermore, mutation analysis of the *p53* and Ha *ras* genes, respectively, did not reveal mutations in their hot spot regions, exons 4–8 for *p53* and codons 12, 13, and 61 for Ha *ras*, in any of the tumors examined. This suggests a *p53*- and Ha *ras*-independent mechanism by which GLI-1 induce these tumors in mouse skin.[191] Thus, AK which acquire activating mutation(s) in HH pathway (as a second muation) will progress to BCC. AK with *TP53* mutations probably has to be refered as SCC.[10,11] If the first mutation is in the HH pathway there is higher probability to develop Trichoepithelioma (TEs), cylindromas, and trichoblastomas and finally BCC.[191,44]

Another fact which desert attention was that PKD1 (PKCμ), and PKCα and PKCε, in fact decresed ERK1/2 activity, do not stimulate it, as it is expected according the scientific data. PKD1 phosphorylates RIN1 on the cell membrane, protein associating with Ras and 14-3-3 proteins (also binding partner of PKD1). Through phosphorylation of RIN1, association with 14-3-3 could be more intence, abrogating its ability to block Ras/Raf-1 interactions. Ras could dissociate and is free to be activated, hence activate Ras/Raf/MEK/ERK/RSK pathway.[67,94,69,95] The data supposs stimulation of ERK1/2 pathway by the three PKC isoforms expressed in keratinocytes. Schonwasser DC *et al.* published data that PKC-α (and PKC-β1, not expressed in keratinocytes) induce desensitizing effect on c-Raf activation, which avoid further activation from growth factors. Probably PKD1 posses similar effect or inhibits ERK1/2 activity. This negative control could demand phosphorylation on particular PKC residues.[96] The observed effect on p-ERK1/2 is additionally confermed by the results of Chiou YS *et al*., who detected increased PKD1 expression (and CD34 expression (stem cell marker)), leading to

decreased p-ERK1/2 levels in two steps according (DMBA)-initiated and 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-promoted skin tumorigenesis protocol in ICR mice. Interesting, the authors detected simultaneous increase in the levels of phosphorylated (activated) phorms of p-PI3K, p-JNK1/2, p-p38, increased expression of p53, p21 nad c-Myc, reversed effects from those expected from scientific data in other cell types (PKD1 inhibit the activity of PI3K, JNK1/2, p38[95]). This paper also proves the ability of PKD1 to activate NF-κB in mouse epidermis of induced carcinogenesis and connected increased expression of proinflammatory and proproliferative genes, including iNOS (inducible nitric oxide synthase), COX-2 (cyclooxygenase-2), ODC (ornithine decarboxylase) and VEGF (vascular endothelial growth factor).[97]

Additional studies are necessary to confirm whether hTERT keratinocytes posses increased EGFR and ERK1/2 expession than that in normal human keratinocytes and whether stimulation of normal epidermal keratinocytes with LPS (specific binding to TLR4 to activate NF-κB[98] or other chronic inflammatory mediators, will lead to increase hTERT, PKD1, NF-κB, ERK1/2 and EGFR expression. It will be interesting whether oral PMDs, possess higher expression of PKD1 and ERK1/2 expression/activity than in normal human keratinocytes vs. SCCs. Increased expression of EGFR is a common feature of plethora of PMDs (see below)[13], there is also data for increased expression and activity of hTERT, NF-kB and COX-2 (see above).[4,5,1]

We speculate that this molecular mechanism (increased NFκB - hTert-PKD1-NFκB-hTert) is probably connected with the progression of chronic inflammation in cancer development.[99,28] It is known that increased activity of NF-kB in inflammation is connected with increased expression of hTERT.[47,98,28] Thus, chronic inflammation could lead to increase expression of hTERT which consequently will lead to $p16^{INK4a}$ mutation (model of hTERT keratinocytes). In this regard hTERT keratinocytes as a cell line model resembles PMDs (premalignant binign deaseases), more than a tumor cell line. However increased PKD1 expression in hTERT keratinocytes, as a consequance of increased hTERT expression and/or $p16^{INK4a}$ mutations, is not a proproliferative signal in this cell line, rather that it is prodifferentiative.[78,79] PKD1 stimulated expression of K10 and Involucrin could be connected with abundance in cytoplasms filaments seen in early hypertrophic diseases, and PKD1 stimulated expression of ERK1/2 and EGFR are closely connected with pathogenetic mechanismes of PMDs.[78,79] On the other hand the molecular mechanisms regulating PKD1 expression are in skin are currently not well known. It is not known also whether PKD1 participates in inflammation process in skin[95] – Fig.8, there are data for its participation in pancreatitis.[100,95]

A paper proving the participation of PKD1 in infalammatory process was published. Group B streptococci (GBS) are one of the leading causes of life-threatening illness in neonates. Proinflammatory responses to GBS mediated through host innate immune receptors play a critical role in the disease manifestation. In a study Upadhyay K *et al.*, investigated the role of protein kinase D (PKD)1 in the proinflammatory responses to GBS. They found that both live and antibiotic-killed GBS induce activation of PKD1 through a pathway that is dependent on the TLR signaling adaptor MyD88 and its downstream kinase IL-1R–associated kinase 1, but independent of TNFR-associated factor 6. Their studies using pharmacological PKD inhibitors and PKD1-knockdown macrophages revealed that PKD1 is indispensable for GBS-mediated activation of MAPKs and NF-κB and subsequent expression of proinflammatory mediators. Furthermore, systemic administration of a PKD inhibitor protects d-galactosamine–sensitized mice from shock-mediated death caused by antibiotic-killed GBS. These findings imply that PKD1 plays a critical regulatory role in GBS-induced proinflammatory reactions and sepsis, and inhibition of PKD1 activation together with antibiotic treatment in GBS-infected neonates could be an effective way to control GBS diseases.[101]

Chronic inflammatory mediators exert pleiotropic effects in the development of cancer. On the one hand, inflammation favors carcinogenesis, malignant transformation, tumorgrowth, invasion, and metastatic spread; on the other hand inflammation can stimulate immune effector mechanisms that might limit tumor growth.The link between cancer and inflammation depends on intrinsic and extrinsic pathways. Both pathways result in the activation of transcription factors such as NF-κB, STAT-3, and HIF-1 and in accumulation of tumorigenic factors in tumor and microenvironment.[28,99]

Nuclear factor-kappa B (NF-κB) regulates the expression of various genes, several genes involved in inflammation and tumorigenesis, including those of the liver. A role for NF-κB has been implicated in the pathogenesis of hepatocellular carcinoma. This transcription factor can regulate hTERT gene transcription, which expression was found to be at high levels in this type carcinoma. In a study, Huang *et al.* showed that LPS (specific binding to TLR4 to activate NF-kappaB) was positive for NFκB p65 mRNA expression and activation, and also up-regulated hTERT mRNA and protein expressions at 36h in a dose-dependent manner. In contrast, MG-132 (inhibiting the activity of 26S proteasome and thereby preventing nuclear translocation of NF-κB) significantly inhibited activation of NF-κB and mRNA expression, and also decreased expression of hTERT at both mRNA and protein levels at 36h in a dose-dependent manner. Furthermore, dexamethasone blocked LPS-induced activation of NF-κB and expression of the hTERT in HepG(2) cells. These findings suggest that NF-κB may modulate hTERT mRNA level, importantly, in protein level in HepG(2) cells and dexamethasone inhibits LPS-induced hTERT via blocking NF-κB.[98]

NF-κB activation in inflammatory cells in response to infectious pathogens, pro-inflammatory

mediators as well as necrotic cell products results in the generation of secretable factors that support growth, survival, and vascularization of pre-malignant and malignant cells. Activation of NF-κB up-regulates cell cycle mediators (cyclin D1, c-Myc), anti-apoptotic (c-FLIP, survivin, Bcl-XL) and adhesion molecules (ICAM-1, ELAM-1,VCAM-17), proteolytic enzymes (e.g.,MMP, uPA), and pro-inflammatory factors (PGHS-2 (COX-2), cytokines) that promote inflammation and tumorogenesis.[28,99] Additioanally, the serine/threonine kinase protein kinase D1 (PKD1) is a stress-responsive kinase and sensor for reactive oxygen species, which can initiate cell survival through activation of NF-κB signaling.[102] PKD1 activates NFkB by the phosphorylation and activation of inhibitory k kinase (IKK). This results in the degradation of inhibitory protein IkB and the release of NFkB from the inhibitory complex, followed by accumulation in the nucleus and induction of downstream target genes. PKD1 may also be involved in IKK independent mechanisms that activate the NFkB pathway within cells.[67,69,95] It was recently demonstrated that KRas-induced activation of the canonical NF-κB pathway is one mechanism of how *PRKD1* (gene of PKD1) expression is increased and identify the binding sites for NF-κB in the *PRKD1* promoter in pancreatic cancer.[103]

The loss of p16 may be an early event in cancer progression, because deletion of at least one copy is quite high in some premalignant lesions. $p16^{INK4a}$ is a major target in carcinogenesis, rivaled in frequency only by the p53 tumor-suppressor gene.[104] Alterations of p16 have been described in a wide variety of histological types of human cancers including astrocytoma, melanoma, leukemia, breast cancer, head and neck squamous cell carcinoma, malignant mesothelioma, and lung cancer. Alterations of p16 can occur through homozygous deletion, point mutation, and transcriptional suppression associated with hypermethylation in cancer cell lines and primary tumors.[105] Methylation of $p16^{INK4a}$ was observed in 44% of 34 patients with oral leukoplakia lesions, and hypermethylation in 76% of OSCCs. These findings demonstrate that methylation is an early event in oral carcinogenesis and that its study may be useful to detect precancerous lesions.[2]

The *p16* gene (also known as *CDKN2A*) encodes $p16^{INK4A}$, which inhibits (inactivating) the CDK4:cyclin D and CDK6:cyclin D complexes. These complexes mediate phosphorylation of the Rb protein and allow cell cycle progression beyond the G_1-S-phase checkpoint. Whereas the *Rb* gene is inactivated in a narrow range of tumor cells, the pattern of mutational inactivation of Rb is inversely correlated with p16 alterations, suggesting that a single defect in the p16/CDK4:cyclin D/Rb pathway is sufficient for tumorigenesis. [105]

The p16 gene expresses two alternative transcripts (p16alpha and p16beta) involved in tumor suppression via the retinoblastoma (Rb) or p53 pathways. Disruption of these pathways can occur through inactivation of p16 or p53, or activating mutations of cyclin dependant kinase 4 gene (Cdk4). We searched for p16, Cdk4 and p53 gene mutations in 20 squamous cell carcinomas (SSCs), 1 actinic keratosis (AK), and 28 basal cell carcinomas (BCCs), using PCR-SSCP. A deletion and methylation analysis of p16 was also performed. Six different mutations (12%) were detected in exon 2 of p16 (common to p16alpha and p16beta), in five out of 21 squamous lesions (24%) (one AK and four SCCs) and one out of 28 BCCs (3.5%). These included four (66%) ultraviolet (UV)-type mutations (two tandems CC : GG to TT : AA transitions and two C : G to T : A transitions at dipyrimidic site) and two transversions. P53 mutations were present in 18 samples (37%), mostly of UV type. Of these, only two (one BCC and one AK) harboured simultaneously mutations of p16, but with no consequence on p16beta transcript. Soufir N et al. data demonstrate for the first time the presence of p16 UV induced mutations in non melanoma skin cancer, particularly in the most aggressive SCC type, and support that p16 and p53 are involved in two independent pathways in skin carcinogenesis.[161]

In a complementary study, it was observed that assembly of cyclin D1/D2–CDK4 complexes was impaired in primary mouse embryo fibroblast (MEF) strains taken from animals lacking the *p21* gene, the *p27* gene, or both. In MEFs from*p21/p27* double-null mice, nuclear import of cyclin D1 is inefficient, and overexpressed D cyclins remain predominantly cytoplasmic. The half-life of unassembled cyclin D1 is significantly reduced from 25 to l0 min.[106] Mutations in both *p21* gene and the *p27 gene are also often observed in* premalignant lesions (see below).

We can speculate that an axis of EGFR- PKD1-NFκB-hTERT in skin and oral epithelium pathology exists, and alteration in this axis is probably connected with the prograssion of chronical inflamated mucosa to precancer in skin and oral mucosa. Since increased expression of hTERT is an early event in the pathogenesis of hyperproliferative skin diseases, (hTERT is considered as a proliferative marker, rather than cancer marker[46,33], its expression is increased in all PMDs.[33,57,48,58,59] Additional studies are necessary to confirm whether other hyperproliferative skin conditions - pre-malignant leucoplakia, erythroplakia, oral lichen planus possesses an increased expression of PKD1 and ERK1/2, as an early consequence of increased hTERT expression. Previously it was detected that expression of total and nuclear EGFR was higher in p16-negative tumors compared to p16-positive tumors[91], which will activate additionally transduction pathways lieing downstream from EGFR. Increase EGFR expression or its ligands EGF, TGF-alpha or AR were detected in all precancer oral lesions (PMDs)[13], and the EGFR copy number is thought to be a useful biomolecular marker to differentiate PMODs from OSCC (see below).

Probably second mutations (first in inhibitors of the cell cycle p16, p21, p27, *Cyclin* D1(amplification) [2] in Kras, p53, EGFR, or hTERT) will reverse benign phenotype of late precancer lesions (PMDs) into benign cancer lesion. Third mutation in the PKD1 gene

(PRKD1), or in E-cadherin gene will increase malignant potential of cancer, activating EMTransition, invasion and metastasis, e.g. will lead to aggressive phenotype. According Ristich VL *et al.* SCCs did not showed increased expression of PKD1[70], although tumour possess increase expression of EGFR, leading to activation of downstream signaling pathways. Thus, down-regulation of *PRKD1* (gene of PKD1) is a very probable reason for conversion of PMDs in SCCs. An analysis of 530 HNSCC tumors from the TCGA via cBioPortal demonstrated low levels of DNA methylation on *PRKD1* gene. Further analysis indicated 13% cases (67 out of 530 cases) of PKD1 had loss of heterozygosity (LOH), while only three cases (< 1%) of PKD1 showed homozygous deletion. Thus, a combination of genetic and epigenetic alterations contributed to the downregulation of PKD1 expression.[71]

PKD1 is activated during oxidative stress through a mechanism that requires nonreceptor tyrosine kinases (c-Abl and Src) and PKCδ (and probably not other PKCs). c-Abl-dependent PKD1 phosphorylation at Tyr463 (in the PH domain) releases intramolecular autoinhibition, and Srcdependent PKD1 phosphorylation at Tyr95 creates a docking site for the C2 domain of PKCδ; PKCδ then phosphorylates the PKD1 activation loop at Ser738/ Ser742. A redox-dependent pathway involving Src and c-Abl also promotes PKD1– PH domain phosphorylation at Tyr432 and Tyr502, but the significance of these modifications is uncertain, because they do not lead to gross changes in PKD1 activity. There is evidence that the reactive oxygen species-activated PKD1 enzyme is localized (although not necessarily restricted) to mitochondria and that it recruits a nuclear factor κB (NFκB) pathway that induces expression of antioxidant/antiapoptotic genes (such as manganese superoxide dismutase) and promotes cell survival. It is noteworthy that the canonical growth factor-dependent PKD1-signaling pathway does not activate NFκB or induce manganese superoxide dismutase (mnSOD), emphasizing that the signaling repertoire and cellular actions of PKD1 can be highly contextual.[119]

Similar mechanism of activation mediated by a Src family kinase cascade was observed by Bolag after UVB exposure and UVB-elicited oxidative stress.[144] Although, according to authors, UVB increases tyrosine phosphorylation of PKD by Src as well as western analysis using an antibody recognizing phosphotyrosine463 (tyrosine residue phosphorylated by Abl, not by Src-authors' remark). This result is also consistent with the ability of the tyr463phe PKD mutant to act in a dominant negative manner to exacerbate UVB's apoptotic effect (and prevent PKD's ability to promote survival).[143] A number of studies have shown that PKD1 opposes the apoptotic effects of oxidative stress (or UVB exposure) in a variety of cells and allow survival of UV-damaged cells. This ability of PKD1 to promote survival would be beneficial in preventing excessive apoptosis with low levels of UVB exposure, causing minimal DNA damage that can be repaired. However, if PKD1 allows survival of cells that have suffered irreparable UV-induced DNA damage, these keratinocytes with DNA mutations could continue to proliferate and form skin tumors. Thus, either a pro-proliferative or pro-survival mechanism could provide a means by which PKD1 could contribute to epidermal tumorigenesis.[144,67] Similar mode of PKD1 activation was observed from Chiou *et al.* in mouse model of papillomas[97] (probably as a result of PKCs depletion from TPA[148], there is no data in human papillomas), and was suppose from us in BCC.[95,44,148]

PKC δ is activated in keratinocytes exposed to UV radiation by caspase-3-mediated cleavage in the hinge domain to generate a constitutively active catalytic fragment, called PKM.[187] The cleavage and activation of PKCδ is involved in UV-induced apoptosis, an important protective mechanism which helps protect the epidermis from cancer by eliminating potentially malignant keratinocytes. Since PKCδ is involved in eliminating UV-damaged keratinocytes via apoptosis, and is down-regulated or inactivated in keratinocytes with activated ras genes, PKCδ is a potential tumor suppressor for skin cancer.[172,185] p38δ mitogen-activated protein kinase (MAPK) is a downstream carrier of the PKCδ-dependent death signal in epidermal keratinocytes. Concurrent p38δ activation and extracellular signal-regulated kinase 1/2 (ERK1/2) inactivation are required for apoptosis. H_2O_2, a known inducer of keratinocyte apoptosis, promotes identical PKCδ and p38δ-ERK1/2 activity changes, leading to similar morphological alterations.[186] Other alterations after UV radiation in skin were recently summarized by us in.[148]

Proves for the participations of PKD1 in inflammatory and tumour promoting events, in accordance with our results and hypothesis, were published Chiou YS *et al.*.[97] Topical application of TPA (A) or DMBA (B) over 12 h, according (DMBA)-initiated and 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-promoted skin tumorigenesis in ICR mice, greatly increased the protein levels of PKD1 and CD34 (stem cell marker), decreased ERK1/2, increased c-Myc, Cyclin B1/CDK1 complexes and Cdc25A. Pretreatment with AcEGCG (peracetylated EGCG) lead to the activation of ERK, the degradation of Cdc25A and the inhibition of cyclin B1/CDK1 complex assembly; these effects cause G2/M phase arrest and block mitotic progression. Pretreatment with AcEGCG at a dose of 1 or 5 μM resulted also in a decrease in the levels of phosphorylated JNK1/2, p38 and PI3K/ Akt compared with the levels in DMBA/TPA-mediated tumors (decreased p-ERK1/2 increased, p-PI3K, p-JNK1/2, p-p38). The authors also observed that the DMBA/TPA stimulation of NF-κB, C/EBPs and CREB-DNA-binding activity was attenuated by pretreatment with AcEGCG in a dose-dependent manner, which transcribe proinflammatory and proproliferative genes, including iNOS (inducible nitric oxide synthase), COX-2 (cyclooxygenase-2), ODC (ornithine decarboxylase) and VEGF (vascular endothelial growth factor). Overall, the authors speculated that AcEGCG exerts antiproliferative and/or antiinflammatory effects in CD34+ skin stem cells and

skin tumors and that the suppression of PKD1 activity and its downstream signaling pathways may be involved in the prevention of skin carcinogenesis.[97]

In this study, Chiou YS *et al.* also analyzed CD34 and PKD1 expression levels in human keratinocytes (HaCaT) and human epithelial carcinoma cells (A431) and found that A431 cells had an ~4-fold induction of CD34 and PKD1 expression compared with HaCaT cells. In addition, CD34 and PKD1 upregulation correlated with a fast proliferative potential. Their results strongly suggest that the overexpression and activation of PKD1 in CD34+ skin stem cells and skin tumors are potential targets for the treatment of skin carcinogenesis.[97] Additionally , when subjected to two-stage chemical skin carcinogenesis protocol, PKD1-deficient mice were resistant to papilloma formation when compared to control littermates.[107] Ristich *et al.* do not detect PKD1 in SCC, and probably down-regulation of its expression could be accepted as a critical point in progression of benigh tumours to SCC[70] (lack of its expression as a cancer marker), two-stage carcinogenesis protocol leads to development of papillomas and consequently SCC.

The Chiou YS *et al*. article is interesting with that that it is the only paper detecting PKD1 effects in mouse keratinocytes on different key kinases. Increased PKD1 levels, after two step carcinogenetic protocol, resulted in decreased p-ERK1/2, and increased p-PI3K, p-JNK1/2, p-p38. The decreased in p-ERK1/2, decreased activity of ERK1/2 is in accordance with our results, which also showed that PKD1 inhibited phosphoryation of ERK1/2 in human keratinocytes[75,74] (and unpublished results with PKD1 antisense oligonucleotide) leading to stimulation of keratinocytes proliferation, contrarily to expected stimulation of ERK1/2 according scientific data. According the same sources increased PKD1 levels has to inhibite phosphorylation (activity) of PI3K, JNK1/2 and p38 in other cell types (PKD1- Substrates and function), but Chiou YS *et al*. detected the reversed effects. The authors also proved NF-κB activation by increased PKD1 levels in mouse keratinocytes in the process of tumour promotion.[97]

In recent report Rashel M *et al.* generated mice with targeted deletion of PKD1 in epidermis to evaluate the significance of PKD1 in normal and hyperplastic conditions, as mentioned above. In addition, the hyperplastic and inflammatory responses to topical phorbol ester were significantly suppressed suggesting involvement of PKD1 in tumor promotion (and inflammation). Consistently, when subjected to two-stage chemical skin carcinogenesis protocol, PKD1-deficient mice were resistant to papilloma formation when compared to control littermates.[107] However, similarly to PKCε over-expression this could promotes the formation of highly metastatic squamous cell carcinomas (papilloma-independent carcinomas - SCC).[108,109,110] PKCε can phosphorylate PKD1 in the activation loop Ser738/Ser742 in human, resulting in its activation[95], although the kinase influences many other cell processes.[111]

Moreover, as it was mentioned above, mTOR major up-stream and down-stream regulator gene expression was assessed in skin biopsies from 15 patients affected by psoriasis, 5 patients with allergic contact dermatitis (ACD), 5 patients with atopic dermatitis (AD) and 3 patients with EGFR-inhibitor-induced skin rash. All analyzed skin diseases showed an increase of mTOR gene expression whereas mTOR up-stream negative regulators were reduced or not enhanced in all of them. mTOR was strongly expressed in all epidermal layers of lesional and non-lesional psoriatic skin. Conversely, pro-inflammatory conditions, *in vitro*, were not able to increase mTOR levels, except for UVB. Similarly, anti-TNF-α therapy was not able to reduce mTOR gene expression in patients with psoriasis. Balato A *et al.* study provides evidence that mTOR is involved in cutaneous inflammatory process, but through a signalling not directly dependent from Thl-Th17 pathway.[112, 95] Activation of PI3K/Akt/mTOR pathway is a central event in many types of cancer[113] and represents a promising target for new treatment strategies.[114,115,44]

PI3Ks are activated by RTKs, such as EGFR, and the catalytic subunit phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP2) to form phosphatidylinositol 3,4,5-triphosphate (PIP3). Interaction of PIP3 with the PH (Pleckstrin Homology) domain of AKT and PDK1 results in a conformational change causing phosphorylation of AKT/PKB by PDK1 and mammalian target of rapamycin complex 2 (mTORC2). This activates AKT that then phosphorylates proteins involved in cell growth and survival. mTOR is a protein kinase that acts downstream of PI3K and AKT and plays an important role in cell growth, survival and protein synthesis regulation. There are two mTOR complexes: mTORC1 activates ribosomal protein S6 kinase 1 (p70S6K, which directs the translation of cell cycle regulatory proteins such as Cyclin D1 and myc[116], TNF-α/mTOR/S6K1 patwhay activates Gli1[117], and inactivates eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1), resulting in protein translation and cell growth, whereas mTORC2 together with PDK1 phosphorylates and activates AKT.[118] PKD1 phosphorylate the p85 regulatory subunit of PI3K (which is inhibited —do not bind RTKs—when it is phosphorylated in the SH2 domen by PKD1.[119] Ras can also activate the PI3K signalling cascade[118,95] and RalGDS (Ras-like guanine nucleotide-dissociation stimulator).[120,95]

mTOR gene expression was significantly increased in psoriasis, allergic contact dermatitis (ACD), atopic dermatitis (AD) as well as in EGFR inhibitor induced cutaneous rash, compared to healthy skin. Psoriasis mTOR expression was also enhanced in non-lesional skin of psoriatic patients but only weakly expressed in the epidermis from healthy individuals. Conversely, mTOR gene expression resulted decreased in peripheral blood mononuclear cells (PBMC) isolated from psoriatic subjects when compared to healthy controls. To confirm the effective enhancement of mTOR in these skin inflammatory diseases Balato A *et*

al. also investigated the expression of mRNA levels of key negative upstream regulators ofmTOR such as TSC 1 and TSC2. TSC I and TSC2 gene expression were not enhanced in psoriasis and ACD and significantly reduced in AD compared to healthy skin; particularly, as regards EGFR inhibitor induced cutaneous rash, TSC1 levels were not significantly increased, whereas TSC2 gene expression was reduced. The results found by investigating TSC1 and TSC2 expression suggest that the augmentation of mTOR in psoriasis, AD as well as ACD could be functionally active because not inhibited by its major negative upstream regulators. Since their experiments showed that mTOR may be involved in skin inflammation they also investigated gene expression of its major downstream effectors such as 4EBPl and S6Kl, which are known as the best output of mTORC1. Neither 4EBPI nor S6KI were significantly augmented in psoriasis, AD, ACD or EGFR inhibitor induced cutaneous rash suggesting that mTOR pathways involved in skin inflammation are different from those traditional and well-known such as those implicated in protein and lipid synthesis.[3] Additionally, Balato A *et al. in vivo* and *in vitro* experiments showed lack of a significant relationship between mTOR, TNF-α and IL-l7A, supporting the hypothesis of alternative signalling pathways for mTOR activation in skin inflammation. Taken together, their results suggested that mTOR is involved in cutaneous inflammatory process, but through a signalling not directly dependent from Th1-Th17 pathway.[3,95]

A recent research by Iversen *et al.* using quantitative RT-PCR (reverse transcription-polymerase chain reaction), determined the mRNA expression of the seven of 14-3-3 isoforms (β, γ, ε, ζ, η, σ and τ) in involved and uninvolved skin from psoriasis, basal cell carcinoma (BCC), atopic dermatitis and nickel induced allergic contact dermatitis. 14-3-3σ mRNA expression was increased in psoriasis and contact dermatitis, but not in BCC. In atopic dermatitis no significant difference between involved and uninvolved skin was found. Increased 14-3-3τ mRNA levels were detected in involved skin from patients with psoriasis, contact dermatitis and BCC. Only 14-3-3τ expression (protein) was significantly increased in involved psoriatic skin compared with uninvolved skin.[121] The docking interaction between 14-3-3τ and PKD1 actually decreases PKD1 catalytic activity, probably through nuclear export of activated PKD1.[119,95] Immunofluorescence staining with 14-3-3τ and 14-3-3σ (stratifin) specific antibodies showed localization of both isoforms to the cytoplasm of the keratinocytes in the various skin sections.[121] The σ isoform is of particular interest because it is specifically expressed in epithelial cells, and its expression is frequently lost during breast and prostate cancer progression. The received data strongly suggest that S11 phosphorylation of Snail is critical for binding to 14-3-3σ and PKD1 is able to phosphorylate S11 *in vivo*[11,95], although there is no data in keratinocytes or in epithelial cancer cell lines. Snail protein is also pulled down with 14-3-3η independent of PKD1.[122, 95-Fig. 4]

Increased expression of PKD1 was detected in ADM (Acinar-to-ductal metaplasia) and in PanIN (pancreatic intraepithelial neoplasia) lesions, but not in adjacent regions of "normal" acinar structures. PKD1 overexpression was previously implicated in pancreatic cancer.[123,124,69,15] Mediators of ADM *in vivo* are activating mutations of Kras, inflammation and persistent activation of the EGF-R. Approximately 95% of all pancreatic ductal adenocarcinoma (PDAC) express either somatic activating mutations of Kras or show increased epidermal growth factor receptor (EGF-R) signaling. In a transgenic animal model, in which an oncogenic mutant of Kras is expressed in acinar cells of the pancreas, ADM and progression to PanIN lesions are observed. These events caused by mutated Kras are further potentiated and lead to pancreatic cancer, when additional pancreatic inflammation occurs.[123,124,103]

In transgenic mice that express TGFα (EGF-R ligand) in the pancreatic epithelium, in areas where acinar cells undergo ADM, the PKD expression pattern was altered. Of the three PKD isoforms, PKD1, PKD2 and PKD3, acinar cells of normal pancreas express only PKD3. As a result of TGFα signaling, PKD1 expression and activity can be detected in regions of ADM, PanIN1 and PanIN2 pre-neoplastic lesions, while the 2 other PKD isoforms are not involved in these processes (their expression decreased in regions of ADM). As the author wrote questions remaining are i) how PKD1 expression is upregulated by both, mutant Kras and EGFR signaling?; and ii) how both pathways can mediate activation of PKD1? Since PKD1 activity downstream of Kras was determined by measuring nPKC-mediated activating phosphorylations, an involvement of the novel PKCs - PKCε and/or PKCδ is most likely, and this is currently under investigation.[123,124]

A more detailed analysis showed that the knockout of PKD1 delays the progression of ADM areas to PanINs. Introduction of wildtype PKD1 increased ADM events in 3D explant cell culture approximately 2-fold and constitutively-active PKD1 approximately 6-fold as compared to virus control or kinase dead PKD1. However, ducts generated by active PKD1 were neither as large, nor as well developed as ducts obtained when metaplasia was induced with TGFα. PKD1 has been shown to activate nuclear factor κ-B (NF-κB) and Notch; and NFκB and Notch both cooperate in some signaling pathways.[103,125,15,100] Since specific small molecule PKD inhibitors exis, PKD1 is a promising new target to prevent ADM and further progression to PanIN pancreatic lesions.[123,124] Finally, increased PKD1 activity can be detected in regions of pancreatitis, in ADM, PanIN1 and PanIN2 pre-neoplastic pancreatic lesions[103] and in pancreatic cancer.[100,126,69]

Human Papilloma Viruses (HPV)

In recent years, the human papilloma virus (HPV) and its link with HNSCC, particularly in oropharyngeal tumors, has been illustrated. Not only can HPV be used as a biomarker of prognostic significance, but also as a preventative target. There are more than 100 subtypes

of HPV, some of which are involved in carcinogenesis and have been designated as high-risk HPVs (e.g. HPV-16 and -18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 66)[127], type 16 is most commonly associated with HNSCC. HPV is a double-stranded DNA virus that encodes several proteins, among which are three oncoproteins: E5, E6, and E7. HPV infection is an early, and probably initiating, oncogenic event in HNSCCs. High-risk oncogenic HPV subtypes have been shown to be capable of transforming oral epithelial cells through the viral oncoproteins E6 and E7. In the USA, about 40-80% of oropharyngeal cancers are caused by HPV, whereas in Europe the proportion varies from around 90% in Sweden to less than 20% in communities with the highest tobacco use. Patients tend to be younger, with no prior history of tobacco and/or heavy alcohol consumption. There is evidence that HPV-positive HNSCC is a sexually transmitted disease. A strong association between sexual behavior (oral sex) and risk of oropharyngeal cancer as well as HPV-16-positive HNSCC has been demonstrated.[8,128] HPV 16 and 18 are well known because they have been shown to significantly increase the risk of cervical cancer in women who have persistent infections with these two types of HPV.[11]

The HPV E6 protein binds p53 and promotes its degradation, whereas E7 protein binds and inactivates pRb liberating E2F. E2F activates genes responsible for cell cycle progression through the G1 to S phase, including cyclin A, E and DNA polymerase, causing inactivation of checkpoints and regulatory pathways, and ultimately promoting cellular proliferation and transformation. These viral oncoproteins determine cell cycle entry and inhibition of p53-mediated apoptosis. HPV-dependent inhibition of pRb promotes p16 accumulation. p16 represents a surrogate marker of HPV-positive HNSCC (see below[8,129,118]). In contrast to HPV-negative tumours, the expression of *CDKN2A*, encoding $p16^{INK4A}$, is highly upregulated, but often inactivated and cyclin D1 is often overexpressed in HNSCC contributing to increased proliferation[116], but its amplification is infrequent.[118] Expression of total and nuclear EGFR was higher in p16-negative tumors (HPV-) compared to p16-positive tumors (HPV+).[90,11]

Doorslaer and Burk showed that oncogenic types papilloma virus (HPV) specifically activate the hTERT promoter, while non-oncogenic types do not.[92] Additionally, *TERT* has been described to have influence in several other molecules and pathways, which modify responses to inflammation, cell death, apoptosis and DNA damage responses, EMT and oncogenesis. *TERT* binds to c-MYC and recruits the complex to heparanase promoter to upregulate heparanase expression promoting invasion and metastasis of gastric cancer cells; furthermore, *TERT*-activated Wnt/β-Catenin signalling promotes c-MYC expression, which could in turn activate *TERT* transcription and expression in a positive feedback loop; It was also described that *TERT* overexpression upregulated the expression and transcriptional activity of a key cell cycle regulator, cyclin D1, in human prostate epithelial cell lines; (iii) finally, it has been shown that *TERT* regulation of ITGB1 in the MDM2-FOXO3a- ITGB pathway is able to promote gastric cancer invasion; (iv) *TERT* is capable of activating the transcription of vascular endothelial growth factor (*VEGF*) in WI-38 and HeLa cells, this activation being independent of telomerase activity and telomere maintenance.[55] hTERT can modulate classical cancer pathways including NF-κB, TGF-β/Smad, and Wnt signaling that all contribute to the metastatic potential and stem cell phenotype of cancer cells.[65]

Resistance to cancer treatment – Ionizing Radiation and Drugs

In a paper Hamakawa *et al.* focused on the 4 molecules, epidermal growth factor receptor (EGFR), cyclooxygenase-2 (COX-2), peroxisome proliferator-activated receptor gamma (PPARγ), and progesterone receptor, that are, respectively, associated with the proliferation and the differentiation of OSCC (oral squamous cell carcinoma) and SGC (salivary gland cancer). Gefitinib ("Iressa," ZD1839), a small molecule EGFR tyrosine kinase inhibitor, can inhibit the proliferation of OSCC cell lines in a dose- and time-dependent manner and lead to cell cycle arrest with accumulation of cells in the G1 phase, and a decrease of cells in S phase. The agent suppressed tumor metastasis in the animal model. Furthermore, a cooperative antiproliferative effect was obtained when cancer cells were treated with radiation followed by Gefitinib. While radiation alone did not significantly affect p38 mitogen-activated protein kinase and MAP kinase kinase (MEK)1/2 autophosphorylation, the combination of gefitinib and radiation completely inhibited the downstream signaling of EGFR. Gefitinib enhanced tumor radioresponsiveness by multiple mechanisms, including the growth inhibition and effects on DNA repair after exposure to radiation. Next, the level of COX-2 expression correlated inversely with increased tumor radiation sensitivity. Treatment with Celecoxib, a COX-2 selective inhibitor, enhanced the radioresponsiveness of HSC-2 cells, which constitutively expressed COX-2. Another promising molecular target is the PPARγ, which is a member of the nuclear superfamily of ligand-activated transcription factors. Recent studies have demonstrated that PPARγ ligands induce cellular differentiation and inhibit cell growth in carcinomas of various types. These data suggest that synthetic PPARγ ligands may be useful for molecular targeting of oral cancer.[130]

Aravindan N. *et al*. studies indicated that ionizing radiation (IR) induces NF-κB-dependent clonal expansion of therapy resistant tumor cells. Functional NF-κB mediates telomerase activity by binding to the κB binding region in the promoter region of TERT, induced also by IR. Elimination of the NF-κB recognition site on telomerase or muting NF-κB compromises IR-induced telomerase promoter activation, confirmed by authors results. The authors investigated whether mitigation of NF-κB-dependent telomerase activation by EGFR tyrosine kinase inhibitor can enhance IR-induced celling killing. SCC-4 and SCC-9 cells exposed to IR with or without Pelitinib (EGFR Tyrosine Kinase Inhibitor) were

examined for NF-κB and hTERT transcription using luciferase reporter assays. Pelitinib inhibited NF-κB activity and potentiates IR-induced cell killing. Furthermore, authors found that Pelitinib inhibited IR-induced TERT transcription, transactivation and telomerase activation in IR-exposed and NF-κB-overexpressed cells. The authors proposed that if IR-induced NF-κB-mediated cell survival supported by telomerase activation, can be inhibited with Pelitinib treatment, one could further enhance therapeutic outcome in squamous cell carcinoma.[53]

In a previous study Tamatani T. *et al* demonstrated that human head and neck cancer cells have significantly enhanced levels of transcription factor nuclear factor (NF)-κB activity compared to their normal counterparts, suggesting that NF-κB plays an important role in the development of this type of cancer. However, it has been reported that chemotherapeutic agents and radiation activate NF-κB activity in cancer cells, thus making the cells chemoresistant and radioresistant. In addition, they have shown that the suppression of NF-κB activity enhanced apoptosis in oral squamous cell carcinoma cells. The authors examined whether Cepharanthin-induced inhibition of NF-κB activity enhances radiosensitivity in human oral carcinoma cells. Cepharanthin is a biscoclaurine alkaloid extracted from the roots of Stephania cepharantha hayata, and is widely used in Japan for the treatment of patients with leucopenia, nasal allergy, and venomous snakebites. Gamma-irradiation (IR) induces NF-κB activity in oral carcinoma cells through the activation of upstream molecules, including Akt and IκB kinase. However, a luciferase assay revealed that cepharanthin suppresses IR-induced NF-κB activity in oral squamous cell carcinoma cells, thereby enhancing the radio-sensitivity. In an *in vivo* study, B88 cells (oral SCC cell line) were s.c. inoculated into the backs of nude mice. Tumor-bearing nude mice received either cepharanthin, IR alone, or a combination of cepharanthin and IR. The combined treatment suppressed tumor growth significantly more than either cepharanthin or IR alone. Cepharanthin inhibited the production of IR-induced IL-6 and IL-8, which are downstream targets of NF-κB. In quantitative real-time RT-PCR, IR also induced the expression of anti-apoptotic proteins (cellular inhibitor of apoptosis protein (cIAP)-1 and -2) in carcinoma cells. Treatment of cancer cells with Cepharanthin combined with exposure to IR decreased cIAP-1 and -2 mRNA expression. These findings suggested that the combination of radiotherapy and Cepharanthin could enhance radiosensitivity in the treatment of human oral cancer.[131]

Telomerase activity (TA), frequently observed in cancer, compensates for telomere shortening thus preventing cell senescence and conferring resistance to therapy. In a study, Papanikolaou V. *et al.* investigated the expression of human telomerase reverse transcriptase (hTERT) and TA and their regulation, as well as apoptotic rates and correlation with the presence of human epidermal growth factor receptor 2 (HER2), in irradiated tumour-derived breast cancer cells. In 50 breast cancer tissue samples hTERT mRNA expression and TA were correlated with cell features (HER2, Estrogen and Progesterone Receptor status). hTERT/TA were found increased only in irradiated HER2-positive cells, which were found to be more radioresistant, while HER2 knockdown led to hTERT/TA downregulation. HER2 was found to mediate hTERT expression through activation of Nuclear Factor-kappa B (NF-κB) and c-myc. The study suggests that following irradiation, HER2 receptor activates hTERT/telomerase, increasing the breast cancer cells' survival potential, through sequential induction of transcription factors NF-κB and c-myc.[132]

However, in a cross sectional study 80 cases of BCC including at least 2 millimeter of surrounding normal skin were selected and analyzed. Immunohistochemical method is used for evaluation of membranous expression of HER2 protein in BCC compared to normal skin. Of 80 cases studied, 34 (42.5%) were female and 46 (57.5%) were male. Membranous staining was found in 44 (55%) of normal surrounding skin and 6 (7.5%) of BCC cases. The increased membranous expression of HER2 in surrounding normal skin compared to BCC was statistically significant. Above findings suggest that HER2 protein could be a factor in developing of BCC and can be used as a negative marker in diagnosis of BCC.[133] Additionally, Liu B *et al.* found that the expression of c-erbB-1 oncogene in all BCC increased by different degrees and the expression of c-erbB-2 oncogene in BCC was significantly reduced or lost when compared to that in normal epidermal cells. Furthermore, apparent negative and positive relationships were observed respectively between the tumor differentiation and the expression of c-erbB-1 and c-erbB-2 oncogenes in SCC.[134]

As it was mentioned above a role for NF-κB has been implicated also in the pathogenesis of hepatocellular carcinoma. This transcription factor can regulate hTERT gene transcription, which was found to be at high levels in this carcinoma. However, positive effects of NF-κB on hTERT protein synthesis in HepG(2) cells were unknown. The authors showed that LPS (specific binding to TLR4 (Toll-Like Receptor 4) to activate NF-κB) was positive for NF-κB p65 mRNA expression and activation, and also up-regulated hTERT mRNA and protein expressions at 36h in a dose-dependent manner. In contrast, MG-132 (blocking the activity of 26S proteasome and thereby preventing nuclear translocation of NF-κB) significantly inhibited activation of NF-κB and mRNA expression. And also reduced the expression of hTERT at both mRNA and protein levels at 36h in a dose-dependent manner. Furthermore, dexamethasone inhibited LPS-induced activation of NF-κB and expression of the hTERT in HepG(2) cells. These findings suggest that NF-κB may modulate hTERT mRNA level, importantly, in protein level in HepG(2) cells and dexamethasone inhibits LPS-induced hTERT via blocking NF-κB.[98]

Activation of NF-κB up-regulates cell cycle mediators (cyclin D1, c-Myc), anti-apoptotic (c-FLIP,

survivin, Bcl-XL) and adhesion molecules (ICAM-1, ELAM-1,VCAM-17), proteolytic enzymes (e.g., MMP, uPA),and pro-inflammatory factors (PGHS-2 (COX-2), LOX, prostaglandins, cytokines – IL-1, IL-6, IL-8, IL-23, TNF, TGF-β, EGF), iNOS, chemokines (CCL2,CCL20), that promote an invasive phenotype. In particular NF-κB provides a mechanistic link between inflammation and tumorigenesis. NF-κB is a major factor which controls apoptosis – based tumor immune surveillance mechanisms of pre-neoplastic and malignant cells. NF-κB also regulates tumor angiogenesis (VEGF) and invasiveness, and may contribute to chemo- and radioresistance of tumor cells (see above).[99,28]

Ionizing radiation (IR) instantaneously causes the formation of water radiolysis products that contain some reactive oxygen species (ROS), ROS are also suggested to be released from biological sources in irradiated cells.[28] The results of Rzeszowska-Wolny *et al*. also showed that ionizing radiation activates cellular processes which produce long-lasting ROS and RNS (Reactive nitrogen species) radicals, which may have different sources in different cell types and could participate in cellular signaling networks important for radiosensitivity and mode of cell death.[135,136] The free radicals generated by ionizing radiation can signal p53 translocation to the nucleus.[136]

Increases in reactive oxygen species (ROS) have been implicated in age-related diseases, including cancer. The serine/threonine kinase protein kinase D1 (PKD1) is a stress-responsive kinase and sensor for reactive oxygen species, which can initiate cell survival through NF-kappaB signaling. It was previously shown that in response to ROS, PKD1 is activated at the mitochondria and regulates the cellular response by activating the NFkB pathway.[67] However, the initial signaling events leading to localization of PKD1 to the mitochondria are not completely known.[142] PKD1 is activated by oncogenic Ras expression and PKD1 promotes Ras OIS (Oncogene-induced senescence) by mediating inflammatory cytokines interleukin-6 (IL-6) and interleukin-8 (IL-8) via modulation of NF-κB activity [44,95]. The authors demonstrate that ROS-protein kinase Cδ (PKCδ)-PKD1 axis is essential for the establishment and maintenance of IL-6/IL8 induction, thus preventing cancer occurrence through induction of cell senescence program. In addition, ablation of PKD1 causes the bypass of Ras OIS, and promotes cell transformation and tumorigenesis. Thus, the authors data provide evidence to support that PKD1 could potentially act as a tumor suppressor to prevent cancer development at an early stage in the context of oncogenic Ras activation in ER:Ras IMR90 cells (human diploid fibroblasts transduced with an ER:RAS).[137] It is known that PKCδ activity modulates also PKD1 activation in oxidative damage in Parkinson's disease (PD) models.[138] PKD1 activation mediated by a Src family kinase cascade was observed by Bollag after UVB exposure and UVB-elicited oxidative stress in mouse keratinocytes.[143,144] From the other site leukocytes are the main source of RNS and ROS acting as chemical effectors in inflammation-driven carcinogenesis.[99]

As already mentioned, HIF-1 (Hypoxia inducible factor-1), the key mediator in hypoxia signaling pathways, is crucially involved in hypoxia-induced tumor development. Recent research of Scherbakov AM *et al* shows that Snail protects breast cancer cell from hypoxia attack, at least partly via β-catenin which up-regulated expression of HIF-1 dependent genes and inhibits apoptosis. Snail1 knock-down enhanced the hypoxia-induced inhibition of cell proliferation giving the direct evidence of Snail1 involvement into cell protection from hypoxia attack. Furthermore, the same authors found that decrease in the estrogen dependency is correlated with increase in Snail1 expression and activity, and demonstrated the Snail1 involvement in the negative regulation of ER, and showed that Snail1 inhibition partially restores the sensitivity of the estrogen-hyposensitive cells to antiestrogen tamoxifen.[139,140] PKD1-mediated phosphorylation of SNAI1 (Snail1,Snail) occurs in the nucleus and generates a nuclear, inactive DNA/SNAI1 complex that shows decreased interaction with its co-repressor Ajuba. Analysis of human tissue samples with a newly-generated phosphospecific antibody for PKD1-phosphorylated SNAI1 showed that regulation of SNAI1 through PKD1 occurs *in vivo* in normal breast ductal tissue and is decreased or lost in invasive ductal carcinoma.[141]

It has been shown previously that the transcription factor HIF-1 is induced in tumor cells not only by different cytokines and prostaglandins, but also by ROS and RNS. This obviously includes radiation-induced activation of HIF-1. HIF-1 is a heterodimeric transcription factor consisting of a constitutively expressed β-subunit and an oxygen-regulated α-subunit. The author investigations revealed no correlation between basal HIF-1α levels and the survival fraction in irradiated tumor cell line simplying that basal HIF-1α levels in human tumor cell lines obviously do not predict their radiosensitivity under normoxia.[141] Administration of the HIF-1 inhibitor YC-1 to hypoxic cobalt-treated cells derived from squamous-cell carcinoma of the larynx effectively inhibited HIF-1α expression, and enhanced the sensitivity of cells to radiation, decreasing the surviving fraction to that of normoxic cells. YC-1 was found to reduce the number of tumor lesions after tumor cell inoculation in nude mice. Compared to radiation therapy alone, inhibition of radiation-induced HIF-1 activation by YC-1 led to a significant reduction in tumor cell growth.[145]

Thus, IR-induces NF-κB, telomerase activity and hTERT transactivation[53,131], leading to radioresistant and chemoresistant cells population in different tumour cells. COX-2 expression, also correlated inversely with increased tumor radiation sensitivity.[130] COX-2 gene is also NF-κB targeted gene. Furthermore**,** EGFR Tyrosine Kinase Inhibitor (Gefitinib**,** Pelitinib)[130,53], NF-kappaB inhibitors (Cepharanthin[131], EGCG[99], COX-2 selective inhibitor (Celecoxib**)**[130], HIF-1 inhibitors (YC-1)[145], and PKD1 unspecific inhibitor

Resveratrol[99] all increase tumor cell sensitivity toward radiotherapy.

Additionally, resistance of Lung Adenocarcinomas to Gefitinib or Erlotinib (EGFR kinase inhibitors) was associated with a secondary mutation in the EGFR Kinase Domain (T790M substitution of methionine for threonine at position 790 - exon 20), deletions in exon 19 and L858R mutation in exon 21. The mutation - T790M was not detected in untreated tumor samples. EGFR mutation (EGFRvIII)[23], corresponds to a deletion of the extracellular domain. This variant has been found in 42% of HNSCC, related to the poor response to monoclonal antibody Cetuximab (competitively inhibits EGFR).[24] Interestingly, EGFRvIII displays ligand-independent signaling, but has low constitutive activity. The low constitutive activity is enough to impart cancer cells with increased signaling, however its growth advantage is due to the fact that these receptors are not downregulated by endocytosis.[12] As currently approved EGFR-targeted treatments do not appear to be efficacious, alternative targeted therapies against EGFRvIII have been developed, including the vaccine Rindopepimut and the monoclonal antibody mAb806 (also known as ABT-806). Rindopepimut did not pass Phase III clinical trials and was discontinued in 2016. ABT-806 has passed Phase I, and an antibody-drug conjugate based on mAb806 called ABT-414 has also been advanced to Phase II (trial identifier NCT02573324) (review:[12]). *K-Ras* mutations have been previously associated with primary resistance to both groups of these drugs[26,30,14], since K-Ras altertions lie downstream of the receptor. Approximately 95% of all pancreatic ductal adenocarcinoma (PDAC) express either somatic activating mutations of Kras or show increased epidermal growth factor receptor (EGF-R) signaling.[8,9] It is thought that oncogenic KRas is an initial event leading to pancreatic cancer. Oncogenic KRas upregulates the epidermal growth factor receptor (EGF-R) and its ligands TGFα and EGF, which leads to additional activation of wildtype KRas; and activity of both pathways are needed for pancreatic tumorigenesis. Major downstream signaling cascades activated by active KRas in pancreatic cancer are the PI3-K/ PDK1/Akt (known also as PKB) and Raf/MEK1/2/ERK1/2 (Extracellular signal Regulated Kinase) pathways.[10] PKD1 activity is also elevated in presence of a mutant Kras, or after EGFR-mediated activation of endogenous wildtype Kras. As a result of such signaling, PKD1 expression and activity can be detected in regions of ADM, PanIN1 and PanIN2 pre-neoplastic lesions, while the 2 other PKD isoforms are not involved in these processes.[10] Pao *et al.* examined 60 lung adenocarcinoma patients and showed that K-ras mutations are associated with a lack of sensitivity to gefitinib or erlotinib. K-ras mutations seem to be resistant to EGFR targeting agents and are reported to be mutually exclusive to EGFR or HER2 gene mutations.[146] In terms of mutations to EGFR-pathway proteins, the efficacy of anti-EGFR therapy has been best studied in cancers with K-RAS mutations, with the consensus that these cancers will display primary resistance to EGFR inhibitors. The FDA now requires an accompanying PCR diagnostic test for K-RAS prior to the prescription of cetuximab or panitumumab for colon cancer.[12]

Oncogenic KRas upregulates the epidermal growth factor receptor (EGF-R) and its ligands, which leads to additional activation of wildtype KRas; and activity of both pathways are needed for pancreatic tumorigenesis.[103] Table with frequency of RAS mutations according to cancer type was recently given by Kodaz *et al.* - Figure1.[31]

Mutations in targeted kinases (proteins) as a cause for drug resistances were also reported for mTOR pahway[120], although PI3K inhibitors or the dual mTOR/PI3K inhibitors led to a significant delay in resistance development in several cancer types including leukemias.[2]

Genome-wide transcriptional profiling shows that aPKC and Smo control the expression of similar genes in tumor cells. aPKC functions downstream of Smo to phosphorylate and activate Gli1, resulting in maximal DNA binding and transcriptional activation. Activated aPKC is upregulated in Smo-inhibitor resistant tumors and targeting aPKC suppresses signaling and growth of resistant BCC cell lines. The results demonstrate aPKC is critical for Hh-dependent processes and implicates aPKC as a new, tumor-selective therapeutic target for the treatment of Smo-inhibitor resistant cancers (probably with Smo and Sufu mutations as well as Gli1/2 amplification).[147,148]

Another reason for chemotherapy resistance are the CSCs (Cancer Stem Cells), which exhibit increased resistance to chemotherapy, because of their resistance to apoptosis and because chemotherapy and radiotherapy strategies mostly targets dividing cells; these CSCs easily escape because they remain mostly in the resting stage of the cell cycle - they are nondividing or slowly dividing.[149,150,151] However, they have the potential to become activated resulting in recurrences or metastases. These cells have the ability of self-renewal, maintaining the CSC reservoir and differentiate into the heterogeneous progeny.[118]

Recent studies revealed that resveratrol induced NPC cells (Nasopharyngeal carcinoma) apoptosis through activating multiple apoptotic pathways. Shen *et al.* found that resveratrol could turn off the metabolic switch, increased the ROS level, and depolarized mitochondrial membranes in NPC CSCs (Nasopharyngeal carcinomas stem cells).[152] These alterations in metabolism occurred concomitantly with the suppression of the CSC properties including the resistance to radiotherapy and chemotherapy, self-renewal capacity, tumor initiation capacity, and metastatic potential in NPC CSCs. Particularly worth mentioning is that resveratrol tackled the nexus of NPC CSCs which resulted in extensive suppression of stemness, EMT, and metabolism-related genes. This extensive suppression in CSCs could also be observed after the authors had ectopically expressed p53, the downstream target of resveratrol. In addition, the suppression of CSC properties by resveratrol could be attenuated by knocking down p53. These findings

substantiated the notion that p53 may serve as a common link between metabolism, stemness, and EMT in CSCs. It was reported that resveratrol can increase the p53 protein level in breast cancer cell line without altering the p53mRNA levels, suggesting that resveratrol may still be useful to treat tumors with a loss of normal p53 function. Besides, resveratrol could significantly activate intracellular Notch-1 and restore wild-type p53 expression in glioblastoma cells. These findings indicate that resveratrol may be an effective drug for treatment of tumors without normal p53 function.[152] Furthermore, resveratrol also inhibited CSC (cancer stem cells) properties in pancreatic cancer, breast cancer, and glioblastoma. Resveratrol could efficiently suppress the invasion and metastasis of tumor cells through reversing the EMT process in lung and breast cancers. It also reduced the self-renewal capacity and stemness gene signatures of CSCs in head and neck cancers.[152]

The major Green tea catechin (polyphenol) EGCG (epigallocatechin-3-gallate) is also chemopreventive, reverse the EMT process in melanoma cells and increases radiosensitivity.[153] Morphological investigations demonstrated that high doses of EGCG (100 and 200 lM) destroyed tumor nest structures and caused cellular degenerative changes, in comparison to untreated control cells. A biological study revealed that EGCG inhibited growth of Oral SCC cells.[154]

The two plant extract Resveratrol (unspecific PKD1 inhibitor) and EGCG (unspecific NF-kB inhibitor) are potent cardioprotectors, which decreases the unwanted side effects of high doses Celocoxib (COX-2 inihibitor), which is offen used in iiradiated and post iiradiated treatment of SCCs, in order to increases tumor cell sensitivity toward radiochemotherapy.[99] At the same time the both substances also suppress expression and activity of COX-2 (PGHS-2)[153,155,156,157] and increase radiosensitivity (see above).

Discussion:

Oral carcinogenesis is a multi-step process. The first step is the development of potentially malignant disorders (PMDs) known as leukoplakia, erythroplakia[33], lichen planus, probably trough mutation in the proteins regulated cell cycle ($p16^{INK4A}$, $p21^{Waf1/Cip1/Sdi1}$, $p27^{kip1}$, *Cyclin D1*(amplification).[2,118] Detection of their mutations could be used for early diagnosis of PMDs. Second mutations in Ras, p53 (TP53) (could be also first), EGFR, hTERT, could be used as markers for early malignant transformation, and probably third in PKD1 and/or E-cadherin genes, leading to activation of EMT, could be used for detection of aggressive alterations. Thus, overexpression of inactivated or mutated forms of p53 in oral epithelial dysplasia has been associated with high risk for transformation to early stage OSCC.[37] Early mutations in p53 were detected in erythroplakia (46%), oral lichen planus (33%), leucoplakia **(**13.3%) and Actinic keratosis, though with low frequencies.[162,158,159,160] In BCC mutations of *p53* (56% of all types of BCCs[2] are known to be late events, whereas silencing of 14-3-3σ takes place early in tumor progression, concomitant with increased expression of PKD1 and Snail and down-regulation of c-myc mRNA[189,44]. In BCCs mutations in $p16^{INK4A}$ (3.5%[161]) are rare and in EGFR are not detected.[11]

Mutations in Ras are not detected in PMDs (with exception of Actinic keratose (AK) (16%)[26,27], Keratoacanthoma (KA)[196] and papillomas[190,44], as a first mutation, without mutations in $p16^{INK4a}$ [44] – Table 1 and Table 2), with low frequencies in SCCs (11% harbor activating Ras mutations)[26], and frequent overactivation in BCCs (mutations ranging from 10 to 30% (50%) according different authors).[38,39,31] Many head and neck cancers are squamous cell carcinomas. More than half a million people are affected every year. The prevalence has continued to gradually increase in recent years. Overall survival in patients with KRAS mutation head and neck tumor is worse. KRAS mutations also demonstrate social differences in head and neck tumors, like many cancers. Though the mutation frequency is 5% in oral cavity tumors in Western societies, it can be seen in up to 18% of cases in Eastern societies. The frequency of KRAS mutation in malignant larynx lesions was 4.8% in one study performed, while there were no HRAS and NRAS mutations. In Eastern societies, the frequency of HRAS mutation in oral cavity tumors can be as much as 35%. KRAS mutation was not detected at all in mouth and hypopharynx cancers in another study conducted. There is KRAS mutation in 11.5% and 3.3% of patients with laryngeal cancer and oropharynx cancer, respectively. NRAS mutation in nasopharynx cancer is reported as 4%, while HRAS mutation is <1% and KRAS mutation is very rare[31] (Table 1). Ras can also activate the PI3K signalling cascade, genetic aberrations of this pathway are also not very common in HNSCC, with mutation in p110α catalytic subunit (encoded by the PIK3CA gene) in 6–20% of HNSCC (amplification).[118,41]

Since increased expression of hTERT is an early event in the pathogenesis of hyperproliferative skin deseases, overexpressed hTERT (under the control of NF-κB[50,51,52,53]) is considered as a proproliferative (proinflammatory) marker, rather than cancer marker. Mutations in HTERT promoter of hTERT gene were detected recently in both BCCs and SCCs, which could be use as a marker for cancer transformation.[25] The quantitative determination especially of EGFR expression could be used as markers for detection of late PML and SCCs, although there are also some conflicting results (see above). It was also shown recently that 42% of HNSCC possess mutations in the gene of EGFR.[24] Thus, detection of mutated genes EGFR and hTERT could be more appropriate for early diagnosis of SCCs.

NF-κB is a key transcription factor that is activated by multiple receptors and regulates the expression of a wide variety of proteins that control innate and adaptive immunity. A number of studies indicate that PKD is a mediator of NF-κB induction in a variety of cells exposed to GPCR agonists or oxidative stress. In view of the increasing recognition of the interplay between inflammation and cancer

development, a possible role of PKD in linking these processes is of importance. However, the precise molecular mechanisms remain incompletely understood. Stimulation of human colonic epithelial NCM460 cells with the GPCR agonist and bioactive lipid lysophosphatidic acid (LPA) led to a rapid and striking activation of PKD2, the major isoform of the PKD family expressed by these cells. LPA stimulated the production of interleukin 8 (IL-8), a potent pro-inflammatory chemokine, and stimulated NF-κB activation. PKD2 gene silencing dramatically reduced LPA-stimulated NF-κB promoter activity and IL-8 production. These results imply that PKD2 mediates LPA-stimulated IL-8 secretion in NCM460 cells through a NF-κB-dependent pathway. PKD2 has also been implicated in mediating NF-κB activation by Bcr-Abl in myeloidleukemia cells. Prostaglandins (e.g., PGE2) produced through COX-2 play a critical role in colon cancer development, and colonic myofibroblasts are major contributors to their generation. Recent results demonstrated that knockdown of PKD1 in these cells prevented the synergistic increase in COX-2 expression induced by the proinflammatory mediators bradykinin and tumor necrosis factor (TNF)-α. Thus these novel results raise the attractive possibility that PKD plays a critical role in mediating COX-2 expression in response to potent pro-inflammatory mediators in human colonic myofibroblasts.[67]

NF-κB also plays a critical role in inflammatory and cell death responses during acute pancreatitis. The PKC isoforms PKCδ and ε are key regulators of NF-κB activation induced by cholecystokinin-8 (CCK-8), an agonist that induces pancreatitis when administered to rodents at supra-maximal doses. PKD was shown to function downstream of PKCδ and PKCε in pancreatic acinar cells stimulated by CCK-8. Specifically, PKD was necessary for NF-κB activation induced by these GPCR agonists in pancreatic cells. These results identify PKD1 as a novel element in the signaling pathways mediating NF-κB activation in acute pancreatitis.[100] PKD has been also identified as one of the critical factors in the development of hypersensitivity pneumonitis caused by microbial agents. Inhibition of PKD1 activation could be an effective way to control acute inflammatory conditions in diverse organs.[67]

Recently it was shown that Group B streptococci (GBS) are one of the leading causes of life-threatening illness in neonates. In a study Upadhyay K *et al*, both live and antibiotic-killed GBS induce activation of PKD1 through a pathway that is dependent on the TLR signaling adaptor MyD88 and its downstream kinase IL-1R–associated kinase 1, but independent of TNFR-associated factor 6. Their studies using pharmacological PKD inhibitors and PKD1-knockdown macrophages revealed that PKD1 is indispensable for GBS-mediated activation of MAPKs and NF-κB and subsequent expression of proinflammatory mediators. Furthermore, systemic administration of a PKD inhibitor protects d-galactosamine–sensitized mice from shock-mediated death caused by antibiotic-killed GBS. These findings imply that PKD1 plays a critical regulatory role in GBS-induced proinflammatory reactions and sepsis, and inhibition of PKD1 activation together with antibiotic treatment in GBS-infected neonates could be an effective way to control GBS diseases.[101]

The low level of PKD1 expression in highly invasive lines was due to epigenetic silencing by DNA methylation[68,69] and this down-regulation of PKD1 leads also to increase expression of MMPs (metalloproteinase), another factor connected with invasive behaviour.[107,163,172] There are conflicting results concerning the use of MMPs expression as markers for tumors lesions, also closely connected with invasion and metastasis. However increase in their expression could be used as a marker for lesions malignant transformation, especially for MMP-9 and MT1-MMP (MMP-14) / MMP-2, degrades type IV colagen in the Basal membranes[10,7,164,165,166,90,167] and major proteinases associated with increase invasive activity[168,146,169] (increased in both SCCs and BCCs). Previously it was suggested that BCCs do not have much metastatic potential because of the retention of high levels of E-cadherin expression. Matrix metalloproteinases capable of cleaving E-cadherin are MMP-3 and -7[7,170] (MMP-9[9,22] and MMP-14[146,170] also according recent data (authors' remark)), which points out how MMPs may mediate invasion, not only by directly degrading matrix, but also by cleaving transmembrane proteins and receptors. E-cadherin expression is reduced in particularly infiltrative BCCs that are also known to produce MMP-7.[7] Absence of E-cadherin expression are also associated with morpheaform and recurrent BCC with MMP-1 immunostai44ning in tumor cells, MMP-9 expression in stromal cells.[171]

As results of Storz and Balaji suggest downregulation of PKD1, is closely connected with induction of EMT and hormone-insensitive phenotype in breast and prostate cancer.[15,29,172,173,174,122] Using EMT markers (vimentin, fibronectin, N-cadherin, vs, E-cadherin; and transcriptional factors - Snail, Slug, Twist; HIF-1α), we could not differentiate late PMDs of early cancer lesions. These markers are useful for detection of aggressive alteration in tumour pathogenesis, which is of importance when a surgical procedure is planned.[10] Several published articles comment the use of E-cadherin expression, together with one of the mesenchymal markers – vimentin or fibronectin, instead of increased β-catenin nuclear staining, for reliable detection of EMTransion, e.g. aggressive alteration.[175]

We can speculate that detection of PKD1 gene mutations in head and neck cancer will detect again aggressive alteration and induction of EMT phenotype, closely connected with increase metastasic abilities and poor prognosis. There is no data for PKD1 expression in the PMDs leading to SCCs, nor for detected mutations in SCCs, PKD1 is down-regulated in SCC, as a result of genetic and epigenetic alterations.[71] Detection of PKD1 gene mutations (and/or, Ras (exc. AK), TP53, EGFR, HERT), will be usefull, at least for early diagnosis of SCCs (SCCs do not express PKD1[70],

however it expresses higher level (copy number) of hTERT, EGFR and c-Myc. BCCs showed increased expression of PKD1 when compared with normal epidermis[70], therefore it could be usefull for early diagnostics of BCCs, normal human keratinocytes express PKD1 in very low levels, detectable only using Quantitative Real Time-PCR.[73,72] However there is no data for PKD1 expression in several precancerous lesions leading to BCCs[10,44], nor for activating mutations in *PRKD1* (PKD1 gene) in BCCs, nor for down-regulation (mutations) in aggressive types of BCCs.[11] PKD1 is down-regulate in SCC[71] and its overexpression promoted the growth of HNSCC tumor xenografts[71] from one site, and from another high *PRKD1* mRNA expression as a single marker (HR 2.00, 95% CI 1.28–3.14, Wald's $p = 0.002$) and positive lymph node status (HR 4.00, 95% CI 2.22–7.37, Wald's p 0.001) independently predicted for unfavorable diseasefree survival (DFS), clinicopathological factors required to accurately identify patients at high risk for recurrence in operable laryngeal cancer.[177] Second late increase in PKD1 expression is connected with high metastatic potential of laryngeal SCC[177] and melonama.[183] Activating mutations in *PRKD1* (PKD1 gene) was detected in 73% of Polymorphous low-grade adenocarcinoma (PLGA) - E710D amino acid substitution (p.Glu710Asp).[176,95,148]

Conclusions:

The development of biomarkers that can play a role in the earlier detection of tumor cells, offering prognostic information can be used as targeted therapies (Table 2). Proteins levels are subjects of complex regulation on different levels –DNA transcription, regulation of the levels and stability of mRNA, and regulation of the halflive of proteins (synthesis, degradation, activity/phosphorylation). Thus, identification of *mutations* as markers for early malignant transformation could be more appropriate not only for early diagnosis of cancer but could potentially influence treatment strategies in head and neck cancer (HNC), since mutations in EGFR and Ras genes are closely associated with resistance to cancer treatment.

PKD1 could be more appropriate target for treatment of PMDs, rather than treatment of cancer lesions. Inhibition of PKD1 activity in ICR mice, using Peracetylated (−)-epigallocatechin-3-gallate (AcEGCG), decreases expression of stem cells marker CD34, reversed the observed alterations, results of the two steps according (DMBA)-initiated and 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-promoted skin tumorigenesis protocol in ICR mice, keratinocytes proliferation and papillomas growth (possessing *Ras* mutations).[97] However, additional studies are necessary to prove or reject this hypothesis, since silencing of PKD1 in hTert (N-hTERT) keratinocytes (PMD cell line, possessing $p16^{INK4a}$ mutation) have decreased the markers of keratinocytes differentiation-K10 and Involucrin.[78] Contrary the knockout of PKD1 delays the progression of ADM (Acinar-to-ductal metaplasia) areas to PanINs (pancreatic intraepithelial neoplasia).[123,124] PKD1 in normal murine mammary gland (NMuMG) epithelial cells is constitutively-active in its basal state and prevents a transition to a mesenchymal phenotype (EMT) (through inhibition of Snail repressed E-cadherin expression).[141]

In normal human and mouse keratinocytes silencing of PKD1 promote keratinocyte differentiation, rather than EMT.[73,178,144,70] In this regard Snail is expressed in a transient manner, in hair placodal cells, during budding morphogenesis of the hair follicle, but is not detectable in the IFE (interfollicular epidermis).[179,180] Basal keratinocytes in the transgenic Snail epidermis display an elongated and spindle-like morphology, which implies an enhanced migratory capacity. Finding that transgenic epidermis exhibited lower levels of E-cadherin in regions expressing Snail, and increased expression of MMP-9, support the notion that Snail induces an EMT for tumor progression.[180] Snail transgenic mice develop spontaneous tumours: Craene *et al.* results indicate that enhanced Snail expression contributes to the stabilisation, expansion and survival of skin stem cells *in vivo* (CD34+), which can result in both skin tumour initiation and malignant progression for a variety of epithelial derived tumour types, such as basal cell carcinoma, squamous cell carcinoma and sebaceous gland carcinoma. Snail expression in a skin-specific p53-null background resulted in accelerated formation of spontaneous tumours and enhanced metastasis.[181] Additionally, Snail expression is induced in chronical UV irradiated skin, by ERK/p38/JNK-AP-1 dependenet pathway, but not TGF-β or IL-1β pathways. Elevated expression of Snail, but not Slug, in response to chronic UV irradiation in human skin may contribute to UV irradiation-induced skin tumor development.[182] If we concider that BCCs posses early increase PKD1 expression, inactivating Snail, the probable reason for developing of BCCs is mutations in 14-3-3σ, making Snail transcriptioanally active.[11,95,44]

Inhibition or silencing of PKD1 in cancer cells is closely connected with induction of EMT (Epithelial to Mesenchimal Transition) and more aggressive cancer phenotype.[122] In this regard reexpression of PKD1, using demethylating agents, was suggested as a treatment strategy in breast and prostate cancer.[29,69,122] In contrast of this scheme is a pancreatic cancer, which shows early high PKD1 expresion in response of mutant Kras, or after increased EGFR-mediated activation of endogenous wildtype Kras. As a result of such signaling, increased PKD1 expression and activity can be detected in regions of pancreatitis, ADM, PanIN1 and PanIN2 pre-neoplastic pancreatic lesions[103] and in pancreatic cancer.[100,126, 69] A recent *in vitro* and *in vivo* animal study involving the use of a new PKD1-specific, small-molecule inhibitor (CRT0066101) showed inhibition of pancreatic cancer growth *in vivo* and suggests the development of PKD1 inhibitors as a novel therapeutic target not only for the treatment of pancreatic cancer[126,69], but also for prevention of progression of precancerous lesions to tumors and even prevention of precancerous lesions[103,123,124] and blocking/prevention of severe pancreatitis in the early stage of the disease.[100] Zhang

et al. data consistently showed that either knockdown or overexpression of PKD1 did not significantly alter the proliferation of HNSCC cells *in vitro*. However, interestingly, induction of PKD1 *in vivo* by Dox (doxycycline) provided a slight growth advantage to the HNSCC tumor xenografts and resulted in a significant increase in final tumor weight in Dox-induced vs. the non-induced tumors. Overexpression of PKD1 promoted the growth of HNSCC tumor xenografts.[71] Thus, reexpression of PRKD1 is not also suitable treatment strategy in HNSCC, since overexpression of PKD1 promoted the growth of HNSCC tumor xenografts[71] from one site, and from another PKD1 down-regulation in HNSCC is not a consequence of promoter hypermethylation.[71]

Late high PKD1 expression was detected not only in laryngeal cancer[177], but in malignant metastatic melonamas, connected with cadherin switch - down-regulated E-cadherin and upregulated N-cadherin expression, increased expression of Cyclin D1. In melanoma cells that express high levels of E-cadherin but very low levels of N-cadherin, PKD1 expression is very faint, in contrary in melanoma cells, that express null or very low levels of E-cadherin but high levels of N-cadherin, PKD1 expression was strong with maximal expression in the most aggressive cell line. PKD1 expression significantly correlated with the mesenchymal features of the melanoma cell lines used in this study and was associated with E-cadherin negative/N-cadherin positive phenotype and high metastatic potential (anchorage-independent growth and migration). PKD1 can induce the activation of NFκB, a transcription factor that can directly bind to N-cadherin promoter and activate its expression. In fact, loss of E-cadherin induces NFκB activity and consequent N-cadherin expression in melanoma cells. Thus, regulation of E-cadherin expression by PKD1 could be enough to induce E- to N-cadherin switch, increase β-catenin nuclear staining and Cyclin D1 expression, promote tumor growth, motility and invasion through a process called epithelial-mesenchymal transition (EMT).[183] Protein kinase C inhibitor Gö6976 but not Gö6983 induces the reversion of E- to N-cadherin switch and metastatic phenotype in melanoma, identifiing the role of inhibited protein kinase D1 in the reversed process called mesenchymal-to-epithelial transition (MET).[183] Inhibiton or knocking-down PKD1 could be useful in metastatic cancers with late increased PKD1 expression/activity.[95]

One possible explanation of the discrepancies, connected with PKD1 function, could be the different mutational status in different cancer types[151] and differences in signal pathways in different cell types.

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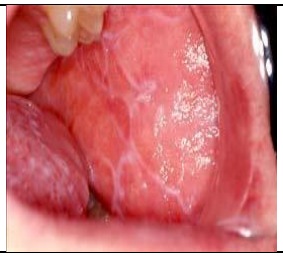
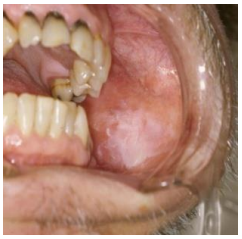
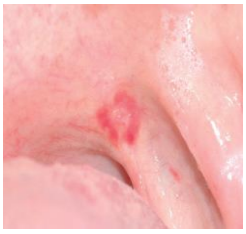
Table 1

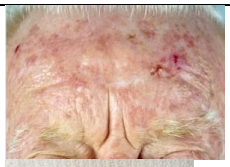
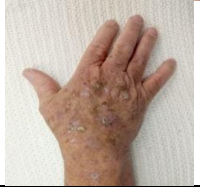
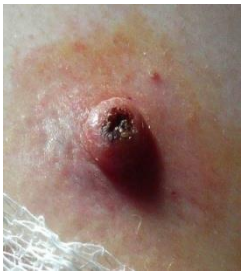
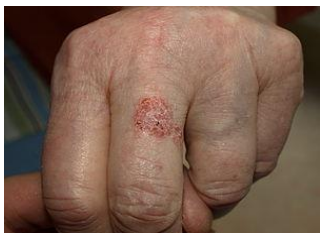
Oral (A) and skin (B) Premalignant Diseases (PMDs)
(images of Trtichoepithelioma and oral papilloma in webpathology atlas).

A: Oral Premalignant Diseases

Hyperplastic and dysplastic oral lesions	**Oral Lichen Planus**	**Oral Leukoplakia**	**Oral Erythroplakia**
			
	Wickham's Striae in the reticular form. https://www.aaom.com/oral-lichen-planus	Leukoplakia on the inside of the cheek. https://en.wikipedia.org/wiki/Leukoplakia	Erythroplakia on the soft palate in a 62-year-old male.[1]
The gold standard for the assessment of oral potentially malignant lesions is microscopic evaluation of haematoxylin and eosin stained sections for the presence of architectural and cytological changes, which are generally referred to as *epithelial dysplasia*. Some texts use the	Oral lichen planus (OLP) is an chronical inflammatory condition that affects mucous membranes inside the mouth. Oral lichen planus may appear as white, lacy patches; red, swollen tissues; or open sores. These lesions may cause burning, pain or other discomfort. Autoimmune disorder (https://www.mayoclinic.org/diseases-conditions/oral-lichen-planus/symptoms-causes/syc-20350869).	WHO "white plaques of questionable risk having excluded (other) known diseases or disorders that carry no risk for cancer". Leukoplakias are commonly homogeneous and most are benign (https://en.wikipedia.org/wiki/Leukoplakia).	erythroplakia is much less common than leukoplakia, erythroplakia carries a significantly higher risk of containing dysplasia or carcinoma in situ, and of eventually transforming into invasive squamous cell carcinoma. Carcinoma is found in almost 40% of erythroplakia, it is mostly found in elderly men around the ages of 65 - 74. It is commonly associated with smoking (https://en.wikipedia.org).[201]

terms squamous intraepithelial neoplasia (SIN) or squamous intraepithelial lesions. In the oral cavity, use of the SIL (squamous intraepithelial lesions) terminology of 'atypical hyperplasia' may lead to confusion because of the large number of common benign hyperplastic lesions, which may be encountered. In oral and maxillofacial pathology therefore, *oral epithelial dysplasia* is regarded as the standard terminology.[202]			

B: Skin Premalignant Diseases

Chronic wounds	**Actinic keratose** (**AK**)	**Keratoacanthomas (KA)**	**Bowen's disease**
	 		
	Actinic keratosis seen on the back of the hands https://en.wikipedia.org/wiki/Actinic_keratosis	1. Keratoacanthoma https://en.wikipedia.org/wiki/Keratoacanthoma	Bowen's disease as seen on a finger https://en.wikipedia.org/wiki/Bowen%27s_disease
Wound that does not heal in an orderly	**Actinic keratosis** (AK) usually presents as **multiple, erythematous**	**Keratoacanthoma** (**KA**) is a common low-grade (unlikely to metastasize or	Bowen disease was first described in the medical literature by a physician named

set of stages and in a predictable amount of time the way most wounds do; wounds that do not heal within three months are often considered chronic (https://en.wikipedia.org).	**or yellow brown, dry, scaly lesions** in the **sun-exposed areas** of the body. It is a lesion of the **middle-aged** and **elderly individuals** with a **male predominance**. It is especially more common in those with **fair complexions** who burn (rather than tan) upon sun exposure. The usual sites of involvement are **face, trunk**, and the **dorsal surfaces of the hands and forearms**. The lesions may coexist with lentigo maligna. The surrounding skin shows additional evidence of sun damage, including **atrophy, hypo- or hyperpigmentation**, and **telangiectasia**. AK is an indicator of **exposure to UV light** and predicts the **risk of developing squamous cell carcinoma** (webpathology atlas). Intraepidermal squamous cell carcinomas with potential progression to invasive squamous cell carcinoma[200] Hypertrophic, atrophic, bowenoid, acantholytic, and pigmented, based on histopathological examination[200]	invade) rapidly-growing skin tumour, with an annual incidence of approximately 150 per 100,000, occurring mostly on sun-exposed areas in fair-skinned patients aged 50 years and older. It is believed to originate from the hair follicle (pilosebaceous unit) and can resemble squamous cell carcinoma. It is dome-shaped, symmetrical, surrounded by a smooth wall of inflamed skin, and capped with keratin scales and debris. It grows rapidly, reaching a large size within days or weeks, pinkish red, dome-shaped papulonodules measuring 1–2 cm in diameter, and if untreated for months will almost always starve itself of nourishment, necrose (die), slough, and heal with scarring, spontaneously regressing within 6–8 months. Keratoacanthoma is commonly found on sun-exposed skin, often face, forearms and hands. It is rarely found at a mucocutaneous junction or on mucous membranes (https://en.wikipedia.org/wiki/Keratoacanthoma). Originally described as a benign tumor mimicking cutaneous squamous cell carcinoma (cSCC), KA is difficult to distinguish from cSCC during its growth, because both show rapid expansion, atypical keratinocyte morphology, and perineural invasion. The regressing phase of KA is histologically distinct.[195]	JT Bowen in 1912. Bowen disease is also known as squamous cell carcinoma *in situ*, and is generally considered an early, noninvasive form of intraepidermal squamous cell carcinoma. [200] (https://rarediseases.org/rare-diseases/bowen-disease/) Bowen's disease is the clinical term for a particular precancerous skin lesion. These lesions rarely cause patients any symptoms, but appear as well-defined scaly patches on sun-exposed skin, commonly in those over 60 years. They occur more in women and most frequently involve the lower legs of those affected in the UK. It is not known why, but the body sites most commonly affected vary across different countries. In general, people with Bowen's disease have an excellent prognosis because the disease is typically slow to develop and responds favourably to treatment. Lesions are usually slow-growing, and although they are not life-threatening, there is a small risk of progression to a skin cancer (estimated to be 3%) known as invasive squamous cell carcinoma. (https://www.cochrane.org/CD007281/SKIN_treatments-cutaneous-bowens-disease)

An early change in benign neoplastic transformation of keratinocytes is the inability to differentiate in response to Ca^{2+} or the PKC activator 12-O-tetradecanoylphorbol-13-acetate (TPA), which is consistent with altered PKCα function in these cells.[203,148] There is no data for early PKCα mutations (methylation) in PMDs.[42,148] There is data for the increased expression and activity of PKD1 only in mouse model of papillomas[97], among PMDs (downregulated in 87% of HNSCC[71]), psoriasis and BCC.[70,95] There are no studies of PKD2 (PKD3) expression of PMDs, although it is possible that PKD2 plays a predominant role in the growth, survival, and motility of HNSCC cells[71,95], nor in BCC.[95]

Table 2

Detected mutations in Premalignant Diseases (PMDs) and benign tumour ($p16^{INK4a}$, Ras, NF-κB, GLI, TP53, HPV DNA).

First mutation in:	Genes:	Premalignant Diseases (PMDs):	Other mutations:	Cancer:
$p16^{INK4a}$	*CDKN2A*	Classical PMDs		
		Oral leukoplakia lesions (hypermethylation in 44% , in 76% of oSCCs)[2] Xeroderma pigmentosum[33] Aberrant p16 expression (nuclear p16 overexpression despite increases in p16 gene promoter methylation) during inflammatory carcinogenesis caused by Candida albicans infection[193] higher p16 expression in papillomas[190,197] and Actinic keratosis[198,199,44] (lack – DD. HPV(-) SCC) Promoter hypermethylation of *CDKN2B* and *TIMP3* was most frequent, of *BRCA2*, *APC*, *CDKN2A* and *CDKN2B* was detected in 2 RRP laryngeal papilloma cases including recurrences, with subsequent progression to SCC. Of the 25 cases, 22 were positive for HPV-6, 2 for HPV-11 and 1 for HPV-16 and 33 in respiratory papillomatosis.[194] Loss of MMP-19 and p16 (negative in the invasive areas) from the epithelium could aid in making the differential diagnosis between well-differentiated SCCs and nonmalignant chronic leg wounds (lack of MMP-7, (MMP-12) MMP-13 in chronic wounds)[166]	*TP53* (79%) *HRAS* (35%) EGFRvIII (42%) *TERT* (50%) *NOTCH1* (14–15%) *PIK3CA* (3.9- 16.1%)[11]	SCC SCC
		Between well-differentiated SCC and keratoacanthoma (lack of MMP-8, presence of MMP-7 and -9 in their epithelial pushing border - SCC) [194]	*TP53* *NOTCH1-2 (22% of SCC)* *PIK3CA* *HRAS* *CDKN2A* *FBXW7*[195]	
Ras	*Ras*	Benign tumours		
		Actinic (solar) keratosis (16%) (*H-Ras, N-Ras*) [26,27,11], Papillomas[190,44] Keratoacanthoma (28.6%)[196]	*SFN* (14-3-3σ) *TP53* (79%) *CDKN2A (*58% in HPV(-)*)* EGFRvIII (42%) *TERT* (50%) *NOTCH1* (14–15%) *PIK3CA* (3.9- 16.1%)[11]	BCC SCC
NF-κB	*NFKB*	Psoriasis (*CARD14* mutation - epidermal regulator of NF-kB[86])		
GLI	*Gli*	Benign tumours		
		Trichoepithelioma (TEs) (*PTCH*[204], *CYLD*[205] mutation), Cylindromas, Trichoblastomas [191,44]	*SFN* (14-3-3σ) (68.3%) *Ras* (10-50%) *TP53 (*38-66%) *TERT* (56-78%)[11]	BCC
p53	*TP53*	Erythroplakia (46%), Oral lichen planus (33%),		SCC

		Leucoplakia (13.3%) Actinic (solar) keratosis (rare) [162,158,159,160]		
HPV DNA		Papillomas Keratoacanthoma (28.6%)*,[196]	$p16^{INK4a}$ positivity [90, 8,129,118 11]	SCC[127,116,92,8,128,11] BCC[10,11]

*RAS oncogene activation and HPV infection seem to represent two independent factors in the development of KA.[196]

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