BULGARIAN MEDICINE ISSN 1314-3387

Българска медицина

Съдържание

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Българска медицина се реферира в международните бази данни Index Copernicus International и EBSCO.

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Review

Протеин Киназа С профил в нормални човешки епидермални кератиноцити, премалигнени заболявания и рак на кожата и в Лицево-челюстната област. Част II: епидермални тумори

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Protein Kinase C profiles of normal human epidermal keratinocites, premalignant diseases and skin and head neck cancer. Part II: Epidermal tumours

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РЕЗЮМЕ:

Протеин Киназа С (PKC, ПКС) семейството от фосфолипид-зависими серин-треонин кинази участват в кератиноцитната пролиферация, диференциация и неоплазмената трансформация. Една от най-ранните промени в ПКС профилът в премалигнени лезии (в Ras трансформирани премалигнени клетъчни линии) е наблюдаваното дезактивиране на PKCδ чрез тирозиново фосфорилиране, свързано с редуциран диференциращ фенотип. Неспособността да се регулира PKCα в

ABSTRACT:

The protein kinase C (PKC) family of phospholipid-dependent serine-threonine kinases has been implicated in keratinocyte proliferation, differentiation and neoplastic transformation. One of the earliest alterations in PKC profile in premalignant lesions (in Ras transformed premalignant cell lines) is observed PKCδ deactivation by tyrosine phosphorylation, linked to a reduced differentiated phenotype. Failure to regulate PKCα in SCC4 may underlie at least part of the failure of calcium to promote differentiation in these cells[3,108].

SCC4 вероятно стои в основата, поне от части, на неспособността на калцият да промотира диференциация в тези клетки[3,108]. PKCε свръхекспресията в мишки, резултира в развитието на свободни от папиломи високо метастатични СЦКи[125]. Yadav V *et al.* съобщават, че загубата на PKC-δ в СЦКи е на транскрипционно ниво. Човешки СЦКи с ниски нива на PKC-δ притежават доказателства за активиране на Ras пътя[114]. В Сквамозни клетъчни карциноми, PKCη се детектира в кератотичните клетки около роговите перли, докато в Базално-клетъчните епителиоми (Базално клетъчните карциноми - БЦК) не се детектира[73]. Анализът на детектираният интензитет показва, че интезитетът на общата и активирана PKCζ се увеличава значително от нормални към малигнени тъкани. Допълнително, само проби от злокачествени тумори показват мембранна детекция на фосфорилирана PKCζ, предполагащо участието ѝ в рецепторното сигнализиране[75,105]. PKD1 е далн-регулирана в СЦКи, в резултат на генетични и епигенетични алтерации, но не хиперметиларана (ниски нива на хиперметилитане в PRKD1 генът)[47,7].

Детектираните мутации при Рак в Лицево-челюстната област - PKCα W58L, PKCδ P568A и PKCε R162H, и PKCγ D193N в меланоми са резюмирани от Antal CE *et al*. [127].

Ключови думи: Протеин Киназа С, ПКС, кератиноцити, възпалителни кожни заболявания, премалигнени заболявания, ПМЗя, папиломи, кожен рак, Рак в Лицево-челюстната област, РЛЧО, Спиноцелуларен карцином, СЦК, Базоцелуларен карцином, БЦК

PKCε overexpressing in mice results in development of papilloma-free highly-metastatic SCC[125]. Yadav V *et al.* have reported that PKC-δ is lost in human SCCs at the transcriptional level. Human SCCs with low PKC-δ had evidence of Ras pathway activation[114]. In squamous cell carcinoma, PKCη is stained in keratotic cells around horny pearls, whereas basal cell epithelioma (Basal Cell Carcinoma) is not stained[73]. Analysis of staining intensity reveals that the expression of both total and activated PKCζ increased significantly from normal to malignant tissue. In addition, only samples from malignant tumors showed membranous staining of phosphorylated PKCζ, implicating its involvement in receptor signaling[75,105]. PKD1 is down-regulated, as a result of combination of genetic and epigenetic alterations, but not hypermethylated (low level of DNA methylation on PRKD1 gene) in SCC[47,7].

Detected mutations in PKCα W58L, PKCδ P568A and PKCε R162H in Head and Neck Cancer and PKCγ D193N in melanoma were reported from Antal CE *et al.* [127].

Keywords: Protein Kinase C, PKC, keratinocytes, inflammatory skin diseases, premalignant diseases, PMDs, papilloma, skin cancer, Head and Neck cancer, HNC, Spinocellular carcinoma, SCC, Basocellular carcinoma, BCC

Introduction:

The protein kinase C (PKC) family of phospholipid-dependent serine-threonine kinases has been implicated in keratinocyte proliferation, differentiation and neoplastic transformation. In order to be determined if Ca^{2+}-mediated keratinocyte differentiation is associated with changes in PKC isozyme gene expression, RNA was isolated from primary mouse keratinocytes grown in medium with 0.05, 0.12, or 1.4 mM Ca^{2+}. Based on northern blot analysis, primary keratinocytes expressed mRNA encoding PKC-α, -δ, -ε, -ζ, and -η, but not PKC-β or -γ. Relatively little change was detected in the level of these transcripts in cells induced to differentiate by exposure to elevated extracellular Ca^{2+}[1,2]. In the normal keratinocytes, the levels of PKCα, PKCδ,

PKCη, and PKCζ increased over the first one to two weeks in a calcium-and time-dependent manner. PKCε decreased in a time-and calcium-dependent fashion over the three-week period[3]. Interestingly, the PKC-ζ transcripts detected in RNA isolated from keratinocytes were approximately 200 nucleotides longer than those from mouse brain, suggesting the existence of an alternative form of this isozyme[4]. PKD1 is hardly detectable in basal skin keratinocytes, but we have proved its expression and functionin cultured normal human keratinocytes, which is similar to the function of the kinase in mouse keratinocytes - proproliferative[5,6,7,8], as PKD3. PKD1 and PKD3 are not expressed in upper epidermal layers, in contrast to PKD2 – Figure 1 part I[6].

Human Epidermal growth factor Receptor (HER) family

EGFR (ERBB1; HER1) is the prototype of a family of four tyrosine kinase receptors, which also includes ERBB2 (neu; HER2), ERBB3 (HER3), and ERBB4 (HER4). EGFR, ErbB2, and ErbB3 are expressed in normal human epidermis (ERBB4 (HER4) is not expressed). Ligand activation of EGFR directly stimulates keratinocyte proliferation, whereas ErbB2 has no known ligand, and ligand activation of ErbB3 is not mitogenic for human keratinocytes. The EGFR pathway is the major mitogenic pathway for them [9].

Aside from the EGF, six other EGFR ligands have been described. These include transforming growth factor-α (TGF-α), amphiregulin (AREG), epiregulin (EREG), betacellulin (BTC), heparin-binding EGF-like growth factor (HB-EGF), and epigen (EPI). EGF, TGF-α, and amphiregulin are specific ligands only for the EGFR. TGF-α is composed of 50 amino acids, and displays 35–40% homology with EGF. BTC, HB-EGF, and EREG show dual specificity for both EGFR and ERBB4. Notably, the neuregulins (NRGs) can bind both ERBB3 and ERBB4, or only ERBB4, depending on the subclass[10].

Ligand binding to EGFR (ErbB1, HER1) induces the formation of receptor homo- (EGFR/EGFR) and heterodimers with other ErbB (HER – Human Epidermal growth factor Receptor) family members (EGFR/ErbB2, EGFR/ErbB3, and EGFR/ErbB4), and the activation of the intrinsic kinase domain, resulting in phosphorylation of specific tyrosine residues within the cytoplasmic tail. Phosphorylated tyrosine residues act as binding sites for a variety of intracellular signal inducers, and stimulate multiple pathways of signal transduction including the RAS/RAF/MEK/ERK, the PLC-γ/PKC, the PI3K/AKT, JAK-STAT, and NF-kB cascades. Thus, the EGF receptor/ligand system comprises expression of EGFR-ligand precursors, their processing by metalloproteases, ligand binding to EGFR, and subsequent activation of EGFR signaling[11,10].

All four members of the ErbB family of protein kinases possess a similar protein kinase domain. ErbB1/2/4 possess protein kinaseactivity while ErbB3 is catalytically impaired. The catalytically important residues within the kinase domain of ErbB1/2/4 are conserved and these three family members closely resemble one another in terms of overall protein kinase structure[12].

As a consequence of an active dimer conformation, the intrinsic kinase domain is activated, resulting in autotransphosphorylation of tyrosine residues in the cytoplasmic tail of the receptor. The process is ATP dependent. The phosphorylated receptor tyrosine residues then serve as docking sites for substrate and adaptor proteins via Src homology 2 (SH2) and phosphotyrosine binding (PTB) domains of signalling proteins, and link the receptor to downstream signal transduction pathways. The cellular responses are due to the pattern of phosphorylation because the specificity of the binding proteins is dependent on amino acids surrounding the tyrosine phosphorylation site. Thus, individual HERs couple to distinct subsets of signalling proteins and the signalling outcome and kinetics depend considerably on receptor dimer composition[13].

ErbB3 is kinase impaired so that induced homodimer formation would fail to stimulate protein kinase activity and downstream signaling. Since the kinase activity of HER3 is impaired, heterodimerization and transphosphorylation by other HER members are required for cell signalling from this receptor[13]. However, kinase-impaired ErbB3 pos-

sesses 1/1000th of the autophosphorylation activity of ErbB1 and the possibility exists that the ErbB3 homodimer is functional[12]. The ErbB3 receptor plays a major role in promoting cell survival. The regulatory subunit (p85) of PI3K binds potentially to a half dozen phosphotyrosines in ErbB3 that lead to the activation of PI3K activity[16].

ERBB4 (HER4) is not expressed in the epidermis[9].

The four ErbB family members are able to form 28 homo- and hetero-dimers. With the 11 growth factors in the EGF-like family and 28 possible dimers, there are 614 possible combinations of receptors-ligands interactions. Including both full-length isoforms of ErbB2 and both isoforms of ErbB3 increases the number of possible com-binations even further. Not all ligands are expressed near cells that possess the ErbB family of receptors so that the number of potential combinations in a given cell is reduced, but still appreciable[12].

In the human epidermis, each ligand of HER1 may have its own effect on development and homeostasis of cutaneous tissues. Although all different ligands activate the same receptor, the regulation of its activation is not identical. Firstly, each ligand seems to activate its own set of HER1 tyrosine phosphorylation. Secondly, since the sorting of ligand-receptor complexes in early endosomes depends on potential ligand-receptor dissociation at acidic pH, some ligands allow the dissociated EGF receptor to recycle, while on the other hand, other ligands do not dissociate, leading both the ligand and the EGF receptor to degradation [18].

HER2 expression is up-regulated in human epidermis, with keratinocyte differentiation and this correlates with the relocalization of the protein into the plasma membrane, especially in keratinocytes of the granular layer. *In vitro,* Poumay Y *and* Mitev V have demonstrated expression of HER2 in cultures of human epidermal keratinocytes and found that the HER2 expression is similarly up-regulated when cultured cells differentiate. Nevertheless, HER2 has been shown to partially induce a malignant phenotype in papilloma-derived keratinocytes, but the effect produced through HER2 was reported as less potent than the induction obtained with the *ras* oncogene[18].

PMDs (premalignant diseases)

An early change in benign neoplastic transformation of keratinocytes is the inability to differentiate in response to Ca2+ or the PKC activator 12-O-tetradecanoylphorbol-13-acetate, which is consistent with altered PKC function in these cells. The PKC isozyme mRNA profile was examined in two benign neoplastic keratinocyte cell lines, 308 and SP-1, which contain an activating mutation of the c-Ha-ras gene (alteration characterisk also for papilloma[23]). Like normal keratinocytes, 308 and SP-1 cells expressed mRNA encoding PKC-α, -δ, -ε, -ζ, and -η. However, the abundance of PKC-ζ transcripts in both cell lines was reduced by 74-89% when compared with normal keratinocytes at similar Ca^{2+} levels. The PKC-ζ transcripts detected in RNA isolated from keratinocytes were approximately 200 nucleotides longer than those from mouse brain, suggesting the existence of an alternative form of this isozyme. In addition, SP-1 but not 308 cells exhibited a sevenfold increase in PKC-η mRNA when cultured in medium with 1.4 mM Ca^{2+}. To address whether these changes were related to the presence of an activated ras gene, RNA was isolated from primary keratinocytes transduced to a benign neoplastic phenotype with the v-Ha-ras oncogene. As with normal, 308 and SP-1 cells, v-Ha-ras transformed keratinocytes expressed mRNA encoding PKC-α, -δ, -ε, -ζ and -η. The level of PKC-ζ transcripts was similar in normal and v-Ha-ras keratinocytes, indicating that reduction of this mRNA in both 308 and SP-1 cells was not a direct result of ras activation. As in SP-1 cells, PKC-η in v-Ha-ras keratinocytes was responsive to extracellular Ca^{2+}, with a four-fold increase in transcript abundance in 0.12 mM Ca^{2+} medium relative to 0.05 mM Ca2+ medium[4]. There is no data for early PKCα mutations (methylation) in PMDs, this is the only Ca^{2+} dependent (binding) isoform in keratinocytes. The alterations in PKC-δ levels are not described, although Calcium also does not regulate PKCα (calcium sentsitive) or δ levels or cause a marked redistribution to membranes[24].

Repeated exposures to UVB of human keratinocytes lacking functional p16INK4a and able to differentiate induce an alternative state of differentiation rather than stress-induced premature senescence. A 2D-DIGE proteomic profiling of this alternative state of differentiation was performed at various times after the exposures to UVB. Sixty-nine differentially abundant protein species were identified by mass spectrometry, many of which are involved in keratinocyte differentiation and survival. Among these protein species was TRIpartite Motif Protein 29 (TRIM29). Increased abundance of TRIM29 following UVB exposures was validated by Western blot using specific antibody and was also further analysed by immunochemistry and by RT-PCR. TRIM29 was found very abundant in keratinocytes and reconstructed epidermis. Knocking down the expression of TRIM29 by short-hairpin RNA interference decreased the viability of keratinocytes after UVB exposure. The abundance of involucrin mRNA, a marker of late differentiation, increased concomitantly. In TRIM29-knocked down reconstructed epidermis, the presence of picnotic cells revealed cell injury. Increased abundance of TRIM29 was also observed upon exposure to DNA damaging agents and PKC activation. The UVB-induced increase of TRIM29 abundance was dependent on a PKC signaling pathway, likely PKCδ. These findings suggest that TRIM29 allows keratinocytes to enter a protective alternative differentiation process rather than die massively after stress[32].

Papilloma

Direct support for a role of EGFR (ERBB1, HER1) 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[33,34]. ERBB2 (HER2) is predominantly expressed in the differentiating epidermal cell layers, in the epithelial cells of the sebaceous glands, and in the outer root sheath of hair follicles. ERBB2 was shown to be activated in EGF-treated epidermal keratinocytes, after treatment of the epidermis with the phorbol ester and tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA), and in the skin of transgenic mice expressing TGF-α in the epidermis, raising the hypothesis that activation of this receptor plays an important role in tumor promotion[35].

Previous studies using keratinocytes from epidermal growth factor receptor (EGFR) - deficient mice revealed that the EGFR is not required for papilloma formation initiated by a mutant rasHa gene, although the tumors that develop are very small. The current study used a combination of bromodeoxyuridine pulsechase, PCNA (proliferating cell nuclear antigen) distribution, and differentiation marker analysis to reveal the following: (a) the EGFR was required to maintain the proliferative population in the basal cell compartment of papillomas; (b) in the absence of EGFR, cycling tumor cells migrated into the suprabasal compartment and initiated the differentiation program prematurely; and (c) these changes were associated with cell cycle arrest. Further analysis of v-rasHa-transformed EGFR-deficient keratinocytes in vitro indicated that such cells migrated more on and attached less to extracellular matrix components. Together, these studies reveal that an essential function for the EGFR pathway in squamous tumors is to maintain a proliferative pool of basal cells and prevent premature terminal differentiation[36].

To study the role of ERBB2 in epidermal homeostasis and skin carcinogenesis, diverse transgenic mouse lines were generated. Constitutive expression of the activated form of ERBB2 (neu*) in epidermal basal cells under the control of the keratin 5 (K5) or K14 promoter resulted in a dramatic phenotype characterized by epithelial hyperplasia, which was particularly severe in the hair follicles. Early mortality because of the severity of the phenotype precluded further analysis and forced the development of additional lines. Doxycycline-inducible, conditional expression of activated ERBB2 in adult animals resulted in hyperplasia of the epidermis and hair follicles; prenatal expression caused perinatal death. More informative results were obtained by the overexpression of wild-type ERBB2 under the control of the K5 promoter. These animals show a milder skin phenotype (nevertheless

including alopecia, follicular and interfollicular epidermal hyperplasia, and enlarged sebaceous glands) and have a longer life span. Analysis of proliferation and differentiation markers indicated an increase in epidermal proliferation and a delay in differentiation. Importantly, spontaneous papillomas, which sometimes converted to squamous cell carcinomas, appeared in homozygous animals as early as 6 weeks of age. K5-ERBB2 transgenic mice were also more sensitive to TPA treatment and to two-stage carcinogenesis. The results of this study indicate that ERBB2 overexpression provides both an initiating and promoting stimulus and demonstrates an important role for this receptor in tumorigenesis of the skin[35,34].

The two-stage carcinogenesis protocol (Topical application of TPA (A) or DMBA (B), according to (DMBA)-initiated and 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-promoted skin tumorigenesis in mice) causes an oncogenic mutation in the H-Rras gene and appeared to be ideal for studing physiological responces to alterations in gene expression against oncogenic Ras signaling in living animals[38], leading to the development of papillomas and consequently SCC. Papilloma formation is mostly connected with increased c-Myc expression, which downregulates $p21^{Cip1}$[39,23]. Sustained activation of c-Myc is sufficient to induce papillomatosis together with angiogenesis-changes that resemble hyperplastic Actinic Keratosis (possessing also Ras mutations (11%)[40,25], a commonly observed human precancerous epithelial lesion. All these premalignant changes spontaneously regress upon deactivation of c-MycER[41]. c-Myc-deficient epidermis is resistant to Ras mediated DMBA/TPA induced tumorigenesis (DMBA-initiated and TPA-promoted skin tumorigenesis in mice). This is mechanistically linked to $p21^{Cip1}$, which is induced in tumors by the activated Ras–ERK (Extracellular signal Regulated Kinase1/2) pathway, but repressed by c-Myc. Acute elimination of c-Myc in established tumors leads to the up-regulation of $p21^{Cip1}$, and epidermis lacking both $p21^{Cip1}$ and c-Myc reacquires normal sensitivity to DMBA/TPA-induced tumorigenesis. This identifies c-Myc-mediated repression of $p21^{Cip1}$ as a key step for Ras-driven epidermal tumorigenesis[39]. Increased c-Myc expression in papillomas is also connected with observed polyploidity (endoreplication – DNA replication in the absence of complete mitosis, connected with G2/M cell arrest) in this lesions, trying to limit division of the cells creating multinucleated cells[31].

According Rashel M *et al.* the hyperplastic and inflammatory responses to topical phorbol ester were significantly suppressed in Protein Kinase D1 (PKD1)-deficient mice suggesting involvement of PKD1 in inflammation and tumor promotion. Consistently, when subjected to two-stage chemical skin carcinogenesis protocol, PKD1-deficient mice were resistant to papilloma formation when compared to control littermates[48]. If PKD1-deficient mice were resistant to papilloma formation when compared to control littermates[48], does it mean that PKD1 influences c-Myc or p21Cip1 expression or both? Our unpublished results have showed that PKD1 do not influences expression and/or phosphorylation (Ser62/Thr58) of c-Myc in normal human keratinocytes[44], but the Chiou *et al.* results have showed increased expression of both as a result of increased PKD1 expression in the two steps cancerogenic protocol[44]. Increased PKD1 expression „downregulates" E-cadherin/β-catenin (stabilization of β-catenin and binding to E-cadherin on the cell membrane, as aresult of T120 phosphorylation of β-catenin,[49] downregulation of nuclear β-catenin) and ERK1/2 signal pathways (PKD1 can phosphorylate RIN1(RAS and RAB Interacting), a protein that associates with Ras and 14-3-3 proteins (also a binding partner for PKD1). Through phosphorylation of RIN1, the association with 14-3-3 could become more intense; therefore, abrogating its ability of blocking Ras/Raf-1 interaction, Ras can dissociate and is free to be activated, such that it can stimulate the Ras/Raf/MEK/ERK/RSK pathway[7]), but upregulate nuclear factor κ-B (NF-κB) and phosphatidylinositol 3-kinase (PI3K), which induce c-Myc. Which is the molecular event which detertmines decreased papilloma formation in PKD1 knockdown mice? According Chiou Y *et al.* in 7,12-dimethylbenz[a]-anthracene (DMBA)/12-*O*-tetra-

decanoylphorbol-13-acetate (TPA)-mediated tumors the levels of both c-Myc and p21 (increased p53) increases (increased protein levels of PKD1, decreased ERK1/2, but increased NF-κB (in papillomas)), and these effects are inhibited by peracetylated (−)-epigallocatechin-3-gallate (AcEGCG)[44].

The skin phenotype of PKCε overexpressing mice is characterized by epidermal hyperproliferation and skin ulceration, beginning at four months of age. However, when tumor formation was induced using a two-stage skin carcinogenesis protocol, papilloma formation was lowered, while progression to SCCs was increased. The papilloma-independent carcinomas (SCC) which develop in PKCε transgenic mice arise from the hair follicle and have increased metastatic potential[56]. Further, the skin of PKCε overexpressing mice was sensitized to UV radiation induced SCC formation[57]. PKCε overexpression, which sensitizes skin to UVR-induced carcinogenesis, suppresses UVR-induced sunburn (apoptotic) cell formation, and enhanced both UV-induced hyperplasia and levels of specific cytokines (tumor necrosis factor α (TNFα), granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), and Interleukin six (IL-6)), implying inhibition of apoptosis and promotion of preneoplastic cell survival. Additionally, PKCε may impart sensitivity to UVR carcinogenesis via its association with Stat3, a transcriptional factor that is constitutively activated in both mouse and human SCC[56]. UVR-induced sunburn cell formation is mediated by Fas/Fas-L and TNFα NFR1 extrinsic apoptotic pathways. The death adaptor protein termed Fas-associated death domain (FADD) is a common adaptor protein for both of these apoptotic pathways. PKCε inhibits UVR-induced expression of FADD leading to the inhibition of both apoptotic pathways. It appears that PKCε sensitizes skin to the development of SCC by UVR by transducting signals, which inhibit apoptosis on one hand, and enhances proliferation of preneoplastic cells on the other hand[58].

The major stem cell population of hair follicle includes interfollicular label retaining cells (LRCs), double positive HSCs (CD34+/α6-integrin+), Mts24+ cells, Blimp1, Nestin, Lgr5+, and Lgr6+ cells. However, the bulge region of hair follicle is considered as the major niche for keratinocyte stem cells. Particularly, the CD34+/α6-integrin+ cells are slow cycling and colocalize with LRCs (label retaining cells- potential initiated cells, that could retain carcinogen-DNA adducts) and confined to bulge region of hair follicles. In terms of their colony forming ability (clonogenicity), CD34+ cells make larger colonies compared to CD34− cells[56].

PKCδ overexpressing mice are extremely resistant to chemically induced tumorigenesis in skin, despite enhanced induction of the proliferation marker ornithine decarboxylase[72], supporting the role of PKCδ in cancer suppression. Thus, the incidence of benign papillomas is reduced and progression towards malignancy slowed down dramatically, though this may refer merely to chemically and not UV radiation-induced carcinogenesis. On the contrary, in mice depleted of PKCδ, apoptosis was suppressed which may enhance tumorigenesis. In fact, this confirmed the anti-promoting function of PKCδ shown in a cell model previously. Finally, the role of PKCδ in establishing immune tolerance, which was demonsrated in transgenic mice, may imply that this isoform could be critical for cell-mediated immunity including tumor cell surveillance[57].

PKC-ζ is not activated nor depleted by TPA (DAG analogue). The level of PKC-ζ transcripts was similar in normal and v-Ha-ras keratinocytes, indicating that reduction of this mRNA in both 308 and SP-1 cells was not a direct result of ras activation[4,57]. PKC-ζ transcripts in benign neoplastic keratinocyte cell lines, 308 and SP-1, was reduced by 74-89% when compared with normal keratinocytes, releasing constraints on Akt/PKB activity, proceeding during skin tumor promotion and progression[4,57].

PKCζ is associated with reduced expression of cyclin D1, sequestration of Akt/PKB, and impaired PI3Kinase signaling[57]. PKCζ can activate the MAPK pathway and nuclear factor NF-κB (IL-1, IL-6, IL-8, and ICAM-1 and VCAM) in result of TNF-α stimulation (activation of innate and acquired immunity in psoriasis)[75]. PKCζ is also crucial for macrophage activation and expression of adhesion molecule ICAM-1,

and metalloproteinase-9 (MMP-9)[75], through ERK1/2 Snail pathway, but not p38[8,42,32,60]. MMP-9 was detected in the epithelium in both acute and chronic wounds (chronic leg ulcers)[76] and found in papillomas (HPV+),[77,78] TE (no data), Actinic keratosis (AK), Keratoacanthomas (KA)[78]. MMP-9 expression has been reported recently in dyskeratotic foci of Bowens' disease and in infiltrative edges of microinvasive carcinomas[79], SCC[79,76], and BCC[79,80]. In addition, MMP-9 was found to be elevated in the Oral Lichen Planus (OLP) inflammatory infiltrating cells[81] in tissue, serum and saliva samples of oral PMDs than in healty controls (distinguish oral leucoplakia and Oral SCC from healthy control)[82], in oral Leucoplakia and Erythroplakia[78]. Salivary MMP-9 could be a useful, non-invasive adjunct technique in the diagnosis, treatment, and follow-up of oral OPMD (leucoplakia, erythroplakia and oral submucous fibrosis (OSMF)) and OSCC[83]. We could not succeed in finding data for MMP expression in oral erythroplakia, with exception for MMP-9[83,78].

Akgül *et al.* have previously shown that HPV8 transgenic mice spontaneously develop papillomatous skin tumors. Histology revealed epidermal hyperplasia, acanthosis and hypergranulosis and in some cases squamous cell carcinomas (SCC). Zymographic and immunoblot analysis of normal skin extracts identified increased amounts of matrix metalloproteinase (MMP)-9, MMP-13 and MT1-MMP in HPV8-positive mice compared with HPV8-negative animals. In situ gelatin zymography of tumor specimens displayed a strong proteolytic activity in papillomas, and SCC putatively attributed to the increased amounts of activated MMP-9 found in tissue extracts. In addition, immunoblot analysis revealed increased amounts of active MMP-13 and MT1-MMP in tumor extracts as compared with control extracts. Immunohistochemical stainings of SCC specimens depicted MMP-13 to be specifically expressed in stromal fibroblasts neighboring the tumor islands, whereas MT1-MMP was detected both in tumor cells and in stromal cells. Taken together, these results implicate a role for MMPs in the development of HPV8-induced cutaneous tumors[77]. Additionally, Van Doorslaer and Burk showed that oncogenic types papilloma virus (HPV) specifically activate the hTERT promoter, while non-oncogenic types do not[88].

Normal epithelium shows increased nuclear p27 expression, which is closely related to differentiation of superficial mature cells. In study of Queiroz *et al.*, 81.25% of the control group cases had p27 expression. In their study, papillomas showed diffuse expression for p27 protein in most cases. This indicates that squamous papilloma is similar to ordinary oral mucosa regarding this immunomarker. By contrast, authors observed lower p27 expression (fewer than 50% of the cells) in 97.06% of the oral SCC. There was immunoexpression of p27 in more than 50% of the cells and 2.94% of the cases only. Reduction of p27 expression, due to the loss of cell cycle regulation, has been shown in the early invasion phase in oral SCC, showing its important role in abnormal proliferation in which its reduction may relate to the ability for tissue invasion by neoplastic cells. Poorly differentiated SCC did not show p27 expression, probably due to the lack of mature cells in that kind of lesion. In moderately and well-differentiated carcinomas, however, there was considerable expression of that protein, related to the growing presence of mature cells. Moreover, p27 expression can be related to a better prognosis in patients with SCC. The authors results are in fully in line with these findings[89,31]. In another study p27kip1 was positive in 23.4%, 26.2%, 25.9% and 4.5% of specimens in the normal skin, AK (Actinic Keratosis), BD (Bowens' Disease) and SCC groups, respectively[90].

ΔNp63α, a proto-oncogene, is up-regulated in non-melanoma skin cancers and directly regulates the expression of both Vitamin D receptor (VDR) and phosphatase and tensin homologue deleted on chromosome ten (PTEN). Since ΔNp63α has been shown to inhibit cell invasion via regulation of VDR, Hill NT *et al.* wanted to determine whether dietary Vitamin D3 protected against UVB induced tumor formation in SKH-1 mice, a model for squamous cell carcinoma development. The authors examined whether there was a correlation between dietary Vitamin D3 and ΔNp63α, VDR or PTEN expression in vivo in

SKH-1 mice chronically exposed to UVB radiation and fed chow containing increasing concentrations of dietary Vitamin D3. Although they observed differential effects of the Vitamin D3 diet on ΔNp63α and VDR expression in chronically irradiated normal mouse skin as well as UVB induced tumors, Vitamin D3 had little effect on PTEN expression in vivo. While low-grade papillomas in mice exposed to UV and fed normal chow displayed increased levels of ΔNp63α, expression of both ΔNp63α and VDR was reduced in invasive tumors(?). Interestingly, in mice fed high Vitamin D3 chow, elevated levels of ΔNp63α were observed in both local and invasive tumors but not in normal skin suggesting that oral supplementation with Vitamin D3 may increase the proliferative potential of skin tumors by increasing ΔNp63α levels[96]. ΔNp63α is overexpressed in up to 80% of primary Head and Neck Cancers (HNC), p63 in 87 – 100% in SCCIS and 100% in BCC[97]. Citro S et al. clearly show an increase of both ΔNp63α mRNA and protein levels in HPV-positive compared to HPV-negative cell lines, which is dependent on the presence of the HPV E6 and E7 oncoproteins[98]. Upon the differentiation of normal keratinocytes, p63 levels rapidly decreased while higher levels were retained in HPV-positive cells. Mighty KK and Laimins LA studies indicate that reducing p63 levels in differentiated HPV-positive cells resulted in the loss of viral genome amplification and late gene expression. p63 regulates the expression of cell cycle regulators, and they determined that cyclin A, cyclin B1, cdk1, and cdc25c were reduced in p63-deficient, HPV-positive keratinocytes, which suggests a possible mechanism of action. In addition, activation of the DNA repair pathway is necessary for genome amplification, and the expression of two members, BRCA2 and RAD51, was altered in the absence of p63 in HPV-positive cells[99].

PITX1 and SOX2 bind to an even more distal site in murine cells and their inhibition reduced ΔNp63 levels. PITX1 and SOX2 were replaced by KLF4 during differentiation, correlating with p63 loss[100].

Spino-Cellular Carcinoma (SCC)

Although the reported percentage of patients with overexpression of EGFR varies, recent data shows that from 80% to 100% of patients with premalignant or malignant oral lesions have high EGFR expression[101]. EGFR itself is overexpressed, amplified, or constitutively activated by ligand interaction or mutation. EGFR gene is often amplified (30% of OSCCs[93] and/or with activating mutations in cancer cells[102]. Amplification of the EGFR is particularly common in human squamous cell carcinomas. Constitutively active EGFR mutants can transform cultured cells. Conversely, dominant-negative constructs for EGFR can reverse the transformed phenotype *in vitro.* Transgenic targeting of transforming growth factor-α to the mammary gland, skin, and liver enhances tumor formation. In these models, there is a strong correlation between EGFR and EGFR ligand-induced hyperproliferation and tumorigenesis[36]. Human and mouse squamous cell carcinomas of the skin overexpress EGFR ligands[36]. Previously it was detected that expression of total and nuclear EGFR was higher in p16-negative tumors compared to p16-positive tumors[103,31, 78-Figure 1].

There is limited data available about PKC expression in SCCHN, and the results are conflicting. Cohen E *et al.* study would suggest that the novel isoforms (δ, ε, and θ) are highly expressed in SCCHN (SCC Head and Neck) cell lines. Furthermore, they reported a successive increase in total and phosphorylated PKCζ expression in normal, dysplastic, and malignant SCCHN tissue. To determine the relative importance of PKC isoforms as mediators of head and neck tumor growth, the authors analyzed their expression in four radioresistant, EGFR-overexpressing SCCHN cell lines (SQ20B, SCC61, SCC25, and JSQ3) by immunoblotting with anti-PKC antibodies. Similar to NHEK cells, the PKC isoforms α, δ, ε, θ and ζ, are expressed at varying levels, whereas isoforms β1, β2, γ, and η do not seem to be expressed in these cell lines. In addition, isoforms α, ε, and ζ are phosphorylated at activation sites to some degree in these lines. It seems that PKCζ is constitutively phosphorylated at Thr410 (in activating loop) in the head and neck cell lines. However, the authors

detected expression of α, δ, ε, θ, and ζ PKC isoforms, as well as phosphorylation of most of these isoforms (α, δ, θ, and ζ) was observed. NHEK cells expressed the phosphorylated forms of PKCθ and PKCδ, but not PKCε. These results reveal that most of the expressed PKC isoforms, with the exception of PKCε, are phosphorylated and potentially activated in growing NHEK (Normal Human Epidermal Keratinocytes) cells. The authors do not detected expression of PKCη, detected by other authors[105]. Additionally, in a human squamous cell carcinoma line, elevations in extracellular Ca2+ rapidly increase PKC in the membrane fraction[106], which is a classical indication of its activation[107], although uncapability of Ca^{2+} to stimulate differentiation in SCC[3,108] (see PKC mutations), was also reported.

Calcium induces both involucrin and transglutaminase-K in normal keratinocytes (NHK), but not in squamous carcinoma cell lines (SCC). The protein kinase C (PKC) agonist phorbol myristoyl acetate potentiates and the PKC antagonist Ro31-8220 blocks the ability of calcium to stimulate the involucrin promoter in normal human keratinocytes, but not in SCC4. Yang LC *et al.* thus examined the ability of calcium to regulate the levels of five PKC isozymes in NHK and two SCC. All five isozymes (-α,-ε, -δ,- η, -ζ) showed little change during culture in SCC4 at any calcium concentration. Calcium and time of culture had partial effects on SCC12B2, a carcinoma that shows partial differentiation characteristics. The authors concluded that although a number of PKC isozymes are regulated during calcium-induced differentiation, PKCα plays a necessary role in mediating calcium-induced differentiation. Failure to regulate PKCα in SCC4 may underlie at least part of the failure of calcium to promote differentiation in these cells[3,108]. Protein kinase C delta (PKC-δ) protein levels are frequently low in chemically and UV-induced mouse skin tumors as well as in human cutaneous squamous cell carcinomas (SCCs). Furthermore, overexpression of PKC-δ in human SCC lines and mouse epidermis is sufficient to induce apoptosis and suppress tumorigenicity, making *PKC*-δ a potential tumor suppressor gene for SCCs. Yadav V *et al.* have reported that PKC-δ is lost in human SCCs at the transcriptional level. The authors used laser capture microdissection to isolate cells from three normal human epidermis and 14 human SCCs with low PKC-δ protein. Analysis by quantitative reverse transcription-PCR revealed that PKC-δ RNA was reduced an average of 90% in the SCCs tested, consistent with PKC-δ down-regulation at the protein level. Analysis of DNA from nine of the same tumors revealed that PKC-δ gene was deleted in only one tumor. In addition, Ras-transformed human keratinocytes, which have selective down-regulation of PKC-δ at both protein and mRNA levels, had significantly repressed human *PKC*-δ promoter activity. Together, these results indicate that PKC-δ gene expression is suppressed in human SCCs, probably via transcription repression. The authors results have implications for the development of topical therapeutic strategies to trigger the re-expression of pro-apoptotic PKC-δ to induce apoptosis in SCCs[113,114].

PKC-δ played as a protective role in SCC partly by down-regulating p63, leading to the suppression of SCC cell proliferation, attenuation of the activity and expression of CSCs (cancer stem cells) in SCC cells[115,22].

In squamous cell carcinoma, PKCη is stained in keratotic cells around horny pearls, whereas basal cell epithelioma (Basal Cell Carcinoma) is not stained. No expression of PKCη is detected in mesenchymal cells at the mRNA or protein level[73].

The skin phenotype of PKCε overexpressing mice is characterized by epidermal hyperproliferation and skin ulceration, beginning at four months of age. However, when tumor formation was induced using a two-stage skin carcinogenesis protocol, papilloma formation was lowered, while progression to SCCs was increased. Further, the skin of PKCε overexpressing mice was sensitized to UV radiation induced SCC formation[57,58].

To determine the in vivo functional specificity of PKC-ε in mouse skin carcinogenesis, Wheeler DL *et al.* generated PKC-ε transgenic mouse (FVB/N) lines 224 and 215 that overexpress - 8- and 18-fold, respectively, PKC-ε protein over endogenous levels in basal epidermal cells. Wheeler DL *et al.* reported that

FVB/N transgenic mouse lines that overexpress PKC-ε protein not only in basal epidermal cells but also in cells of the hair follicle developed papilloma-independent squamous cell carcinoma (SCC) elicited by 7,12-dimethylbenz(a)anthracene initiation and 12-O-tetradecanoylphorbol-13-acetatepromotion or by repeated ultraviolet radiation exposures. The susceptibility to the development of SCC was proportional to the level of expression of the PKC-ε transgene. The authors reported that PKC-ε FVB/N transgenic mice (line 215) that overexpress in epidermis 18-fold PKC-ε protein in more than their wild-type littermates spontaneously develop a myeloproliferative- like disease (MPD) in 100% of PKC-ε transgenic mice[120].

Consistent with authors previous report, by 6 months of age, PKC-ε transgenic mice exhibited extreme hyperkeratosis, alopecia, suppurative dermatitis, and development of scales most remarkable over the tail base, ears, face, and the dorsal skin. Transgenic mice (overexpressing PKCε protein) revealed that PKCε was not expressed in the tumor itself; however, the uninvolved tissue surrounding the SCC exhibited intense PKCε expression. Also, human SCC, similarly to mouse SCC, do not expressed PKCε in the tumour, while surrounding uninvolved epidermis showed high PKCε expression. PKCε expression could be used as a marker for increased susceptablility for metastazis[61,62,63]. SCC developed in PKCε transgenic mice is metastatic and originates from the hair follicle[56]. Thus, the interersting question is why UVB exposure do not induce BCC development in UVR-induced carcinogenesis? – because mice do not develop BCC. At 6 months of age the PKC-ε transgenic mice showed marked hepatosplenomegaly. Both the liver and spleen weights varied from two to six times larger from their control wild-type littermates at 6 months of age[63].

Benign neoplastic keratinocytes express significantly reduced levels of PKCζ transcripts in comparison to normal keratinocytes, though no correlation was found between expression levels and ras activation in the tumorigenic cell lines. Nevertheless, down-regulation of PKCζ may contribute to skin tumorigenesis by releasing constraints on Akt/PKB activity, proceeding during skin tumor promotion and progression. Analysis of staining intensity reveals that the expression of both total and activated PKCζ increased significantly from normal to malignant tissue. In addition, only samples from malignant tumors showed membranous staining of phosphorylated PKCζ, implicating its involvement in receptor signaling. Inhibition of PKCζ, but not other PKC isoforms, blocks EGF-stimulated MAPK (ERK) activation, DNA synthesis and proliferation in normal keratinocytes, and the majority of head and neck cell lines[75,105].

Protein kinase C (PKC) ζ has been implicated as a mediator of epidermal growth factor (EGF) receptor (EGFR) signaling in certain cell types. Because EGFR is ubiquitously expressed in squamous cell carcinoma of the head and neck (SCCHN) and plays a key role in tumor progression, the authors determined whether PKCζ is required for tumor cell proliferation and viability. Examination of total and phosphorylated PKCζ expression in normal oral mucosa, dysplasia, and carcinoma as well as SCCHN tumor cell lines revealed a significant increase in activated PKCζ expression from normal to malignant tissue. PKCζ activity is required for EGF-induced extracellular signalregulated kinase (ERK) activation in both normal human adult epidermal keratinocytes and five of seven SCCHN cell lines. SCCHN cells express constitutively activated EGFR family receptors, and inhibition of either EGFR or mitogen-activated protein kinase (MAPK) activity suppressed DNA synthesis. Consistent with this observation, inhibition of PKCζ using either kinase-dead PKCζ mutant or peptide inhibitor suppressed autocrine and EGF-induced DNA synthesis. Finally, PKCζ inhibition enhanced the effects of both MAPK/ERK kinase (U0126) and broad spectrum PKC inhibitor (chelerythrine chloride) and decreased cell proliferation in SCCHN cell lines. The results indicate that (a) PKCζ is associated with SCCHN progression, (b) PKCζ mediates EGF-stimulated MAPK activation in keratinocytes and SCCHN cell lines, (c) PKCζ mediates EGFR and MAPK-dependent proliferation in SCCHN cell lines; and (d) PKCζ inhibitors function additively with other inhibitors that target similar or complementary signaling pathways.

In particular, inhibition of PKCζ in SCCHN cells potentiates the action of MEK and PKC inhibitors. Because U0126 blocks MEK activation of MAPK, whereas PKCζ inhibits Raf kinase activation (2), the two inhibitors act at different steps along the pathway but seem to be additive[105].

Baso-Cellular Carcinoma (BCC)

Mukhopadhyay and colleagues generated a mouse strain, which expresses physiological levels of an activated KRas ($KRas^{G12D}$) allele along the midline epidermis and hair follicles (Msx2-cre; $Kras^{G12D}$). The single $KRas^{G12D}$ allele induces proliferation in the basal keratinocytes, sebaceous gland and ORS, manifested in redundant skin folds, progressive hair loss, and spontaneous papillomas arising mainly on non-hair-bearing areas. While Kras activation has a marked effect on the hair follicles, changes in downstream RAS/MAPK effectors were minor and only transcriptional changes, but no effects on cell signaling were observed. Surprisingly, even these small changes led to reduced levels of Sonic hedgehog (Shh), which controls hair follicle morphogenesis in embryonic skin and telogen-anagen transition in postnatal skin. In line with this, Shh blockade results in the arrest of hair follicle morphogenesis or deregulates telogen–anagen transition. Thus, the reported phenotypes of Ras gain-of-function mutations might partially result from decreased Hedgehog pathway activity[40].

In mammalian 293T cells (human kidney embryonic), coexpression of constitutively active PKCα reduced the activity of Gli1 in a dose-dependent manner, whereas constitutively active PKCδ increased the activity of Gli1, although this required higher expression levels. Regulation of mutant Gli1 protein localized exclusively to the nucleus was similar to that of the wild-type protein, indicating that nuclear-cytoplasmic shuttling is not a determinant of Gli1 control by either PKC isoform. Furthermore, PKC regulation of Gli1 did not involve activation of mitogen-activated protein kinase signaling. Finally, Neill GW et al. show that exogenous Gli1 does not alter the expression of PKCα in human primary keratinocytes, suggesting that loss of this isoform in BCC is not via Hedgehog signaling. As BCCs have been proposed to originate from the ORS (outer root sheath), loss of PKCα expression may be relevant to tumor formation; this may, in part, be because of the predicted increase in Gli1 transcriptional activity[122].

PKCη is not detected in basal cell epithelioma (Basal Cell Carcinoma)[73], we could not find data concerning PKCε and PKCζ expression in BCC. If we concider that there are data for PKCι expression, not expressed in normal keratinocytes, probably PKCζ is not expresed of in this cancer, as its telomeric position could lead to loss of gene[123] as a result of aneuploidity observed in BCC *(Table 2).*

PKCι has previously been demonstrated in epithelial cells to interact with the polarity protein Par6 leading to normal mitotic spindle alignment, normal cell polarity and Rac1 activation to support transformed growth. In rhabdomyosarcoma primary cells, ATM inhibits PKCι–Par6 binding and Rac1 activation leading to an increase in cells in G2 phase of the cell cycle. Aside from its interaction with polarity proteins, a further role for PKCι as an oncogenic kinase via cell intrinsic Hedgehog (HH) signalling has emerged. This additional role was identified from a proteomic screen of basal cell carcinoma (BCC) cells where PKCι was revealed as an interacting partner to missing-in-metastasis (MIM), a scaffold protein necessary for Hedgehog signalling. PKCι was found to directly bind to and phosphorylate GLI1, the major transcription factor of the HH pathway, at residues within its zinc finger DNA binding region. This trigger transcription of GLI1 dependent genes, among which is Snail[126], one the six (Snail (Snai1), Slug (Snai2), Twist, ZEB1/ZEB2 and E47[22]) transcriptional factors activating EMTransition (characterized with expression of mesenchymal cell markers – low E-Cadherin and high Vimentin, Fibronectin[31], neurothrophinreceptor B (TrkB)/BNDF (tyrosine kinase receptor, acting through AKT - invasion and migration of HNSCC) – increased Twist and N-Cadherin, CD44v6[130]). When these residues were mutated to non-phosphorylatable alanines (S243A and T304A) there was decreased DNA binding detected by chromatin immunoprecipitation (ChIP) and thus PKCι would appear to phosp-

horylate GLI1 to potentiate transcription of HH signalling components. As PKCι is itself a GLI1 target gene, this sets up a positive feedback system that can amplify HH signalling independent of upstream inputs (Smoothened (SMO) and Suppressor of Fused (SuFu) mutations, activating[22,25] and inactivating (missense)[133], respectively) and lead to tumour growth. Of course this is a BCC model, but HH signalling may be activated by a number of genomic aberrations in rhabdomyosarcomas. In fact both BCCs and rhabdomyosarcoma are found in transgenic mice with germline mutations of the tumour suppressor gene Patched1 (PTCH) which leads to the activation of HH signalling. In humans, these mutations of PTCH, a gene located on chromosome 9q22.3, causes Gorlin Syndrome, a condition that in addition to BCC and RMS predisposes to ovarian fibromas, meningiomas and medulloblastoma[134]. Additionaly, it is thought that Snail is not only implicated in the early onset of the tumours but also contributes to invasiveness, as has been observed in a subset of human BCCs. In all panel of basal cell carcinoma (BCC) samples examined, 16.6% of them were Snail-positive. However, Snail expression was evident only at invasive fronts, where cells seem to undergo EMT[135]. The main function of Snail is inhibition of E-cadherin expression, β-catenin transcriptional activation and EMT induction[130,31]. The rest functions are summarized in Table 4[22].

Although BCC are with basal origin, EGFR is expressed at a significantly higher level in SCC than in BCC[140]. EGFR activity maintains proliferation levels of the basal layer and its inhibition causes differentiation of the keratinocytes and induction of suprabasal markers K1 and K10[40]. By immunohistochemical analysis of a panel of 20 human BCC using a clinically approved diagnostic anti-EGFR antibody, Schnidar H et al. can show that all BCC tested expressed EGFR. Five BCCs showed EGFR expression at levels slightly lower than normal skin, eight tumors gave signals comparable with normal skin, and seven tumors showed higher EGFR levels than normal skin. This was partially confirmed by qPCR analysis of EGFR mRNA expression in BCC samples compared with normal human keratinocyts, or N/TERT-1 keratinocytes. Similarly, Ptch _/_ (ASZ001) and Ptch _/_; p53_/_(BSZ2, CSZ1) mouse BCC cell lines express elevated levels of EGFR mRNA when compared with Ptch+/+ keratinocytes. In agreement with the mRNA expression data, all BCC cell lines express significant levels of total and activated tyrosine – p-EGFR[141]. Additionally, ERK1/2 is one of the major kinases lying downstream of EGFR and transdusing EGFR signal into the cells. Only 5/12 (48%) BCC examined stained positive for pERK more than in normal skin, in comparision with 100% positivity of cSCC (N=11)[142]. Schnidar H *et al.* have previously shown that activated ERK is not highly expressed throughout the entire tumor mass of BCC, although it can be readily detected in small subregions of the tumors, such as the peripheral palisading cells with high proliferative activity, as well as in infiltrating cells. The authors speculate that the synergistic interaction of EGFR/MEK/ERK and GLI act in distinct subpopulations of BCC cells is associated with tumor growth and a more aggressive phenotype[143].

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 and erly feature of BCC, increased also in TE[144,145], and is linked to the induction of epidermal SC markers in immortalized N/Tert-1 keratinocytes. GLI1 overexpression 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. Finally, ERK activity was predominantly negative in 13/14 BCCs (superficial/nodular), indicating that GLI1 does not routinely co-operate with ERK to induce the formation of this common skin tumour[53]. On the other hand, a negative effect of GLI1 on EGFR and ERK activation can be observed in keratinocytes when cultured under conditions that allow induction of epidermal stem cell markers by GLI1, further underlining the context-dependent regulation of GLI protein activity[141]. As determined by

Snail messenger RNA and E-cadherin protein expression this is not associated with epithelial–mesenchymal transition (EMT), and GLI1 actually represses induction of the EMT marker vimentin in EGF-stimulated cells. GLI1 opposes EGFR signalling to maintain the epithelial phenotype[53]. However this is not the exact model for studing BCCs, since N/Tert-1 keratinocytes possess mutation in p16INK4A, which is not typical for BCCs[146,147,31].

Analysis of BCC (basal cell carcinoma) lesions of Ristich et al. showed increased expression of PKD1 (PKCμ) when compared with normal epidermis, but not in SCC lesions (squamous cell carcinoma). For BCCs samples in which comparison was possible, the tumours exhibited elevated PKD immunoreactivity relative to the basal layer of the normal epidermis. This result suggests that BCCs possess greater amounts of PKD than normal basal keratinocytes. 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[150]. Answer of this question will be received when we study PKD1 expression in precancer (pre-BCC) lesions like AK and TE. Thus, another curently adequate question, received its answer, lack of PKD1 expression in SCCs despite increase expression of EGFR (increased NF-κB and c-Myc[7], PKD1 inducers), is a consequense of PKD1 gene downregulation, as a result of combination of genetic and epigenetic alterations, connected with the progression of precancer to cancer[44,150,47,31,7]. 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[47].

Surprisingly, BCC do not express the callassical and novel PKC isoforms – α, δ, η, ε (and ζ), which could activate PKCμ/PKD1, upregulated in BCC. Thus, the only PKC mediators of EGFR signaling in BCC are PKD members, if PKD2 and PKD3 are expressed in BCC – there is no data (see[7]). IL-1- and oncostatin M (OSM)-induced phosphorylation of protein kinase D (PKD) in human chondrocytes is strongly associated with signalling via the atypical PKCι isoform. Consequently, *PRKD3* silencing significantly reduced the expression of MMP1/13, as well as reduce serine phosphorylation STAT3 and all three MAPK groups. Reduction AP-1 genes *FOS* and *JUN,* critical for the induction of many MMPs including *MMP1/13. ATF3* (AP-1 factor activating transcription factor 3) was also reduced concomitant with the observed reduction in *MMP13* expression[101].

Major oncogenic drivers of lung adenocarcinoma (LADC) include activating mutations in epidermal growth factor receptor *(EGFR),* chromosomal translocations that generate oncogenic *EML4-ALK* fusions, and activating mutations in *KRAS.* PKCι is overexpressed in *KRAS* LADC and PKCι expression predicts poor outcome. Genetic silencing of *PRKCI* inhibits transformed growth and invasion of *KRAS* LADC cells *in vitro,* and tumor development *in vivo.* Furthermore, lung-specific genetic disruption of *Prkci* in the *LSL-Kras^{G12D}* mouse LADC model blocks tumor initiation by inhibiting clonal expansion of putative lung cancer stem cells. A synthetic lethality screen identified a small molecule oncrasin that selectively inhibits oncogenic *KRAS* LADC in a PKCι-dependent manner. These studies establish *PRKCI* as a critical oncogenic effector of *KRAS* in LADC. Functional characterization of LADC TICs revealed a requirement for NOTCH[170].

Tumor necrosis factor α (TNF-α), doxorubicin and other genotoxic chemotherapeutic agents also activate PKD1, but the role of PKC in these signaling pathways is unknown[172] (see- PKD1 activation). Whereas a PKD mutant with Ser744 and Ser748 altered to alanine was resistant to activation in response to cell stimulation, mutation of both sites to glutamic acid residues (to mimic phosphorylation) generated a constitutively active PKD[171,7].

Src family kinases (SFKs) play an important role in cancer proliferation, survival, motility, invasiveness, metastasis, and angiogenesis. Among the SFKs, c-Src and c-Yes are particularly over-expressed or hyper-activated in many human epithelial cancers. Western blotting and immunohistochemical staining sho-

Table 1: Role of different PKC isotypes in Head and Neck Cancer (explanations in the text; transcriptional regulation of PKCs in: [22-Table 5] effects on focal adhesion (FA) and WNT/β-catenin in: [22]).

PKC isoforms		BCCs and SCCs	References
PKC α	Ras-transformed	*v-ras* oncogene is attributed to increased activity of PKCα and activation of AP-1 transcription factors (DAG). Ras gain-of-function mutations might partially result from decreased Hedgehog pathway activity.	Cataisson C *et al.*, 2003[24] Doma E *et al.*, 2013[40]
	SCC	Failure to regulate PKCα in SCC4 may underlie at least part of the failure of calcium to promote differentiation in these cells. PKCα knockout mice - impairment of epidermal hyperplasia in response to tumor promoter application, but an increased sensitivity to tumor formation, probably because of the induced inflammation.	Denning MF, 2004[72] Yang LC *et al.*, 2003[3] Hara H *et al.*, 2005[151] Bollag W, 2009[152] Cataisson C *et al.*, 2003[24]
	BSS	PKCα is absent in basal cell carcinomas. It reduces the activity of the Gli1 transcription factor in the Sonic hedgehog signaling pathway (activated in BCCs).	Neill GW *et al.*, 2003[122]
PKC δ	Ras-transformed	Following *ras* transformation and in response to various growth factors such as TGFα and EGF - PKCδ is deactivated by tyrosine phosphorylation (inactivated), linked to a reduced differentiated phenotype. *ras* transformation – PKCδ is down-regulated (at both protein and mRNA levels) or inactivated. In TPA stimulated murine or UV irradiated transformed human keratinocytes, induction of PKCδ tyrosine phosphorylation has been also correlated with increased activity. Apparently, PKCδ can be diferentially phosphorylated at several distinct tyrosine residues having opposing effects on its activity and the physiological outcome.	Denning MF, 2004[72] Breitkreutz D *et al.*, 2007[57] Yadav V *et al.*, 2010[113] D'Costa AM *et al.*, 2006[114] Pestana A *et al.*,2017 [28]
	SCC	PKCδ transgenic mice are resistant to chemical carcinogenesis despite enhanced induction of the proliferation marker ornithine decarboxylase - a role in tumor suppression. PKC-δ played a protective role in SCC partly by down-regulating p63 (decreasing CSCs in SCC) and induces apoptosis. Human SCCs with low PKC-δ had evidence of Ras pathway activation. Lost in SCCs, in ras-transformed keratinocytes via a TGFα/EGFR autocrine loop.	Denning MF, 2004[72] Zhang D *et al.*, 2017[115] Teicher B, 2006[153] D'Costa AM *et al.*, 2006[114] Yadav V *et al.*, 2010[113]
	BCC	Not expressed in BCCs. Constitutively active PKCδ increased the activity of Gli1.	Neill GW *et al.*, 2003[122]
PKC η	Ras-transformed	As in SP-1 cells, PKC-η in v-Ha-ras keratinocytes was responsive to extracellular Ca^{2+}, with a four-fold increase in transcript abundance in 0.12 mM Ca^{2+} medium relative to 0.05 mM Ca^{2+} medium (see below).	Dlugosz AA *et al.*, 1992[4]
	PMDs	SP-1 but not 308 cells (benign neoplastic keratinocyte cell lines) exhibited a sevenfold increase in PKC-η mRNA when cultured in medium with 1.4 mM Ca^{2+}, without change in expression in NHK.	Dlugosz AA *et al.*, 1992[4]
	SCC	Loss of PKC η increases tumor incidence in the DMBA/TPA chemical carcinogenesis protocol (much higher sensitivity to carcinogenesis), indicating PKC η also has a role in tumor suppression. PKC-η gene expression is suppressed in human SCCs (reduced an average of 90%), probably via transcription	Denning MF, 2004[72] Breitkreutz D *et al.*, 2007[57] Kashiwagi M *et al.*, 2002[73]

		repression. Stained in keratotic cells around horny pearls.	Yadav V *et al.*, 2010[70]
	BCC	Not detected in basal cell epithelioma (Basal Cell Carcinoma).	Kashiwagi M *et al.*, 2002[73]
PKC ε	Ras-transformed	In ras transformed keratinocytes, PKCε transcription remains unchanged.	Breitkreutz D *et al*, 2007[57]
	PMDs	DMBA and that initiated keratinocytes harboring **Ha-ras mutations** require Stat3 for proliferation and clonal expansion during tumor promotion with TPA – stimulation of c-Myc.	Macias E *et al.*, 2013[71]
	SCC	Over-expression promotes the formation of highly metastatic squamous cell carcinomas. PKCε associate and phosphorylate Stat3 on Ser 727. PKCε depletion prevented Stat3Ser727 phosphorylation, Stat3 DNA binding, and transcriptional activity, constitutively activated in mouse and human SCC, connected with invasion PKCε overexpressing mice was sensitized to UV radiation induced SCC formation - the papilloma-independent carcinomas (SCC) arising from the hair follicle and having increased metastatic potential. Development of SCC seems to be associated with PKCε-mediated induction of TNFα cytokine. and developing of a myeloproliferative-like disease (MPD) in 100% of PKC-ε transgenic mice. Human SCC, similarly to mouse SCC, do not expressed PKCε in the tumour, while surrounding uninvolved epidermis showed high PKCε expression. Could be used as a marker for increased susceptablility for metastazis and /or targeted in SCC.	Denning MF, 2004[72] Breitkreutz D *et al.*, 2007[57] Teicher B, 2006[153] Singh A *et al.*, 2013[56] Wheeler DL *et al.*, 2005[63] Aziz MH *et al.*, 2007[61] Aziz MH *et al.*, 2010[62] [63,59,56,116,117,118,119]
	BCC	There is no data. *Prkce* (PKCε gene) is induced by GLI; STAT3 is expresses in BCC, but in order to be activated STAT has to be phosphirylated on Tyr^{705} by JAK (IL-6) or SRC and Ser^{727} by PKCs (PKCε[52]), necessary for maximal Stat3 transcriptional activity[71,61,62]. Phosphorylation of STAT3 at Tyr705 regulates MMP-9 production in epithelial ovarian cancer.	- Louro ID *et al.*, 2002[126] Singh A *et al.*,2013[56] Aziz MH *et al.*, 2007[61], 2010[62] Jia ZH *et al.*, 2017[154]
PKC ζ	Ras-transformed	The level of PKC-ζ transcripts was similar in normal and v-Ha-ras keratinocytes, indicating that reduction of this mRNA in both 308 and SP-1 cells was not a direct result of ras activation (see below).	Dlugosz AA *et al.*, 1992[4] Breitkreutz D *et al.*, 2007[57]
	PMDs	Increased significantly in psoriasis. PKC-ζ transcripts in benign neoplastic keratinocyte cell lines, 308 and SP-1, was reduced by 74-89% when compared with normal keratinocytes. Benign neoplastic lesions - reduced levels of PKCζ; releasing constraints on Akt/PKB activity, proceeding during skin tumor promotion and progression.	Zhao Y *et al.*, 2008[75] Dlugosz AA *et al.*, 1992[4] Breitkreutz D *et al.*, 2007[57]
	SCC	Increased in squamous cell carcinoma and downregulation of PKCζ could inhibit cancer cell DNA synthesis and proliferation trough downregulation of EGFR-ERK1/2 activity, similarly to their inhibitors. PKCζ is also crucial for macrophage activation and expression of adhesion molecule ICAM-1, and metalloprotease-9 (MMP-9).	Cohen EE *et al.*, 2006[105] Zhao Y *et al.*, 2008[75] Ivanova PV *and* Maneva AI, 2019[7]
	BCC	There is no data. If we concider that there are data for PKCι expression, not expressed in normal keratinocytes, probably lack of expression (telomeric possition).	

PKCι		Not expressed in normal human keratinocytes; PMDs and SCC. Src can activate PKCλ/ι.	Denning MF, 2004[72] Mackay HJ, Twelves CJ, 2007[155]
	BCC	Interacting partner to missing-in-metastasis (MIM); a scaffold protein necessary for Hedgehog signalling; PKCι was found to directly bind to, phosphorylate and activates GLI1, the major transcription factor of the HH pathway (expressed in BCC), induceced by positive feedback mechanism PKCι and Snail expression. Targeted kinase in the treatment of BCC.	Martin-Liberal J *et al.*, 2014[134] Atwood CX *et al.*, 2013[138]
PKCμ/ PKD1	Ras-transformed	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, Snail-dependent induction). PKD1 blocks EMT (Snail – Ser11) and cell migration (MMPs; SSH1L (Slingshot 1L - inactivation) and PAK4 (p21-activated kinase 4 - activation), with a net effect of inhibiting cofilin; filopodia formation and length through phosphorylation of VASP (Enabled/Vasodilator-stimulated phosphoprotein); focal adhesion dynamics by targeting PIP5K1γ (phosphatidylinositol-4-phosphate 5-kinase type-1γ Ser^{441}) and VASP and β-catenin[157], inhibition of MTA1 (metastasis-associated protein 1) via polyubiquitin-dependent proteosomal degradation in prostate cancer [158]).	Chiou YS *et al.*, 2013[44] Durand N *et al.*, 2016[156] Ivanova P, Maneva A, 2018 [31,7] Storz P, 2018[157] Ganju A *et al.*, 2016[158]
	PMDs	Upon wounding however, PKD1-deficient mice exhibited delayed wound re-epithelialization correlated with a reduced proliferation and migration of keratinocytes at the wound edge. 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. 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 (increased p-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 and increased levels of p53, p21 and c-Myc). 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	Rashel M *et al.*, 2014[48] Chiou YS *et al.*, 2013[44]

		growth factor). When subjected to two-stage chemical skin carcinogenesis protocol, PKD1-deficient mice were resistant to papilloma formation when compared to control littermates Increased expression in psoriasis; In hTert keratinocytes, also called N/Tert-1 or N-hTERT keratinocytes, near 9 fold increase of PKD1mRNA, increase also of PKD1 protein. We expected increased expression in other PMDs, connected with activation of inflammatory processes.	Rashel M *et al.*, 2014[48] Ristich V *et al.*, 2006[150] Ivanova P *et al.*, 2008, *Arch Derm Res*[5] Ivanova P *et al.*, 2007, *CRABS*[26,27] Ivanova PV *and* Maneva AI, 2018[31] Ivanova PV *and* Maneva EESJ, 2020, subm[7]
	SCC	Down-regulated in in 87% in HNSCC, as compared to the normal and adjacent normal tissues, Low levels of DNA methylation (combination of genetic and epigenetic alterations), contrary to prostate, breast gastric, and colon cancers. 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. Increased PKD1 expression after Doxycyclin treatment, not influencing proliferation and motility of HNSCC. Dox-treated PKD1-c1 (positive PKD1 clones) mice also showed elevated p-EKR1/2 and reduced IκBα, indicative of the activation of the MEK/ERK1/2 and the NF-κB signaling pathways. In contrast, the PI3K/Akt signaling pathway was not affected since p-Akt level was not altered. Accordingly, IHC staining showed increased cell proliferation (Ki67) in tumor explants of the Dox-treated PKD1-c1 group as compared with the controls Thus, overexpression of PKD1 promoted the growth of HNSCC tumor xenografts. PKD1 may be a potential target for microenvironment-directed tumor biotherapy. Chen J *et al* found that hypoxia not only induced the expression of HIF-1α, but also induced the expression and activation of PKD1 in the same SCC25 cells. Knock-down of PKD1 decreased the growth, as well as the expression of HIF-1α, glucose uptake, lactate production and glycolytic enzyme (GLUT-1 and LDHA) expression, as well as reduced the phosphorylation of p38 MAPK, while the percentage of apoptotic SCC25 cells was increased. They found that PKD1 is associated with the activation of p38 MAPK signaling and the activation of p38 MAPK signaling is necessary for HIF-1α accumulation and nuclear translocation. PKD1 is a potential therapeutic target for oral squamous carcinoma, knock-down of which inhibit the growth and promote the apoptosis of SCC-25 cells via downregulating Bcl-2 expression and downregulation of the expression of P-gp (unspecific multidrug resistance). High *PRKD1* mRNA expression as a single marker and positive lymph node status, independently predicted for unfavorable diseasefree survival (DFS), clinicopathological factors required to accurately identify patients at high risk for recurrence in operable laryngeal cancer.	Ristich V *et al.*, 2006[150] LaValle CR *et al.*, 2010[159] Zhang L *et al.*, 2018[47] Zhang L *et al.*, 2018[47] Chen J *et al.*, 2018[160] Wang JN *et al.*, 2019[161] Fountzilas E *et al.*, 2013[162]
	BCC	Increased expression in human BCCs PKD1 phosphorylates Snail on Ser11, binding 14-3-3σ, and translocates form nucleous to cytosol, becoming	Ristich V *et al.*, 2006[150] Ivanova P *and* Maneva A[15]

		transcriptioanally inactive – inhibit EMT. Early 14-3-3σ mutations (hypermethylations), leading to increased nuclear Snail. Nonphosphorylated Snail is a more effective repressor for E-cadherin expression and inducer for EMT[163].	Ivanova P *and* Maneva A[31] Du C *et al.*, 2010[163] Ivanova P *et al.*, 2021[22]
	Other HNC	73% of PLGAs (Polymorphous low-grade adenocarcinoma) posses specific hotspot mutation, resulting in an E710D amino acid substitution (p.Glu710Asp), resulting in increased kinase activity (activating mutation), but expression of the mutant protein reduced cellular migration (reduces invasion). Consistent with these findings, the presence of the *PRKD1* hotspot mutation was significantly associated with metastasis-free survival.	Weinreb I *et al.*, 2014[164]
		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.	Fountzilas E *et al.*, 2013[165]
		In melanoma cells that express high levels of E-cadherin but very low levels of N-cadherin, PKD1 expression is very faint. In metastatic melanoma cells increased PKD1 expression significantly correlated with the mesenchymal features of the melanoma cell lines used, associated with E-cadherin negative/N-cadherin positive phenotype (cadherin switch) and high metastatic potential (anchorage-independent growth and migration).	Merzoug-Larabi M *et al.*, 2017[166]
PKD2	Ras-transformed	There is no data. PKD2 and PKD3 tend to drive EMT and cell migration.	- Durand N *et al.*, 2016[156]
	SCC	PKD2 mRNA was upregulated in seven out of ten tumors *vs.* normal in patient-paired HNSCC tissue specimens. It is possible that PKD2 plays a predominant role in the growth, survival, and motility of HNSCC cells, and these functions have compensated the loss of PKD1 in tumors.	Zhang L *et al.*, 2018[47]
		PKD2 and PKD3 promoted the activity of uPA and MMP-9 in prostate cancer (MMP-7 and MMP-9 in pancreatic cancer).	Zou Z *et al.*, 2012[167]
	BCC	There is no data.	-
PKD3	Ras-transformed	There is no data. PKD2 and PKD3 tend to drive EMT and cell migration.	- Durand N *et al.*, 2016[156]
	SCC	PKD3 was minimally expressed in the control and in almost all HNSCC cell lines examined (increased PKD2 or PKD3 expression in tumor vs. normal tissue).	Zhang L *et al.*, 2018[47]
		PKD2 and PKD3 promoted the activity of uPA and MMP-9 in prostate cancer (MMP-7 and MMP-9 in pancreatic cancer).	Zou Z *et al.*, 2012[167]
	BCC	There is no data.	-

Table 2: Expression of different PKC isotypes in inflammation, PMDs, papillomas, Bowens' Diseases (BD), Actinic Keratosis (AK), Trichoepithelioma (TE-we could not find data), and skin Head and Neck Cancer – SCC and BCC.

PKC isoforms	-α	-δ	-η	-ε	-ζ	-ι	-μ (PKD1)
inflammation	+ act.	/	/	/	+	-	+ (??)[7]
PMD (*ras* transformed)	/	-	/↓-↑ $[Ca^{2+}]_o$	/	↓	-	↑ (psoriasis)
papillomas	/	↓ (↑/-)	/(↓)	/(↑)	/↓	-	↑
BD (SCC *in situ*)	?	- (loss)[77]	?	?	?	?	+ (mRNA ↑+[163])
SCC	+ (mut. in C1) failure of Ca^{2+} to promote differentiation	↓ /- (ras transformation)	-/+	+ (-t/+str) ↓ p-ε	+ ↑	-	- (+) (mRNA ↑↑+[168])
AK	?	+	?	?	?	?	+ (mRNA ↑+[168])
BCC	-	-	-	? (Jansen AP *et al.*, 2001 [125], [65,169])	-	+ (↑↑)	+ (↑↑)

/ without alterations in expression; ↓ decreased expression; ↑ increased expression; + expressed; - absent (loss); ? There is no data

wed that c-Src was expressed in all malignant skin tumors, but not in normal skin, while c-Yes was expressed in malignant melanoma (MM) and squamous cell carcinoma (SCC), but not in basal cell carcinoma (BCC) and normal skin[46]. Arun et al. have detected increased c-Abl, SRC – induced PKD1 activity in mouse keratinocytes after UVB exposure, the main pathogenetic cause for the development of BCC[43,8,177,31,25]. Additianally, Montagner A *et al.* unveil a cascade of events involving peroxisome proliferator-activated receptor (PPAR) β/δ and the oncogene Src, which promotes the development of ultraviolet (UV)-induced skin cancer in mice. UV-induced PPARβ/δ activity, which directly stimulated Src expression, increased Src kinase activity and enhanced the EGFR/Erk1/2 signalling pathway, resulting in increased epithelial-to-mesenchymal transition (EMT) marker expression. Consistent with these observations, PPARβ/δ-null mice developed fewer and smaller skin tumours, and a PPARβ/δ antagonist prevented UV-dependent Src stimulation. Furthermore, the expression of PPARβ/δ positively correlated with the expression of SRC and EMT markers in human skin squamous cell carcinoma (SCC), and critically, linear models applied to several human epithelial cancers revealed an interaction between PPARβ/δ and SRC and TGFβ1 transcriptional levels. Taken together, these observations motivate the future evaluation of PPARβ/δ modulators to attenuate the development of several epithelial cancers[178].

Finally, several other signaling pathways are presumably involved in BCC tumorigenesis. Mutations of the tumor suppressor gene p53 have been shown in 40% of sporadic BCC and were correlated with aggressive behavior. P53 mutations are present in approximately 56% of all types of BCCs, also frequent in SCCs, mutated in 79% of the head and neck cancers and overexpressed in 47% of precancerous lesions[93]. About 71% of the p53 mutations detected in aggressive and nonaggressive BCCs and SCCs were UV signature mutations[31]. In addition, loss of p53 has been shown to accelerate tumorigenesis of BCC in Ptch1+/− mice, likely through Gli1 activation[133]. Another study showed dysregulation of Ras in 100% and mutations in up to 50% of BCCs[200]. PTEN might be the critical target for UV-induced skin tumorigenesis. Deletion of 10q23, where PTEN is located, was found to be an infrequent event in human BCC[201]. Mutations in HTERT promoter of hTert gene (UV-signature) were detected recently in both BCCs and SCCs, which could be used as a marker for cancer transformation[202,31]. Additionally, mutations in 14-3-3σ by early

methylation was detected in 68.3% of BCCs (early alterations in BCCs:[31], not dected in SCC[203,204,31,7]).

An schematic representation of deregulations of signalling pathways and transcription factors in SCC (Table 2 in:[31,25]) and melanoma are given inThe Cancer Genome Atlas Network, 2015.

Analysis connected with alterations especially of PKD1 during progression from inflammation, through precancer to cancer were recently published from us - reviews[7,23,22,25,31], by other authors[6,48,44,168,47,150], and in other cancers[205,206,163,49].

Protein Kinase C in Cancer

In cancer cells, PKC isozymes are involved in cell proliferation, survival, invasion, migration, apoptosis, angiogenesis, and anticancer drug resistance through their increased or decreased participation in various cellular signaling pathways. During cancer cell proliferation and survival; for example, PKC isozymes stimulate survival or proliferation associated signaling pathways, such as Ras/Raf/MEK/ERK or PI3K/Akt (also known as PKB)/mTOR pathways, but suppress the expression of cancer suppressor-associated or apoptotic signals such as caspase cascade or Bax subfamily. However, the activation statues of PKC isozymes and the downstream signaling cascades can be affected by different internal and external cellular conditions. This is particularly true during short- or long-term 12-Otetradecanolyphorbol 13-acetate (TPA) treatment, whereby short-term TPA treatment increases PKC activation, but long-term treatment downregulates PKC isozyme function (review:[207]).

Through target protein phosphorylation, PKC isozymes can directly or indirectly participate in diverse biological phenomena, such as cell cycle regulation (e.g., MARCKS, p53, and p21 ($p21^{Cip1}$ or $p21^{Waf1}$)), cell adhesion (e.g., adducins and integrins), DNA synthesis and transcription (e.g., transcription factor C/EBP and glycogen synthetase kinase 3β (GSK3β)), cell motility (e.g., RhoA and integrins), apoptosis (e.g., Bad and Bcl-2), drug resistance (e.g., P-glycoprotein (P-gp; also known asMDR1 or ABCB1)), and cell growth and differentiation (e.g., basic fibroblast growth factor (bFGF), epidermal growth factor receptor (EGFR), v-raf-1 murine leukemia viral oncogene homolog 1 (Raf1), and H-Ras, ERK1/2)[207]. Additionally, PKC isozymes have been found to regulate multiple cellular processes of direct relevance to T cell development and function, including differentiation, migration, survival, apoptosis, endocytosis, and secretion/exocytosis[208].

Furthermore, the expression patterns and functions of PKC isozymes in cancer cells largely depend on the type of cancer being investigated; however, the mechanism is not clear. For example, PKCδ acts as an antiapoptotic regulator in chronic lymphocytic leukemia (CLL) but as a proapoptotic regulator in acute myeloid leukemia (AML). PKCα shows proliferative functions in several types of cancer, but has antiproliferative functions in colon cancer cells. Importantly, PKC isozymes that are specifically overexpressed in certain types of cancer can be used as diagnostic or therapeutic targets. Thus, understanding the role and expression of individual PKC isozymes in each type of cancer may help to elucidate important cues for discovering novel drugs and for developing diagnostic or therapeutic tools. The PKC isozymes and their roles in multiple types of cancer cells as summarized in Table of review of[207].

Mutations in Protein Kinase C in Head and Neck Cancer

Cancer-Associated Protein Kinase C Mutations Reveal Kinase's Role as Tumor Suppressor[127] according the only in the field published paper summarizing detected PKCs mutations in different cancer types. We extracted data and will comment several mutations detected in Head and Neck cancer.

Analysis of cancer types most frequently harboring PKC mutations revealed that, although PKC isozymes are mutated across many cancers, PKC mutations are enriched in certain cancers. Namely, PKC isozymes are mutated in 20%–25% of melanomas, colorectal cancers, or lung squamous cell carcinomas, but in <5% of ovarian cancers, glioblastoma, or breast cancers. Additionally, nPKC isozymes are most commonly mutated in gastrointesti-

nal cancers (pancreatic, stomach, and colorectal), which have a lower mutation burden than melanomas and lung cancers, highlighting their importance in this type of cancer. The majority of PKC mutations are heterozygous, with an allele frequency varying from 0.05 to 0.67 for the mutations characterized. This indicates that PKC mutations can be truncal events in regards to tumor heterogeneity and exist in a majority of the cells within a tumor or can be branchal events acquired later in tumorigenesis as the tumor progresses to a more aggressive stage. This is consistent with PKC mutations being co-driver events that enhance tumorigenesis mediated by primary drivers[127].

The authors analysis of 46 mutations present within eight of the PKC genes revealed that ~61% (28) of them were LOF and none were activating. A lack of identification of activating mutations is not an artifact of authors assays, as activating PKC mutations that increase PKC affinity for DAG or decrease autoinhibition are readily detectable (data not shown). LOF mutations were identified within cPKC (α, β, γ), nPKC (δ, ε, η), and aPKC (ζ) isozymes and occurred within the C1, C2, and kinase domains as well as the pseudosubstrate and C-terminal tail[127-Figure 4B]. Strikingly, several LOF PKC mutations (e.g., PKCβ A509V, PKCγ P524R, and PKCα W58L, H75Q, and G257V) acted in a dominant-negative manner by decreasing global endogenous PKC activity[127].

Antal CE *et al.* next evaluated 21 kinase domain mutations, two of which were within PKCδ: D530G in colorectal cancer and P568A in head and neck cancer. Asp530 functions as an anchor for the kinase regulatory spine, a highly conserved structural element of eukaryotic kinases; not surprisingly, the D530G mutant was kinase dead and not primed by phosphorylation. Mutation of the conserved Pro568 to Ala also prevented a response to natural agonist stimulation but maintained some PDBu-stimulated activity, as a small pool of this mutant was phosphorylated[127-Figure 3].

Strikingly, all three PKCη mutations examined (K591E, R596H, and G598V) altered its subcellular localization by pre-localizing it at the plasma membrane prior to stimulation [127-Figure 3D]. However, despite constitutive membrane association, these mutants had reduced basal and stimulated activity as read out by a phospho-(Ser) PKC substrate antibody because they were not processed by phosphorylation. The authors have previously shown that unprocessed nPKC isozymes have exposed C1 domains that induce constitutive membrane association[127].

The PKCε R162H pseudosubstrate mutation showed reduced agonist-stimulated and basal activity[127].

LOF mutation that prevented processing of the atypical PKCζ was also found within the APE motif (E421K), that is involved in substrate binding and allosteric activation of kinases[127].

From Figure 4(D) of Antal CE *et al.,* 2015[127] is obvious that the frequency of mutations of PKC isoformes in Head and Neck cancer is higher than of cancers with less than <5% mutations in PKCs (of breast, ovaries, prostate and glioblastoma), but much lower than those with high freqence of PKC mutations (melanoma, of pancreas, lung and uterus, according Figure 4). In the table are not showen mutations in two PKC isoforms – PKCε and PKCι, the last of which is not expressed in normal human keratinocytes. Although according Table 3 mutations of PKCε (R162H) is detected, leading to low kinase activity in Head and Neck cancer it is with low allele frequency - 0.15[127]. PKCζ gene is positioned at the end of 1 chromosomes and could be lost as a result of aneuploidity, observed in BCC (*Table 1*[7]). Melanomas with detected mutation in PKCγ (which is not expressed in human keratinocytes) are fromed by melanocytes, another cell type in human epidermis, with different PKC expression profile from keratinocytes.

Increased PKD1 expression is detected in metastatic melanomas, connected with downregulated E-cadherin and upregulated N-cadherin expression, the cadherin switch which has been shown to promote tumor growth, motility and invasion through a process called epithelial-mesenchymal transition (EMT). In T1 and G1 melanoma cells that express high levels of E-cadherin but very low levels of N-cadherin, PKD1 expression is very faint. In I5,

Table 3: Mutations in PKCs in Head and Neck cancer (extraction of Table1: LOF PKC Mutations in cancer and

Mutations PKCs	Aktivity	Domain	Cancer(s)	Residue Importance	Allele Frequency	Other Mutations	Other genes' mutations
αW58L	None-c	C1A	Head and neck	DAG binding, conservred in all C1a domains	0.22	γW57splice θ W171* (at the end of chromosomes Table 1- PKC gene module)	TP53 (23/50) SEC22B (8) REG3A(8) CDKN2A(7) BLID(7)
δP568A	None-c		Head and neck	Conserved in all PKC isozymes	0.16	δ P568S β P561H γ P575H	TP53 (22/47) K-Ras (13) OR4K1 (11) CDKN2A (9)
εR162H	low		Head and neck	Non-conserved	0.15		TP53 (18/57) POM121L12 (12) **KRAS** (11) FGFR1OP2 (10)
γD193N	None-c	C2	Colorectal /melanoma / ovarian	Ca^{2+} binding site	0.28		TP53 (52/102) CDKN2A (17) **KRAS** (16) POM121L12 (16) OR4K1 (16) OR4A5 (16) DNAJC5B (12)
PKCη							TP53 (17/51) **KRAS** (11) RPL10L (9)
PKCζ							TP53 (15/28) POM121L12 (6)
PKCι							TP53 (26/48) CDKN2A (10)

C - Kinase-dead.

M2 and M4T2 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 (i.e. M2 cells). Furthermore, 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). Additionally, PKD1 knockdown in M4T2 metastatic melanoma cells significantly induced down-regulation of N-cadherin and up-regulation of E-cadherin, supporting the role of PKD1 in E-cadherin to N-cadherin switch. 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[166].

KRAS phosphorylation has been reported recently to modulate the activity of mutant KRAS protein *in vitro.* In this study, Barceló C *et al.* defined S181 as a specific phosphorylation site required to license the oncogenic function of mutant KRAS *in vivo.* The phosphomutant S181A failed to induce tumors in mice, whereas the phosphomimetic mutant S181D exhibited an enhanced tumor formation capacity, compared with the wild-type KRAS protein. Reduced growth of tumors composed of cells expressing the nonphosphorylatable KRAS S181A mutant was correlated with increased apoptosis. Conversely, increased growth of tumors composed of cells expressing the phosphomimetic KRAS S181D mutant was correlated with increased activation of AKT and ERK, two major downstream effectors of KRAS. Pharmacologic treatment with PKC inhibitors impaired tumor growth associated with reduced levels of phosphorylated KRAS and reduced effector activation. In a panel of human tumor cell lines expressing various KRAS isoforms, the authors showed that KRAS phosphorylation was essential for

survival and tumorigenic activity. Furthermore, they identified phosphorylated KRAS in a panel of primary human pancreatic tumors. Taken together, Barceló C et al. findings establish that KRAS requires S181 phosphorylation to manifest its oncogenic properties, implying that its inhibition represents a relevant target to attack KRAS-driven tumors[217].

Discussion:

PKC enzymes (PKCα, PKCβ, PKCγ, PKCδ, PKCθ, PKCε, PKCη, PKCι, and PKCζ) are encoded by nine genes (PRKCA, PRKCB, PRKCG, PRKCD, PRKCQ, PRKCE, PRKCH, PRKCI, and PRKCZ), which can be classified into three sub-classes based on their requirements for DAG and calcium (Figure 1). The sub-classes are classical or cPKCs (PKCα, PKCβ, PKCγ) activated by calcium and diacylglycerol, novel or nPKCs (PKCδ, PKCθ, PKCε, PKCη) activated by diacylglycerol but not calcium, and atypical or aPKCs (PKCι and PKCζ), which are not activated by either calcium or diacylglycerol[215].

Different PKC enzyme expression profiles have been identified in normal human and mouse keratinocytes. Human keratinocytes express cPKCα, nPKCδ, PKCε, PKCη, aPKCζ, and PKCμ/PKD1[5] in very low mRNA and protein levels, PKD2 and PKD3[6]. Mouse keratinocytes express several other PKC isoforms, including cPKCβII[152] and γ[218], nPKC θ[74] and the aPKCι/ λ[57,40]. In the normal keratinocytes, the levels of PKCα, PKCδ, PKCη, and PKCζ increased over the first one to two weeks in a calcium-and time-dependent manner. PKCε decreased in a time-and calcium-dependent fashion over the three-week period[3].

In papilloma: The two-stage carcinogenesis protocol (Topical application of TPA (A) or DMBA (B), according to (DMBA)-initiated and 12-O-tetradecanoylphorbol-13-acetate (TPA)-promoted skin tumorigenesis in mice) causes an oncogenic mutation in the H-Rras gene and appeared to be ideal for studing physiological responces to alterations in gene expression against oncogenic Ras signaling in living animals[220], leading to the development of papillomas and consequently SCC. DMBA/TPA treatment is necessary for BCC formation from patched deficient epidermal cells in Ptch(flox/flox)CD4Cre(+/-) mice[221]. Papilloma formation is mostly connected with increased c-Myc expression, which downregulates $p21^{Cip1}$[39,23]. Sustained activation of c-Myc is sufficient to induce papillomatosis together with angiogenesis-changes that resemble hyperplastic actinic keratosis (possessing also Ras mutations), a commonly observed human precancerous epithelial lesion. All these premalignant changes spontaneously regress upon deactivation of c-MycER[41]. c-Myc-deficient epidermis is resistant to Ras mediated DMBA/TPA induced tumorigenesis (DMBA-initiated and TPA-promoted skin tumorigenesis in mice).This is mechanistically linked to p21Cip1, which is induced in tumors by the activated Ras–ERK (Extracellular signal Regulated Kinase1/2) pathway, but repressed by c-Myc. Acute elimination of c-Myc in established tumors leads to the up-regulation of $p21^{Cip1}$, and epidermis lacking both $p21^{Cip1}$ and c-Myc reacquires normal sensitivity to DMBA/TPA-induced tumorigenesis. This identifies c-Myc-mediated repression of $p21^{Cip1}$ as a key step for Ras-driven epidermal tumorigenesis[39].

Proves for the participations of PKD1 in inflammatory and tumor promoting events, in accordance with our results and hypothesis[31,25], were published Chiou *et al.*[44]. Topical application of TPA (A) or DMBA (B) over 12 h, according to (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 (−) epigallocatechin-3-gallate) leads 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, and p-p38; and increased levels of p53, p21, and c-Myc (in papillomas)). The authors also obser-

ved that the DMBA/TPA stimulation of NF-κB (p-IκBα, p-p65), 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 inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), ornithine decarboxylase (ODC), and vascular endothelial growth factor (VEGF). Overall, the authors speculated that AcEGCG exerts antiproliferative and/or anti-inflammatory 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[44]. Interesting the authors do not observed significant alterations in the phosphorylation status of Ser744/748 of PKD1, although detected increase in PKD1 activity, similarly to the activation of the kinase from UVB[43,8].

PKCα, δ, and ε have been transgenically targeted to the epidermis of FVB/N mice with the keratin 5 promoter producing distinct phenotypic changes. Although undisturbed skin was normal in PKCα or δ mice, PKCε mouse epidermis was slightly hyperplastic, suggesting this isoform contributes to keratinocyte proliferation. When the PKC activator 12-*O* tetradecanoylphorbol-13-acetate (TPA) was applied to transgenic skin, sustained hyperplasia was greatest in PKCε epidermis, confirming a proliferative influence of this isoform. Major differences among the three transgenic lines were detected in skin tumor induction studies using 7,12-dimethylbenz[a]anthracene as initiator and TPA as the promoter. K14-PKCδ mice developed few papillomas or carcinomas, whereas K14-PKCε mice were very sensitive to carcinoma formation, developing malignant tumors even in the absence of TPA application. K14-PKCα transgenic mice did not differ from nontransgenic mice in tumor yield. Wang and Smart developed K14-PKCα transgenic mice on a C57B/6 background and demonstrated that skin tumor formation was not influenced by overexpression of PKCα. However, TPA treatment of the K5-PKCα mice caused severe intraepidermal and dermal inflammation, intraepidermal neutrophilic inflammation, degeneration of hair follicles, and a disruption and sloughing of the epidermis, changes not detected in the other PKC transgenic strains[24].

PKCδ overexpressing mice are extremely resistant to chemically induced tumorigenesis in skin, despite enhanced induction of the proliferation marker ornithine decarboxylase[72], supporting the role of PKCδ in cancer suppression. Thus, the incidence of benign papillomas is reduced and progression towards malignancy slowed down dramatically, though this may refer merely to chemically and not UV radiation-induced carcinogenesis. On the contrary, in mice depleted of PKCδ apoptosis was suppressed which may enhance tumorigenesis. In fact, this confirmed the anti-promoting function of PKCδ shown in a cell model previously. Finally, the role of PKCδ in establishing immune tolerance, which was demonsrated in transgenic mice, may imply that this isoform could be critical for cell-mediated immunity including tumor cell surveillance[57].

PKCη is not expressed in basal proliferative keratinocytes[72,67,68]. PKCη is primarily distributed to the uppermost granular layer, absent in the spinous layers[57,73,74]. However loss of PKC η increases tumor incidence in the DMBA/TPA chemical carcinogenesis protocol (much higher sensitivity to carcinogenesis), indicating PKC η has also a role in tumor suppression[73].

PKC-ζ is not activated nor depleted by TPA. The level of PKC-ζ transcripts was similar in normal and v-Ha-ras keratinocytes, indicating that reduction of this mRNA in both 308 and SP-1 cells was not a direct result of ras activation[4,57]. PKC-ζ transcripts in benign neoplastic keratinocyte cell lines, 308 and SP-1, was reduced by 74-89% when compared with normal keratinocytes, releasing constraints on Akt/PKB activity, proceeding during skin tumor promotion and progression[4,57]. PKCζ is also crucial for macrophage activation and expression of adhesion molecule ICAM-1, and metalloproteinase-9 (MMP-9)[75], through ERK1/2 Snail pathway but not p38[8,42,32,60]. MMP-9 was detected in the epithelium in both chronic wounds (chronic leg ulcers)[76] and found in papillomas[77].

The Akt/protein kinase B (PKB) pathway protects keratinocytes from the toxic effects of ultraviolet light (UV). In experimental mouse multistage skin carcinogenesis, Akt activity

increases in benign squamous papillomas, and this increase persists through premalignant progression and malignant conversion. Overexpression of Akt in neoplastic keratinocyte cell lines enhances their tumorigenicity and produces a more aggressive malignant phenotype[55]. Activation of PKCδ and PKCε provide a negative regulation for Akt phosphorylation and kinase activity in mouse keratinocytes and serve as modulators of cell survival pathways in response to external stimuli (dephosphorylation of Akt on Ser-473), whereas PKCα enhanced phosphorylation of Akt on Ser-473 (Akt kinase activity). PKCη showed no obvious dose-dependent effect on the level of phospho-Akt-Ser-473[55]. Down-regulation of PKCζ in benign neoplastic keratinocytes may contribute to skin tumorigenesis by releasing constraints on Akt/PKB activity, proceeding during skin tumor promotion and progression[57]. Data in the literature supposed that 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[50,7,23].

In ECs PKD1 stimulates PI3K/Akt connected with VEGF synthesis[51,52] (Figure 8[7] and Figure[123]).

In Spinocellular carcinoma: To determine the relative importance of PKC isoforms as mediators of head and neck tumor growth, Nagao *et al.* analyzed their expression in four radioresistant, EGFR-overexpressing SCCHN cell lines (SQ20B, SCC61, SCC25, and JSQ3) by immunoblotting with anti-PKC antibodies. Similar to NHEK cells, the PKC isoforms α, δ, ε, θ and ζ, are expressed at varying levels, whereas isoforms β1, β2, γ, and η do not seem to be expressed in these cell lines. In a human squamous cell carcinoma line, elevations in extracellular Ca^{2+} rapidly increase PKC in the membrane fraction[106], which is a classical indication of its activation[107], although uncapability of Ca^{2+} to stimulate differentiation in SCC[3,108] (see also PKC mutations). Muatations in PKCα W58L (kinase dead), in C1 region (DAG binding region), were detected in HNC, leading to failture to be activated by DAG and TPA after membrane translocation[127]. Failure to regulate PKCα in SCC4 may underlie at least part of the failure of calcium to promote differentiation in these cells[3,108]. The skin phenotype of PKCε overexpressing mice is characterized by epidermal hyperproliferation and skin ulceration, beginning at four months of age. However, when tumor formation was induced using a two-stage skin carcinogenesis protocol, papilloma formation was lowered, while progression to SCCs was increased. Further, the skin of PKCε overexpressing mice was sensitized to UV radiation induced SCC formation[57,58] and development of papilloma-free highly-metastatic SCC[125].Yadav V *et al.* have reported that PKC-δ is lost in human SCCs at the transcriptional level. Human SCCs with low PKC-δ had evidence of Ras pathway activation. The expression of activated Ha-ras in mouse keratinocytes induces tyrosine phosphorylation and enzymatic inhibition of PKC-δ, and has been linked to a reduced differentiated phenotype[114]. In squamous cell carcinoma, PKCη is stained in keratotic cells around horny pearls, whereas basal cell epithelioma (Basal Cell Carcinoma) is not stained. No expression of PKCη is detected in mesenchymal cells at the mRNA or protein level[73]. Analysis of staining intensity reveals that the expression of both total and activated PKCζ increased significantly from normal to malignant tissue. In addition, only samples from malignant tumors showed membranous staining of phosphorylated PKCζ, implicating its involvement in receptor signaling. Inhibition of PKCζ, but not other PKC isoforms, blocks EGF-stimulated MAPK (ERK) activation DNA synthesis and proliferation in normal keratinocytes, and the majority of head and neck cell lines[75,105]. PKD1 is down-regulated in SCC, although not as a result of hypermethylation in the majorities of HNSCC. 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 [47,7]. PKD2 mRNA was upregulated in seven out of ten tumors vs normal in patient-paired HNSCC tissue specimens. It is possible that PKD2 plays a predominant role in the growth, survival, and motility of HNSCC cells, and these functions have compensated the loss of PKD1 in tumors. Authors detected increased PKD2 or PKD3 expression in tumor vs. normal

tissue, PKD3 was minimally expressed in the control and in almost all HNSCC cell lines examined[47]. Thus, loss of PKD1 (and/or increased PKD2) expression could be used as a marker in differentiation diagnose between papilloma (benign lesions) and SCC[6,5,44,47,7].

Increased activation of epidermal growth factor receptor (EGFR) family members such as HER2/ErbB2 can result in more aggressive disease, resistance to chemotherapy and reduced survival of head and neck squamous cell carcinoma (HNSCC) patients. The frequency of HER2 over-expression varies between 6% and over 80% depending on tumor type and is associated with shorter disease-free and overall survival. Intriguingly, the activation status of HER2 but not EGFR predicts resistance to the EGFR inhibitor Gefitinib in HNSCC, suggesting that interactions between family members are important for unknown reasons. HER2 and ADAM12 (proteolytic shedding of EGFR ligands including HB-EGF (Heparin-Binding EGF)) expression were increased in oral SCC cells derived from a recurrence compared to cells from the primary tumor from the same site. To understand the molecular mechanisms underlying HER2 regulation of ADAM12, authors investigated the signaling pathways directing ADAM12 production in SCC cells. Inhibition of Phosphatidyl Inositol-3-Kinase (PI3K) or mammalian Target of Rapamycin (mTOR) decreased ADAM12 transcripts in HER2-expressing SCC cells, while transfection with AKT increased ADAM12 mRNA (HER2 up-regulated ADAM12 expression through both PI3K and JNK pathways). Experiments utilizing ADAM12 transfection or siRNA targeting of ADAM12 revealed that the protease increased both the migration and invasiveness of oral SCC cells. Surprisingly, ADAM12 also increased HER2 message, protein levels, and activity through an Ets1-dependent mechanism. Collectively, these results reveal a novel positive activation loop between ADAM12 and HER2 that may contribute to HNSCC progression[104].

Inhibition of Wnt signal pathway by GLI could be the main reason for decreased Myc protein expression in Bowen's disease (OVOLV1 see below) and in BCC (leucoplakia is under question) and down-regulation of GLI (inactivating mutations in HH/PTCH/Smo) could be the main reason for progression of Bowens' disease into invasive SCC. Activating mutations in hedgehog signaling pathway genes, especially PTC1 and SMO, are pivotal to the development of basal cell carcinomas[78].

BRCA1 and BRCA2 mutation carriers have elevated risks of breast and ovarian cancers. The risks for cancers at other sites remain unclear. Melanoma has been associated with BRCA2 mutations in some studies, however, few surveys have included non-melanoma skin cancer. Ginsburg OM *et al.* followed 2729 women with a BRCA1 or BRCA2 mutation for an average of 5.0 years. These women were asked to report new cases of cancer diagnosed in themselves or in their family. The risks of skin cancer were compared for probands with BRCA1 and BRCA2 mutations. Of 1779 women with a BRCA1 mutation, 29 developed skin cancer in the follow-up period (1.6%). Of the 950 women with a BRCA2 mutation, 28 developed skin cancer (3.0%) (OR = 1.83 for BRCA2 versus BRCA1; 95% CI 1.08-3.10; P = 0.02). The odds ratio for basal cell carcinoma was higher (OR = 3.8; 95% CI 1.5-9.4; P = 0.002). BRCA2 mutation carriers are at increased risk for skin cancer, compared with BRCA1 carriers, in particular for basal cell carcinoma. In summary, this study suggests that there may be a higher risk of non-melanoma skin cancer in BRCA2, versus BRCA1 carriers. This observation may be due to a higher than expected risk among BRCA2 carriers, or to a lower than expected risk among BRCA1 carriers. The most common risk factor for skin cancer is ultraviolet (UV)—induced DNA damage, which is repaired predominantly by nucleotide excision repair. Squamous cell carcinomas of the skin often contain cyclobutane pyrimidine dimers and the removal of these dimers depends in part on an interaction between BRCA1 and P53. While no similar data currently exist for BRCA2, it would interesting to study this phenomenon in BRCA2-deficient model systems [132].

Gu J *et al.* results have showen PRKD1 mRNA significantly differed among the control group - normal skin (0.64 ± 0.09), SCC group (5.37 ± 1.06), BD group (2.69 ± 0.72） and

AK group (2.43 ± 0.46） （F = 21.37, P < 0.05), and was significantly higher in the SCC, BD and AK groups than that in the control group (P < 0.05), as well as in the SCC group than that in the AK and BD groups (both P < 0.05). However, no significant difference in the PRKD1 mRNA was observed between the BD group and AK group (P > 0.05). Immunohistochemical study showed that the total PKD1 protein and pPKD1/Tyr463 in the SCC and BD groups were mainly expressed in the cytoplasm and cell membrane of spinous layer cells and atypical cells, and their rates were significantly higher than those in the AK group and control group (all P < 0.01). The pPKD1/Ser916 was only slightly expressed in some cancer nests of well-differentiated SCC tissues, but not in poorly-differentiated SCC, AK, BD tissues and normal skin tissues. In the SCC group, the rate of PKD1 increased with the increase of the pathological grade of SCC, and the PKD1 was positively correlated with pPKD1/Tyr463 (r c c = 0.479, P < 0.05). Western blot results were consistent with immunohistochemical findings. Conclusion PKD1 and pPKD1/Tyr463 may be involved in the development and differentiation of skin tumors derived from stratified squamous epithelium, and PKD1 may exert promotive effects on the formation of cutaneous SCC by activating the Tyr463 phosphorylation site [168].

Conclussions:

Different PKC enzyme expression profiles have been identified in normal human and mouse keratinocytes. Human keratinocytes express PKCα, PKCδ, PKCε, PKCη, PKCζ, and low levels of PKCμ/PKD1[5], PKD2 and PKD3[6]. Mouse keratinocytes express several other PKC isoforms, including cPKCβII[152] and γ[218], nPKC θ[74] and the aPKCι/ λ[57,74] there is no data for other PKD isoforms expressed in mouse keratinocytes. In the normal keratinocytes, the levels of PKCα, PKCδ, PKCη, and PKCζ increased over the first one to two weeks in a calcium-and time-dependent manner. PKCε decreased in a time-and calcium-dependent fashion over the three-week period[3].

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, which is consistent with altered PKC function in these cells. Among PKCs PKCα is connected with Ca^{2+} induced differntation in human keratinocytes, as a Ca^{2+} responsive PKC isoform, although alterations in its expression or early mutations in PMDs are not reported. Alterations in protein levels of PKCη and PKCζ were reported in v-Ha-ras transformed keratinocytes[4], both of them down-regulated. Interestingly, alterations in PKCα activity are not detected, the only Ca^{2+} - dependent isoform in keratinocytes. The both cell lines do not express PKC-δ, characteristic for ras transformation.

Previously we have detected increased high expression of PKD1 in hTert (N/Tert-1 or N-hTERT) keratinocytes, which can be concider as a premalignant cell line, possessing p16INK4a mutations[5,26,27,25,78]. There has to be molecular mechanism of EGFG down-regulation, in commitment of differentiation, different from keratins expression (K1,K10). One possible explanation is connected with a specific interplay between calmodulin and PKCδ in the regulation of the morphology of and trafficking from the early endocytic compartment of EGFR [247]. PKCδ is responsible to inhibit the recycling of the EGFR from the early endocytic compartment [247], which could be the mechanism connected with down-regulation of EGFR in commitment of differentiation. PKC-δ played a protective role in SCC partly by down-regulating p63 (decreasing CSCs in SCC) and inducing apoptosis[115].

We could not succeed in finding data concerning PKC isoforms expression in Actinic keratosis, precursor lesion of BCC (and SCC as well), with exception of PKCδ (lack in BCC and SCC -marker) and PKD1 (loss in SCC, although results of Gu J et al. are different ?[168]). PKD1[252] and PKCι[138] are the only PKC isoforms expressed in Basocellular Carcinoma[7,23], there is no data for its expression in BD, AK and TE. There is no data for the other two PKD isoforms - PKD2 and PKD3 in three lesions – *Table 1* and *Table 2*. There is no data for PKCε expression in BCC (if it is expressed, induction by GLI[126]), its loss (mutations, hypermethylation) could be connected with low metastaic rate of this cancer, except increased PKD1 expression[23].

Ghr expression, suppressed on inhibition of Erbb2, is a marker for the progression from Actinic Keratosis to squamous cell carcinoma. Significant correlations have been shown between *Mmp9,* reduced by the Erbb2 inhibitor, and Erbb2 expression with respect to clinicopathological parameters in head and neck squamous cell carcinoma, and oral squamous cell carcinomas have higher expression of *Mmp9.* These data support a multifaceted role for Erbb2 in skin cancer development and progression[166].

Benign trichoblastic neoplasms are negative for AR proteins, further aiding in their distinction from BCC, which often expressed them. In a study done by Katona *et al.*, immunohistochemistry for AR and CK20 was performed on 15 DTE and 31 mBCC. AR expression was seen in 13% (2/15) of DTE and 65% (20/31) of mBCC cases. CK20-positive Merkel cells were identified in 100% (15/15) of DTE and 3% (1/31) of mBCC. The expected pattern of AR-, CK20+ immunophenotype was present in 87% (13/15) of DTE cases. In mBCC, 61% (19/31) was AR+, CK20-. No DTE was AR+, CK20- and no mBCC was AR-, CK20+. Another study concludes that immunohistochemistry for ARs and CK20 is helpful, but interpretation is difficult in some DTEs when few cells are immunopositive for these markers. This can be especially true in small biopsy specimens, which is a particular problem as these lesions are often present on the face[259].

CD10 staining pattern may be a useful adjunct marker in distinguishing between TE and BCC. In a study done by Pham et al., 12/13 cases (92%) of TE showed positive stromal immunoreactivity. Of these, eight cases also demonstrated positivity of the papilla, and two showed positivity of the basaloid cells. No TE demonstrated epithelial expression alone. On the other hand, expression of CD10 by basaloid cells was identified in 20/23 (87%) cases of BCC. Stromal positivity was also identified in three cases of BCC[259].

Lum *and* Binder studied the proliferative rate of basal-cell carcinoma and TE in small biopsy specimens. They found that BCCs qualitatively showed a greater proliferative fraction (using the antibody, Ki-67) compared to TE (50.0 vs 13.0%), as well as over-expression of p53. BCCs marked by p21 demonstrated scattered nuclear positivity compared to the virtual absence of staining in the TE. Bcl-2 stains BCC in a diffuse pattern, whereas all of the TEs in one study showed staining of the outermost epithelial layer[259, 23,22].

One of the earliest molecular changes during the reprogramming process includes the activation of Wnt/β-catenin signaling, on which the development of BCCs critically depends (cytoplasmic distribution of β-catenin is important for the TE (Trichoepitheliomas - PTCH[260], CYLD[261] (regulation of JNK pathway[262])[23,22] mutation, Cylindromas, Trichoblastomas - HH/Smo mutations, GLI overexpression[144][25-Table 2,23]), and TF (Trichofolliculomas) formation, benign skin neoplasms originating from hair follicle cells, E-cadherin showed loss of membrane-type expression with relocalization into the cytoplasm of tumor cells, predominantly in TE, the levels of c-myc and cyclin D did not differ from the protein expression in control hair follicle and sebaceous cells[121].

JNK1/2 activates Jun/Fos, and enhances their interaction with phosphorylated ATF2, which then enhances SHH/Gli induced tumorigenesis. In SCC, MKK4/7 activates JNK1 and JNK2. JNK1 induces apoptosis, whereas JNK2 promotes carcinogenesis in an AP1-dependent manner. SCCA1 promotes SCC via inhibition of JNK1 and CYLD inhibits SCC via suppression of JNK2/AP1 cascade. In melanoma, the MALT1, MKK4/7, and JNK/AP1 signaling cascade promotes melanoma cell proliferation and migration, whereas CYLD inhibits it[262].

Acknowledgement:
With thanks to the Editorial Board for corrections and ask for additional remarks.

Compliance with Ethical Standards:
The authors declare that they have no conflicts of interests.

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Психологически последствия и характер на реакцията при въздействие на тежък стресогенен фактор – загуба, както и степента на влияние на травматичното събитие

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Psychological consequences and nature of the reaction under the influence of severe stress factors - loss, as well as the degree of influence of the traumatic event

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РЕЗЮМЕ:

В съвременния свят човек става обект на негативното въздействие на различни интензивни стресови събития, които застрашават неговото психично благосъстояние. Практиката показва, че най-тежко приемани и най-интензивни като дълбинност на преживяването са кризите след загуба - на здраве и значим/любим човек (смърт и/или раздяла, развод). Изучаването на психологичните последствия от преживяване на силен стрес при загуба (здраве, смърт, раздяла/развод) е изключително актуално – веднъж, поради тежестта и честото срещане на този вид стресор и втори път, поради неговата способност да дестабилизира психическото

ABSTRACT:

In the modern world the person becomes an object of the negative impact of various intense stressful events that threatening his mental well-being. Practice shows that the most difficult for acceptance and most intense in the depth of the experience are the crises cause by loss - of health and significant/loved one (death and/or separation, divorce). The study of the psychological consequences of the experience of severe stress after loss (health, death, separation/divorce) is extremely relevant – first due to the severity and frequency of this type of stressor and second, due to personal ability to destabilize the mental functioning not only for the individual, and the whole family system. Everyone

функциониране не само на отделния индивид, а и на семейството като цяло. Всички въвлечени в този вид кризисно преживяване са изправени пред предизвикателството да се променят и адаптират към новите условия – необратими по своя характер. Настоящата ситуация на Ковид пандемия, представлява едно такова предизивкателство.

Ключови думи: загуба, раздяла, смърт, криза.

involved in this type of crisis is faced to the challenge of changing and adapting to new conditions - irreversible in nature. The current situation of the Covid pandemic is one such challenge.

Key words: loss, separation, death, crisis.

Introduction

Personality crises

Personality crises are related to micro-traumas in life, to psychological characteristics and trials (crises in marital relationships, intergenerational relations, adjustment crisis, sexual relations, acceptance of sexuality, etc.). People try to manage the crisis with their available coping mechanisms, but they ran out and eventually failed. In such cases, there is often an over-engagement with the life situation or with the body (experience of symptoms): headache, palpitations, conversion symptoms – and this way they stop dealing with the crisis. Very often, in a state of severe personality crisis, the individual may focus on the health of others, for example, child or partner. (Boncheva, 2013).

Situational crises

Situational crises are referred to losses: health, death, disasters, abuse, material losses, etc. They have the meaning of macrotraumas. Every unprocessed crisis turns into a trauma. There is an absolute blockage of available resources. Emotions have the power of affect - they are strong, short-lived, and inappropriate to the situation. In these cases, the mind is blocked because affect dominates. The person no longer has access to his/her available resources. (Boncheva, 2013). The words crisis, stress and trauma are often confused due to lack of understanding of their exact definitions and parameters. The definitions of these three concepts often overlap even in the scientific literature. Also, the individual's response to these conditions is unique and is determined by personality, temperament, character, protective factors, coping strategies, adaptive abilities, support system, number, intensity, and duration of stressors.

Roberts (2005) describes the crisis as an acute disturbance of psychological homeostasis, in which the old coping strategies cannot be used, the individual falls into a state of distress and his functioning is impaired. A crisis is a subjective reaction (response) to a stressful or traumatic life event or a series of situations that are perceived by the individual as upsetting and threatening. This leads to a violation of the stability of the affected person and his ability to adapt, using his previous psychological experience. Each crisis consists of the following elements:

- Dangerous or traumatic event.
- Vulnerable or unbalanced condition.
- Precipitating factor.
- Active state of crisis - determined by the way the individual experiences.
- Resolving the crisis (Roberts, 2005)

Method

Participants

The research is based on the practices of the authors (psychologist - psychotherapist and psychiatrist - psychotherapist). We included in the research 60 people, 28 men (46.7%) and 32 women (53.3%) in the age range 30 - 55 years. At the age of 30 - 45 years - 29 (48.3%), 13 (21.6%) men and 16 (26.7%) women. At the age of 46 - 55 years - 31 (51.7%), 15 men (25.0%) and 16

women (26.7%). All subjects applied for specialized help after experiencing loss: 20 (33.3%) of the subjects (8 men and 12 women) after loss of health; 18 (30.0%) of the people after death of a significant person (8 men and 10 women) and 22 (36.7%) of them after separation /divorce (12 men and 10 women), (Table 1).

Table 1. Distribution by sex, age, and crisis event

Loss	Age	Men		Women		Total	
Health	30 – 45	2 (3,4%)	8	5 (8,3%)	12	7 (11,7%)	20
	46 - 55	6 (10,0%)	(13,3%)	7 (11,7%)	(20,0%)	13 (21,6%)	(33,3%)
Death	30 – 45	3 (5,0%)	8	4 (6,7%)	10	7 (11,7%)	18
	46 – 55	5 (8,3%)	(13,3%)	6 (10,0%)	(16,7%)	11 (18,3%)	(30,0%)
Separation/	30 – 45	8 (13,3%)	12	7 (11,7%)	10	15 (25,0%)	22
Divorse	46 – 55	4 (6,7%)	(20,0%)	3 (5,0%)	(16,7%)	7 (11,7%)	(36,7%)
Total		28 (46,7%)		32 (53,3%)		60 (100%)	

The study is an attempt to assess the psychological consequences and the nature of the reaction to a severe stressor - loss, as well as the degree of influence of the traumatic event. The main problem in the study is the influence of the professional social - psychological support and the personal maturity of the patients, as well as their complex importance for reducing the negative psychological consequences.

Instruments

1) Derogatis Symptom Check List - Derogatis SCL - 90 - R (Derogatis Symptom Check List - 90 - Revised), (L. R. Derogatis, 1977, 1994). Reliability of the study (Cronbach's Alpha) - 0.86. SCL - 90 - R is a self-evaluating, symptomatic questionnaire, which begins after a factor analysis of the Hopkins Symptom Checklist (HSCL), (L. R. Derogatis et al, 1974). As a result, 5 significant factors were derived (LR Derogatis et al, 1971) - somatization, obsessions, interpersonal sensitivity, depression, and anxiety (in its original version), subsequently (after revision) 4 more were added. (LR Derogatis et al, 1976). Currently, SCL - 90 - R contains 9 scales, which are used to assess certain mental disorders, study the mental state, experiences, and therapeutic dynamics of patients with somatoform disorders, eating disorders and other disorders. It should be noted that the questionnaire contains two indicators reflecting the severity of symptoms (GSI - General Symptom Index) and the presence of symptomatic distress (PSDI - Symptomatic Index of Positive Distress).

2) Statistical methods:

• U crit of Man Whitney - for reliability in the differences of the studied indicators by groups and for establishing significant differences in the studied indicators;

• Churchl - Wallis Test (H-criterion) - for comparison and evaluation of statistical significance in more than two distributions of a given trait;

• Cronbach's Alpha - to measure the reliability of the internal consistency of the rocks.

Results:

Analysis of the results of the Short Symptom Questionnaire - Derogatis SCL - 90 - R

In terms of intergroup differences - gender and age, compared based on subscales and the General Symptom Index (GSI). Statistically significant differences were found on the GSI index and scales: "Somatization" (SOM), "Social contact insecurity" (INT), "Depression" (DEP), "Anxiety tension" (ANX) and "Aggression and hostility" (AGG). / VOC).

In all subscales, apart from "Aggression and hostility", higher values are found in women. Probably, this is due to the higher sensitivity of women to their condition compared to men, rather than should be taken as an indicator of deteriorating health. (Table 2).

Table 2. Gender Comparative analysis

	SOM	INT	DEP	ANX	AGG/HOS	GSI
Mann-Whitney U	3227,500	3795,500	3543,000	3393,000	4576,000	1868,500
Wilcoxon W	8277,500	9900,500	9648,000	8443,000	9626,000	6918,500
Z	-5,172	-3,890	-4,459	-4,798	-2,109	-8,268
Asymp. Sig. (2-tailed)	,001	,001	,001	,001	,035	,001

The results by age indicator, total for all surveyed people showed that in 29 people (48.3%) from the age group 30-45 years there is a moderate to a high degree on a scale: "Uncertainty in social contact" (INT), (u = 2.74; p < 0.006); "Anxiety tension" (ANX), (u = 3.07; and "Aggression and hostility" (AGG / HOS), (u = 1.90; In the age group 46 -55 years - 31 subjects (51.7 %), with moderate to high degree are the scales: "Somatization" (SOM), (u = 3.55; p < 0.001) and "Depression" (DEP), (u = 5.17; = p <0.001).

The obtained results allow that with increasing age, both genders are prone to a stronger feeling of bodily dysfunction - cardiovascular, gastrointestinal, respiratory, and other symptoms. This also includes complaints such as: headache, other pains, general autonomic symptoms, and discomfort - the somatic equivalent of anxiety. More pronounced in this age group (46 -55 years) are the negative affective symptoms - lack of interest in life, motivation, and luch of energy, which go along with a sense of hopelessness, and painful emotions and experience - depression, sadness, despair, guilt, etc.

Regarding intragroup differences based on gender and age, statistically significant differences were found only in the surveyed women - 16 (26.7%) aged 30 -45 years and the same (16 - 26.7%) aged 46 -55 years. Significant differences in the scales: "Anxiety stress" (ANX), (u = 5, 62; p < 0,001) and "Uncertainty in social contact" (INT), (u = 4, 45; p <0,001) , which are more pronounced in women aged 46 -55 years. Statistically significant differences on these scales in this age group allow us to assume that the crisis events for these are related with normativ crisis (menopause). During the crisis, two main social roles of a woman disappear - the ability to bear and give birth to children and the role of a sexual partner (libido decreases with age). This is probably the reason for the psycho-somatic symptoms and the feeling of insecurity and inferiority in contacts with other people. For men, the intragroup variable "age" was irrelevant to the scale values.

The results by nature of loss (health, death, separation/divorce) showed statistically significant differences on the following scales:

• Depression (DEP), (Kruskal-Wallis 2 = 39.73; p <0.001), severe in men in the age group 46 -55 years, after separation/divorce from the partner.

The age range gives us reason to assume that the probable cause is the overlap of two crisis events - andropause and abandonment. A very typical symptom of andropause is the feeling of general weakness, lack of vitality, and energy, and concentration, decreased libido, low self-esteem, feeling of loneliness, guilt and hopelessness. In general, moderate to high severity on the Depression scale was found in all other subjects with loss of health and death.

• Somatization (SOM) - moderate to severe in women 30 - 45 years - (2 = 29.97; p <0.001) and 2 = 31.43; p <0.001 - in women 46 - 55 years of age, as well as in 25.0% (Church-Wallis 2 = 20.05; p <0.001%) of men - all in the age group 46 - 55 years.

• Aggression and hostility (AGG / HOS) - a strong degree is found in 21.6% of men in the age group 30 - 45 years, after separation and divorce (2 = 27.11; p <0.001), and in 26.7% of women in the same age group (2 = 15.92;

p <0.05). We believe that the high score on this scale is a result of deteriorating relationships at the end of a relationship, which are usually with a strong negative charge - insult, anger, accusations and self-pity. Based on a moderate to high degree on this scale, which is observed in all subjects experiencing loss of death (30.0%), we also assume that at the time of the study are in the second stage of grief - "Anger" (E. Kubler-Ross, D. Kessler, 2021). This stage comes after the "Denial" and is characterized by feelings of anger, rage, envy, or resentment.

• Unsecure in social contact (INT) – a strong degree is observed in all subjects after loss of health (33.3%), respectively in women aged 30 - 45 years (8.3%) - 2 = 15.92; p <0.001; women aged 46 - 55 years (11.7%) –2 = 9.84; p <0.05; for men aged 30 - 45 years (3.4%) - 2 = 10.44, p <0.05 and for men in the age group 46 - 55 years (10.0%) - 2 = 9.45; p <0.05. Moderate to a high degree is also observed in all the study group who are experiencing separation / divorce.

Probably the bad news about the disease determines the feelings of personal inadequacy and inferiority, especially compared to others. Self-condemnation, anxiety, and noticeable discomfort in the process of interpersonal interaction complement the content of this scale. A well-developed sense of self-awareness leads to a sharp sense of loss of habitual social roles, and from there to presumed (possibly real) negative expectations and attitudes of others.

Discussion

In terms of crisis state, the experience of loss is the most difficult and complex process. The factor "Suddenness" intervenes in the scenario, which includes surprise (shock), negation and their subsequent stages, filled with high-intensity emotions that leed to the experience lonelyness, scary and hopeless. The loss of a sense of "connection to reality" is one of the main characteristics of going through this type of personal crisis. As a rule, it is accompanied by despair, loneliness, fear, anxiety, pessimistic attitude to life and others.

The most pronounced characteristics of the crisis of loss (especially of health) are loneliness, self-doubt, despair, aggression and anger towards others, hostility towards them and the feeling that others are also hostile, belittling and devaluing, which is determined by nature of the 'I' sub-structure affected by the crisis (loss of health). The main center of the "I-social" is the integrative status, the social roles, the "social mask" of the individual - therefore, the feeling of loneliness, self-doubt, anger when compared to others and expected hostility (fantastic and / or real) are most clearly represented in this type of crisis (loss of health), and in turn can serve as its indicators.

Conclusions

Loss of a loved one is a situation that anyone can face. Each person defines this event in his/her own way, giving it its own unique meaning. The main thing with this type of loss is that it is irreversible. The way that person reacts to this type of loss is influenced by a whole range of different factors: the situation of loss itself; the significance of the lost person, the place he/she occupies in the life; the presence or absence of support in this period; personality (with all personality traits); knowledge and perceptions of death.

When the grieving person realizes that the loss is real and irreversible, he may fall into a state of depression or severe sadness. The feeling of loss is acute and is accompanied by loneliness and hopelessness. The experience is intensified by remorse, regret, constant recall of the situation and analysis of what he could have done differently, to change the outcome, it leads to strong sense of guilt.

Guilt, from the whole spectrum of emotions is one of the most painful and common feelings. In situations of this type, guilt can arise from completely different sources, for different reasons. The person can find a number of reasons that make him feel guilty.

After the death of a significant loved one, when the individual realizes that the situation will never be the same as before. He feels "tangled," "stuck in a dead end," "in a maze." It takes time for the grieving person to fully realize what has happened and to feel "who he / she is now", to see the new, completely different perspective of his / her life.

Separation/divorce is one of the most common and significant phenomena in interpersonal relationships. The individual loose usual forms of connection/contact with a loved one. This suggests the negative significance of separation as a factor in forced loneliness.

Situations of this nature have the status of a crisis, as they are associated with limited and/or missing opportunities to meet the need for emotional contact - one of the basic needs of man.

In summary, it can be said that among the many and varied life events, a special place is occupied by loss. Its significance is determined by the fact that it accompanies a person throughout his life. It is not able to predict how many losses one will experience and what psychological consequences this will result.

The "vicious circle of loneliness" in situations of separation directly reflects on self-esteem and the attitude to maintain positive interpersonal relationships. Low self-esteem is a major factor in negative prognosis in interpersonal relationships. The decrease in general social activity is presented as apathy, stiffness, and feeling that others do not understand. The sufferring of separation/divorce understands the impossibility of quickly establishing new and equivalent lost relationships. This is probably the moment when the subject realizes and experiences deeply the feeling of love for the lost person. In this regard, the value of the past, a happy time of communication with a loved one, increases significantly. Here we are talking about the phenomenon of "subjective residence in the past" or "subjective turning to the past" - a person recreates the most positive moments of a broken relationship and receives a kind of satisfaction.

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Спектър на фрустрационните реакции при юноши в периода между 12-14 години

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Frustration reactions spectrum in adolescents in period between 12-14 years

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РЕЗЮМЕ:

През целия си живот индивидът постоянно е изправен пред личностни или нормативни кризи. Използването на натрупаният емоционален или рационален опит понякога позволява да се разработят адаптивни стратегии за преодоляването им. В случаите, когато това не може да бъде направено, се достига до състояние, изискващо синтез на нов адаптационен опит или развитие на Вътрешен конфликт. Настоящото изследване има за цел да изследва реакциите на подрастващите в периода на нормативна криза, в ситуации на разочарование и общото ниво на агресивни тенденции, както и наличието на връзка между реакциите на фрустрация и агресивно поведение. В периода 2018 - 2020 г. в нашата практика сме консултирали 109 подрастващи и техните родители. Информирано съгласие за включване в изследването на характеристиките и динамиката на реакциите в ситуации на разочарование е получено от техните родителите.

Ключови думи: фрустрация, юноши, агресия, криза

ABSTRACT:

Throughout his life, the individual is constantly faced with personal or normative crisis. The uses of the accumulated emotional or rational experience sometimes is able to develop adaptive strategies for overcoming it. In cases where this cannot be done, it reaches a state requiring the synthesis of a new adaptive experience or the development of Inner conflict. The present study is aimed to exam the reactions of adolescents as a period of normative crisis, in situations of frustration and the general level of aggressive tendencies, as well as the presence of a link between reactions of frustration and aggressive behavior. In the period 2018 - 2020, in our practice we have consulted 109 adolescents and their parents. Informed consent for inclusion in the study of the characteristics and dynamics of reactions in situations of frustration was obtained from the parents.

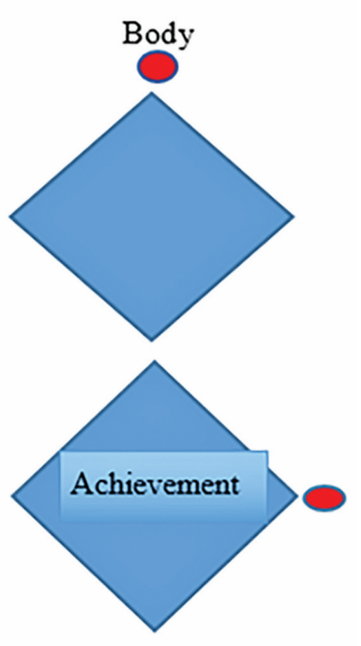
Keywords: frustration, adolescents, aggression, crisis

Introduction

Puberty is often called a crisis. During this period, are occur significant changes in the development of personality, which lead to a critical change in the thinking, behavior, emotions interests and attitudes of others. Puberty is a turning point in human development. It marks the transition from childhood to adulthood, completes one period and helps to adapt to the next. The normative crises are time of qualitative changes, the result of which is the transition to a new, higher level of development and functioning.

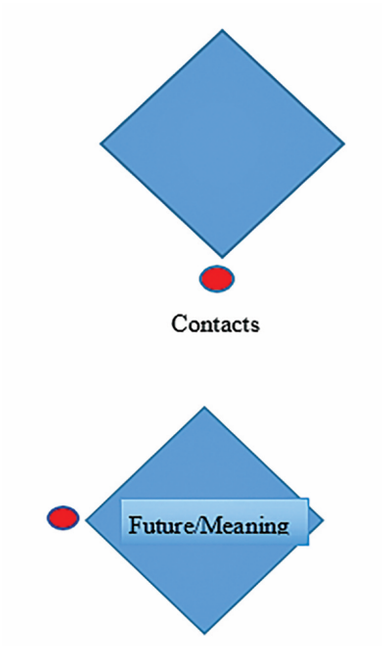
In every step of the development, the individual must face the solution of various "psychological tasks", which to pass "successfully" require a certain resource to deal with. If this happens, the individual resolves the corresponding crisis, "accumulates" new "psychological experience" and moves to a higher level of personal functioning. Adequate role models and new coping strategies have been developed (Boncheva, 2013). In each normative crisis there are: objective factors, most often changes coming from the environment or physiological processes and a subjective factor - deficit, way of surviving and resolving the crisis.

In an attempt to summarize the prezentation of what is happening to the adolescents, we focused on one of the tools of Positive and Transcultural Psychotherapy -"Balance Model" and "Conflict Processing Model", according to which the person develops himself (in abilities) and distributes the energy of its activity in four main areas: "Body" - a tool of the senses; "Activity" - a tool of logic and rationality; "Contacts" - an instrument of tradition and "Fantasy / Sence of life" - an instrument of intuition. (N. Peseshkian, 1999) (fig. 1).



Beginning of puberty, the appearance of secondary sexual characteristics, rapid growth and change in the body, sudden changes in mood, accompanied by a feeling of influx of strength and energy to helplessness.

Deficiencies: Loss of interest in previous/old activities, decreased productivity of cognitive functions and reduced performance. Striving to prove their uniqueness by any means, incl. problematic behavior and rejection of norms.
Resources: Differentiated attitude towards the learning content, expanded volume and selectivity of knowledge preferences and abilities. Development of volitional qualities: from the basic dynamic - strength, speed of reaction, through qualitative - the ability to withstand greater and longer load - endurance, perseverance, patience, to complex and differentiated volitional qualities – concentration consistency of concentration and perseverance.
The need for self-determination gives impetus to increased cognitive and creative activity - curiosity, experimenting with different activities, participation in different clubs and schools.



Deficiencies: Stubbornness, rudeness, disobedience, arbitrariness, devaluation, ignoring and/or sharp opposition to the authority of the adult. Frequent mood swings - from irritability, "explosiveness", aggression, and negativism, to tearfulness, indifference, indifference, and apathy. The relationship with the adults is strongly conflictual, with an active pursuit of separation, expressed in disregard for norms, contesting rules rejecting and not respecting restrictions, rebellion against sanctions.
Resources: Expansion of communication skills, formation and development of organizational skills, business skills, entrepreneurship, discipline, responsibility, expanding the range of strategies for dealing with conflict situations.
Contrary to the content of contact with adults, the desire for contact with peers develops - the circle of friends is the place to learn new models and practice them. In the group of friends, everyone experiences and shares the same thing. Reflection.

Deficiencies: Feeling a lack of meaning in life, fear of the future, insecurity, helplessness, hopelessness. Strong internal contradictions between desire and possibilities.
Resources: Broadening the horizons and enriching and restructuring the system of values - in the field of communication - selective attitude and evaluation of others, and self-esteem.

Figure 1. An instrument of intuition (Addapted by the Authors from N. Peseshkian).

Emotionally, puberty could be defined as a period with the most intense changes. The feelings and emotions of the adolescent are under extreme pressure and the range of polar feelings is extremely wide. The feeling of "being adult", as a specific new formation, is the key in self-awareness of adolescents - a structural and meaningful center in the young person's experiences. On the one hand, it is an expression of the new life position in relation to oneself to others and the surrounding reality, on the other hand, it determines the direction and content of his social activity and the system of new aspirations and the feelings they experience. Not all adolescents defend their sence of adulthood in the same way, and there is no "copy-paste" scheme for adolescent behavior (there are many factors related to adolescent):

- Level of their independent activity
- Knowledge
- Cognition (activity and achievement)
- Emotions, as a test of love, trust, patience, time, faith, and security
- Sphere of contacts - relationships and communication
- Family model - internal and external borders, upholding the rules and norms, the relationship between parents and especially the attitude towards children. Practice shows that children in whom the relationship with adults (parents) is based on unconditional execution, obedience, and sanctions, and not on trust, support, security and encouragement, the period of puberty crisis is much more difficult (Arabadzhiev & Tomcheva, 2021).

The most important feature of puberty is the fundamental changes in the field of self-awareness of the adolescent. It is essential for the whole subsequent development and formation of the personality. During this period, young people actively develop their own independent system of standards for self-esteem and self-attitude, and the ability to look at their own world is developing more and more. Dilova (1999) defines self-esteem as "a conscious evaluative-emotional attitude towards one's own personality". Rosenberg (1965), "evaluations that the individual makes and maintains in relation to himself and expresses

in the form of approval or disapproval." An attempt for a complete definition of the general self-assessment was proposed by Silgidjian (1978, p. 36), "a system of personal values or qualities that the person realizes as belonging to him, experiences and evaluates their significance, as well as the need to implement them in social behavior".

During the "crisis of puberty", the susceptibility of adolescents and adolescents to frustration is very strong. Young people are faced with many challenges, on the one hand there is the flourishing of creative, cognitive, and intellectual abilities, and logical approaches to solve problematic situations, on the other hand entering adulthood is accompanied by emotional instability, stress and frustrating situations related to the psychological difficulties of growing up.

The key of growing with minimal emotional damage is the formation of psychological resilience in young people, based on confidence in their own strengths and skills, ability to accept and cope with challenges, flexibility of solving problems and overcoming difficult situations. In other words, it is a matter of forming frustrating tolerance (Arabadzhiev & Tomcheva, 2021). Rosenzweig (2006) is focused on the adaptive function of frustration tolerance, understanding it as the individual's ability to experience frustration without losing psychobiological adaptations.

Method

Participants

In the period 2018 - 2020, in our practice we have consulted 109 adolescents all male at age 12-14 and their parents. Informed consent for inclusion in the study of the characteristics and dynamics of reactions in situations of frustration was obtained from the parents.

Aim of the study: To study the reactions of adolescents at age 12-14 years-old in situations of frustration and the general level of aggressive tendencies, as well as the presence of a link between reactions of frustration and aggressive behavior.

Instruments

Primary psychotherapeutic interview – 5-steps model of Method of Positive and Transcultural Psychotherapy (Peseschkian H., 2000).

Rosenzweig Picture Frustration test (Rosenzweig S., 1945; Bulgarian standardization Mechkov, 1979). This projective test, was designed to measure characteristic modes of responding to frustration, in which the respondent is presented with 24 cartoon drawings, each depicting one person saying something frustrating to the other, the second person being shown with a blank speech bubble. The respondent's task is to fill in each of the 24 blank speech bubbles with the first response that comes to mind. The score is based on nine factors, derived from combinations of three types of aggression (obstacle-dominance, ego-defense, and need-persistence) and three directions of aggression (extraggression, imaggression, and intraggression).

Aggression questionnaire (Buss and Perry, 1992). The Buss–Perry Aggression Questionnaire (BP-AQ) is a 29-item, four-factor instrument that measures physical aggression, verbal aggression, anger, and hostility.

Results:

During the first psychotherapeutic interview was found the conflict content:

- High level of anxiety *("Things in the contact with my/our son depend on me, I can't handle it - I'm helpless and that's a problem!")* - in 60.5% of the parents of young adolescents (12-14 years).
- Disappointment, dissatisfaction, discouragement *("I/we can't do it; We are supposed to be good parents, but it doesn't work! I don't understand what's going on!")* is the experiences of 47,7% of parents of young adolescents.
- Outrage, irritation, accusations *("He/she is not what we expected! "He/she behaves elementary and none of our efforts work!)* showed 21.1% of the parents of young adolescents.
- Insult, aggression *("I/we give him / her everything that a good parent is supposed to, and we expect to get good behavior! "He/she tries to overcome with his/her behavior, but it*

will not happen - with punishments and restrictions we will "cure" his/her stubbornness!) is observed in 15.5% of parents of young adolescents.

The content of the problems they share could be conditionally divided into several main groups: Relationships with adults; peer relationships; problems at school; dissatisfaction with oneself and dissatisfaction with others (Table 1).

To track the age specifics of frustration reactions in young adolescents, we used the Rosenzweig Picture - Frustration Test. The reliability of the results (Cronbach's Alpha) - 0.77 in the age group 12 - 14 years. This group showed the highest values in external accusatory reactions to deal with frustration (category "E"). Regarding the object to which the reaction is directed, the highest values are observed in the reactions fixed to self-defense (category "ED"). (Table 3). Compared to the normative range (Mechkov, 1979) the reactions of the group are normal (Code 3).

With Rosenzweig's *Picture Frustration test* in 61.3% of 12 - 14-year-old was found, anxiety, tendency to rely on rigid stereotypes of activity and inability to assess the situation. Adolescents are fixated on the conflict as an event/obstacle (factor E – extrapunitive responses) and through vulnerability, a desire to impose themselves and "have a say" try to attract attention to themselves. In frustrating situation, 58.0% of young adolescents, tend to react with acute rejection of what is happening, a desire to subordinate reality to their needs, striving for dominance and intolerance to "Foreign will" (factor E - extrapunitive responses). The other factor with a greater emphasis on young adolescents is the impulsive response to circumstances (factor M – impunitive responses) 61.1% of 12–14-year-old react with carelessness, frivolity, irresponsibility and/or underestimate or underestimate the situation. Such behavior is subject to

Table 1. Problems through the perspective of young adolescents.

Problems	Age group 12 – 14-years old
Relationships with adults (parents and significant adults)	Conflicts with parents: "They forbid me to go out!"; "They don't like my friends and we have fights over them!"; "They threaten me with punishment!"; "They constantly scold me and insult me!" "They accuse me of being disobedient, lazy and irresponsible!"; "They want things to happen just their way - They don't understand me!"; "I have no right to want or to do anything – because I am young!"; "They don't buy what I want, but what they have decided!"; "They don't love me!"
Relationships with peers	Rejection, isolation, harassment, aggression, ridicule
Problems at school	Lack of interest and resistance - "My parents chose the school, let them study!"; bad grades - "They deliberately give me bad grades!"; punishments; Rebellion against the rules: "The rules are designed to be broken!"
Dissatisfaction with themselves	Body; appearance; abilities
Dissatisfaction with others	Others are evil, dissatisfied, vindictive, hate me, insult me and gossip.

Table 2. Values of the mean in the group of 12-14 years.

Age		E	I	M	OD	ED	NP
12 – 14 years	Mean	11,17	4,833	7,684	5,99	10,92	6,77
	N	57	57	57	57	57	57
	Std. Deviation	3,853	1,9419	2,9725	2,304	2,904	2,591

emotional breakdowns, and frequent change of wall and asthenic states. In the characteristic of frustration reactions in adolescents (12-14 years) with a strong statistical significance (39.9%) proved factor "e", which on the one hand is an indicator of claims and expectations towards the other to remove frustration, on the other hand means activity, tendency to delegate responsibility and leadership. In the examined young people, the factor "e" shows significance in combination with the factors in:

- "I" (intropunitive responses) – a sign of self-criticism, focus on one's own inferiority, sense of guilt, remorse, sometimes self-blame and self-discreditation with the characteristic behavior of politeness and irrational conformism.
- "M" (impunitive responses) – tendency to be indifferent in situations of frustration, devaluation or demonstrated indifference, which is probably the selective use of psychological defense „reaction's formation" to deal with the fear of new frustration or to contain a repressed aggressive impulse built in the adolescent's perceptions of subjective unacceptability of aggressive behavior – 53.7% in young adolescents.

Buss and Perry Aggression Questionnaire (AQ) Results:

The questionnaire reliability (Cronbach's Alpha) for the individual subscales varies between 0.73 and 0.79, reaching 0.77 for the overall score of aggression. The average values of the studied variables are higher in the group and found in all components of aggression, except for the scale "Hostility". (Table 3).

12–14-year-olds show a willingness to use these two forms of aggression to achieve their own. Young adolescents openly show their irritability, dissatisfaction, anger, and irritability. Still limited cognitive abilities affect the meaning of behavior, and the lack of tolerance in achieving the desired and the strength of the impulse that guides the actions of adolescents gives aggression a more protective character. Limited self-control and the emerging self-esteem, expressed mainly in sensitivity to negative evaluation and the accompanying emotions and experiences, predispose to aggressive actions.

The low value on the scale "Hostility" is an indicator that the actions of young adolescents are not determined by prolonged and persistent negative attitudes towards the surrounding reality (people and events), but rather the result of an emotional state of provocative nature, such as anger.

Hostility is an antagonistic attitude towards people, which includes a cognitive, affective, and behavioral component. The affective component is represented by several interconnected emotions such as: anger, irritation, resentment, disgust, contempt, and others. The cognitive component contains negative beliefs about the world and others - mistrust, suspicion, contempt, prejudice and cynicism, behavioral component includes a diverse repertoire of actions, most often hidden - passive-aggressive actions, unwillingness to cooperate and compromise, avoid contact (communication), cold attitude towards others, etc. (Barrett et al., 2007).

Passive - aggressive behavior is, perhaps the worst way to show anger - as opposed to the open and spontaneous way, to strongly

Table 3. Average value for the components of aggression

Age		OA	FA	VA	A	H
12 -14	Mean	98,74	34,49	22,77	22,89	18,84
	N	57	57	57	57	57
	Std.	13,915	6,596	6,921	5,554	3,895

relieve the tension that usually follows the trajectory: dissatisfaction - irritation - anger - rage. The adolescent and the young adults are not yet able to understand his insidious and destructive ability, and they do not realize that their resistance and perseverance prevent the imprisoned anger from being released. Examples of such behavior are procrastination, stubbornness, suspicion, resentment, anger, deliberate "inability" to make the expected or repeated failure to perform the required tasks. On a conscious level, the young man believes that in this rank he defends himself and "lets them understand." On an unconscious level, such behavior is aimed at infuriating and / or upsetting authorities (parents, teachers, educators).

"There is no smoke without fire!" - what is the spark that ignites the aggressive behavior of a growing person?

Discussion

The summary information from the primary psychotherapeutic interview shows that to establish himself in his new social position, the young man tries to go beyond his current style of contacts. His efforts are focused on finding ways to realize his "growing" opportunities, the pursuit of autonomy and independence (experienced as freedom), to develop his individuality and to receive recognition from adults, whose model he repeats, and to whom he wants to show his readiness to take a place in the "world of the great."

The sphere of communication with peers is very emotionally charged. And if in the second normative crisis (of the first grader) the successful outcome is the good contact with just one person – "My friend! Peace in the group" (Bontcheva, p.101), then in the crisis of puberty the leading motive in the behavior of the young man is to consolidate his place and to establish himself in the group of peers. Friendship during this period is complex and controversial, and friends are a source of social and emotional significance. The assessment that he expects and receives from his peers acquires paramount importance and displaces from the adolescent's field of vision the relationship with the significant adult, but the content of the contact retains its strong emotional charge. The inner struggle of the growing person is, on the one hand, a strong desire for autonomy, independence, and freedom, and on the other hand the need to feel and receive love, patience, attention, trust and time (primary current abilities) to feel secure and stable and to experiences himself as significant and valuable.

This is a "cornerstone" in the contact between parents and young adolescents. Shared problems show where the discrepancy is - parents have expectations for success, achievement, order, accuracy, courtesy, discipline, responsibility (secondary current abilities), and the young adolescent and adolescents have needs for support, help, cooperation and partnership. Parents demand and blame, and /or helplessly withdraw from active contact with their children, while adolescents either protest violently or remain grimly silent and act in their own way.

Unmet needs cause frustration. The rapid pace of physical and cognitive development leads to the formation of new needs, and the narrowed psychological horizon of "Here and now!" determines the framework in which the adolescent insists on getting what he wants. Based on the psychological features of the period of crisis of puberty, it is evident that adolescents, due to their vulnerability and

not-strengthened self-image, choose demonstrative behavior - from open aggression, active-offensive position, striving for dominance and intolerance to requirements, to impulsiveness and poorly judged action decisions. Successful self-defense in their experience is the activity through accusations, demands / expectations of the other to take responsibility for what is happening, rejecting and denying their own guilt and / or participation trying to "equalize forces".

Conclusions

1. Ego-protective type of reactions dominates, followed by the reactions fixed to the satisfaction of needs.

2. Anxiety, tendency to rely on rigid stereotypes of activity and inability to assess the situation are found. Young adolescents fixate on the conflict as an event / obstacle and through vulnerability, a desire to impose themselves and "have a say" try to attract attention to themselves. Young adolescents tend to react with acute rejection of what is happening, a desire to subordinate reality to their needs, striving for dominance and intolerance to "Foreign will"

3. Young people in this group are focused on their inner world and believe that the world should be what they want it to be. They lack enough experience to judge and accept opinions that differ from their own. They try to hide the uncertainty in their abilities and skills, relying on protective mechanisms.

4. The crisis of puberty is characterized by the fact that the adolescent acquires new opportunities and strengths that were previously absent or were a process of development. Moving to a higher stage of development, the young person feels that he already has much more strength, independence and will to solve the problems that until recently were solved by adults.

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Скрининг за ранна диагностика на хипофарингеален плоскоклетъчен карцином

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Sceening for an early diagnosis of hypopharyngeal squamous cell carcinoma

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РЕЗЮМЕ:

Ранната диагностика на рака на хипофаринкса, води до по-големи шансове за излекуване на заболяването и повишаване преживяемостта при това заболяване. Към настоящият момент няма разработен и предложен скрининг за рак на хипофаринска. Целта на предлаганият от нас скрининг за ранна диагностика на рака на хипофаринска, е за постигане на следните резултати: а/ Да се намали броя на хората, които да развият рак на хипофаринска; б/ Да се намали броя на хората, които умират от това заболяване; в/Да се намерят хората н риск, които имат нужда от скрининг по-често, спрямо хората с нисък риск;

Водещ принцип в нашата стратегия за превенция и скрининг за рак на хипофаринска, е насочен към справяне с предотвратимите рискове за рак на хипофаринска.

Ключови думи: хипофарингеален карцином, скрининг, превенция, рискови фактори, ранна диагностика.

ABSTRACT:

The early diagnosis of squamous cell hypopharyngeal carcinoma leads to greater, or at least better results, concirning the survival and treatment itself. Up to the moment there is not a valid such screening method for that cancer. So, the aim of the suggested by us is as follows:

- to reduce the number of the people with potentially hypopharyngeal cancer;
- to reduce the number of the people, dying from that disease;
- to find out people with higher risk from getting such illness, in comparision to people with lower risk.

Key words: hypopharyngeal cancer, screening, prevention, risk factors.

Introduction:

The screening methods are used for an early diagnosis of an oncological disease, just before the first symptoms to appear. Meanwhile all over the World the scientific researches lead to develop many tests, later being used as a screening method for a certain type of cancer. Unfortunately, up to the moment there is not such a helpfull screaning one for the hypopharyngeal cancer. Still now, that process is being diagnosted in a rather advanced stage - III, or IV, and very, very rarely - at the beginning - Ist and IInd. That cancer usually makes rapid methastases, after a surgical treatment carries away bad prognosis and low survival rate. The screening test, suggested by us, is a measurement, giving a chance to identify the people,exposed being on a high hypopharybgeal cancer risk.

The aim of the suggested by us screening for an early diagnosis of the hypopharyngeal carcinoma is to gain the following results:

1. To reduce the number of the people, later performing such cancer;

2. To reduce the number of the people, dying from that;

3. To find out people with high risk, needing such screening procedure, in comparision to the people with a lower risk.

The acknowledge of the people about the screening role,early diagnosis process,ability and willingness to cure o.s., as well as the clinical discoveries - all these features do play an important role in the strategy for making better results in the whole diagnostic process of the hypopharyngeal carcinoma.

Our leading principle in the strategy towards prevention and screening of hypopharyngeal cancer is declared towards overcoming the potential and curative risks of the cancer. Tobacco-smoking comes to be the most serious, but on the other hand - a curative risk for that cancer.So, stopping smoking makes the prevention from cancer valid and possible. Here come the other curative risks - such as too much alcohol drinking and smoked food consuming. Something more- thinking and speaking about healthy way of living mean a mixture between the following:

- feeding with rich in quality food;

no smoking at all, as well as alcohol drinking;

- an active sports facilities and respectively-activities for any age;

In order to gain that healthy way of life, we intend to inform the EU - institutions for receiving a psychological support for the population of Bulgaria, thus the final result will be accomplishing the already mentioned priorities.

In conclusion we may say, that the screening of an early diagnosis for a hypopharyngeal cancer comes to be an attractive strategy in increasing the percentage of the successfully survived people.

A patient's screening pathway for an early diagnosted hypopharyngeal carcinoma:

The following document is filled up by the physician in order to be an useful one, during the observation period of time for any of the followed-up patients with predominant oncological - hypopharyngeal disease:

- Screening N of the patient.....
- Date of examination.....
- Name of the patient.....
- Date of birth.....

I. A principle model for screening of people with high risk for a hypopharyngeal carcinoma, using an individual pathway-card for examination and estimation of the indexes during the follow-up period of time:

1. Tobacco smoking for a long period of time-both active and passive:

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2. Daily consuming of too much alcohol:

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3.Age over 55:

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4. Sircumstances from genetic point of view. A presence of malignant illness in that same localization in the family members:

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5. Sircumstances from professional point of view- azbest exposion, as well as other chemical substances-inhalating,working in a coal - mining basin, etc.

6. Specific treatment of any other malignant head and neck disorders:

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7. Any specific invasions by Ebstein - Bar virus:

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8. Any specific invasions by HPV:.

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9. A presence of steady ill teeth in the oral cavity:

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10. A presence of chronic inflammatory processes in the upper respiratory tract - sinuitis, pharyngitis,stomatitis aphtosa, tonsillitis chronica, reccurent oral cavity pains:

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11. Any habitats in consuming specific food - like smoked ones, or others:

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12. Are there any cicatrices in the oral cavity - as a result of active intoxications, leading to a local immune regression, or as a result or an influence of virus chronic activities:

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II. Symptoms:

1. A presence of enlarged neck lymph nodes, or a node. This is one of the first symptoms, accompaning a hypopharyngeal cancer - to be seen in more than 50% of the patients:

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2. A presence of local oral cavity inflammatory process, lasting more than it should be, more and more, after a treatment:

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3. A presence of pain, going from the oral cavity towards the ear:

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A pattern for an early diagnosis of a hypopharyngeal carcinoma:

1. A hystorical evidence of the illness, supported by a local ENT-status with byopsy, predominantly:

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2. Hypopharyngoscopy, if needed- a bronchoscopy:

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3. X-rays of lungs and chest:

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4. Computer Tomography, or IMR of the primary tumour:

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5. Computer Tomography or IMR of the upper mediastinum:

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6. Ultra-sound examination of the abdomen:

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7. An appropriate teeth status, with treatment, if possible:

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8. A sternum punction for declaring the stage of the lymphoms and the G2 and G3 squamous cell carcinomas:

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9. Any kind of laboratory measurements, like: peripheral and biochemical substances in the blood, beta 2 microglobulins, etc.

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10. Laboratory examination of urine:

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Conclusions:

1. The screening method for an early diagnosis of a hypopharyngeal carcinoma supports the physicians in finding out it in an early stage.

2. The early diagnosis helps and leads to greater chances in the treatment itself, thus- to longer survival.

3. As far as the risk in the screening are concirned, they are the following:

- false positive data - sometimes the screening may show a presence of tumour formation, afterwords it proves to be a benignent one;

- hyperdiagnosis - sometimes unneccessary examinations and measurements may be prescribed. Usualy they go together with false positive results;

- false negative results: sometimes the screening methods reveal no presence of a malignant tumour.As a reult-the patient is not going to be treated, the way he (she) should be, thus this leads to a later precise diagnosis, lower survival, etc.

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Нео-адювантната химио-терапия при авансирани хипофарингеални плоскоклетъчни карциноми и спасителни мерки при рецидиви на тумора

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Neo-adjuvant chemo-therapy, performed in advanced hypopharyngeal squamous cell carcinoma , helpful deeds in recurrent cases

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РЕЗЮМЕ:

Изследвахме ролята на нео-адювантната химиотерапия за възможен частичен или пълен клиничен отговор за последващ оперативен подход при авансирани плоскоклетъчни карциноми. Нео-адювантната химио-терапия е изпълнена с:- Platinum † Taxane или с трите препарата:- Platinum † Taxane † 5-Flurouracil (5 FU), в три седмичен режим.От общия брой 59 изследвани пациенти, в IVa клиничен стадий бяха 69% от случаите, в IVb клиничен стадий 21% от случаите и 10% от случаите в III-ти клиничен стадий. В резултат на проведената нео-адювантна химиотерапия и резултатен клиничен отговор, се извършиха хирургически интервенции при 10 пациента /

ABSTRACT:

We checked the role of neo-adjuvant chemo-therapy as far as the whole curative process of that disease is concerned – generally, or partially – from the effect point of view, as well as the need of following operative approach in advanced hypopharyngeal squamous cell carcinoma. The neo-adjuvant chemotherapy is done by: C-platinum, Taxane, or the three medicaments – Platinum, Taxane, 5-Fluroruracil (5FU) – all in a period of 3 weeks. The total number of the patients was 59 , among them 69% were in IV a clinical stage, 21 % - IV b,10% - in III clinical stage. The clinical result after these procedures is as it follows – summarised clinical answer - in 66% of the patients, among

33.3% от случаите/ от общо подлежащите 33 пациента за оперативно лечение. Органосъхраняващи операции се извършиха при 19 от пациентите / 57.5% от случаите/. Нео-адювантната химиотерапия е полезна процедура за приложение при авансирани хипофарингеални плоскоклетъчни карциноми.

Ключови думи: хипофарингеален карцином, химиотерапия, рецидиви, нео-адювантна химиотерапия, спасителни мерки.

which – 60% - partial clinical response, and 6% - general, full one . As a result of all that, surgical interventions were performed in 10 patients (33.3)% from all 33 people, suspected for an operation at all . Organic-saved operations have been done in 19 people (57.5%). The final result is that the neo-adjuvant chemo-therapy is a helpful procedure in advanced hypopharyngeal squamous cell carcinoma .The mean period of time, registered by us for a disease – recurrence is 10.3 months (a wide interval – 2.1 – up to 61 months). We checked that 79 % of the patients do survive 3 years period of time – after surgical operations and chemo-radio-therapy. We did not fell upon a person with 3 years survival after a chemo-radio-therapy, only .

Key words: hypopharyngeal squamous cell carcinoma, chemotherapy, recurrences, cumulative survival, neo-adjuvant chemo-therapy, helpful deeds.

Introduction:

We made a thorough retrospective and prospective follow-up of 59 patients, not well treated, being with advanced carcinoma – during the period 2010-2020 – in Complex Oncological Centre – Plovdiv – for the sake of estimating the role of neo-adjuvant chemo-therapy in order to achieve a full, or partial clinical answer, to perform a certain operative treatment, as well, or not.

As far as the good results of all these helpful deeds are concerned, among people with recurrences, we checked precisely 49 patients, treated for that in COC – Plovdiv, during the same period – 2010-2020. Among all these people, 45% could be connected with some helpful procedures on a recurrence base. According to the types of the recurrences – 85 % of all the cases are local ones, 23 % - regional, in 19 % - distant recurrences.

Material and methods:

We made a thorough retrospective and prospective follow-up of 59 patients, not well treated, being with advanced carcinoma, during the period 2010-2020 in COC- Plovdiv. The aim of the study was to determine the role of neo-adjuvant chemo-therapy in achieving full or at least partial clinical answer for a following surgical treatment in advanced hypopharyngeal squamous cell carcinoma. That neo-adjuvant therapy is done with the substances – C-Platinum, Taxane, or the 3 of them- Platinum, Taxane and 5- Flurouracil (5FU), for a period of 3 weeks. For estimating the good results of all these helpful deeds, we checked precisely 49 patients with recurrences of hypopharyngeal squamous cell carcinoma. So, we calculated the mean 3 years survival rate of people with performed already operative treatment, together with chemo-therapy, or 3 years survival rate for people with performed chemo-therapy, only.

Results:

We made a follow-up examination of 59 patients, not well treated,with advanced hypopharyngeal carcinoma, during the period 2010-2020 in COC – Plovdiv. The aim of the study is to determine the role of the neo-adjuvant chemo-therapy in accordance with a future full or at least partial clinical effect, as

well as forwarding operation – in advanced hypopharyngeal squamous cell carcinoma.

Thus, the neo-adjuvant chemo-therapy is performed the role of the neo-adjuvant chemo-therapy in accordance with a future full or at least partial clinical effect, as well as forwarding operation – in advanced hypopharyngeal squamous cell carcinoma.

Thus, the neo-adjuvant chemo-therapy is performed by the substances: C-Platinum, Taxane, 5-Flurouracil (5FU)- for a period of 3 weeks. The mean age of the patients is 55 years. In most of the patients with advanced hypopharyngeal carcinoma, the tumour itself engages the structure, called „sinus pyriformis". In our study hat concerns 83 % of the examined people. In accordance to the clinical stage, the situation is as it follows :69% - belonging to IV a, 21% - to IV b, 10% - to III clinical stage. (table 1)

Finally, after performing the neo-adjuvant chemotherapy, the whole summarised clinical answer was to be seen in 66% of the cases, among which – 6% - with full clinical response from the treatment, and 60% - with partial one. As a result of that full response, forwarding operative treatments have been performed later – in 10 people (33,3%) from all 33 patients, suitable at all. Organic-saved operations have been done in 19 people (57.5%) of all, suitable for that.

Conclusion:

The neo-adjuvant chemo-therapy could be a helpful procedure in advanced hypopharyngeal squamous cell carcinoma.

Nevermind the progress, registered in last years in both types of treatment – operative and radio-chemo-therapy, this kind of cancer

Table 1: Neo-adjuvant chemo-therapy in 59 patients with advanced hypopharyngeal squamous cell carcinoma – according to the clinical answer and operative possibilities:

number	Hypopharyngeal squamous cell carcinoma	Indexes	
		cases	%
1.	Clinical stage :		
	- Total number	58	100
	- In III clinical stage	6	10
	- In IV a clinical stage	41	69
	- In IV b clinical stage	12	21
2.	Neo-adjuvant chemo-therapy :		
	Total summarised answer	39	66
	Among them :		
	- Partial clinical answer	35	60
	- Full clinical answer	4	6
3.	Performed operative treatment – after the chemo-therapy :		
	- Total number of patients , suitable for an operation	33	100
	- Performed operative treatment	10	33.3
	- Performed organic-saved operations	19	57.5

Table 2: Helpful procedures, performed in recurrent hypopharyngeal carcinoma, following primary surgical and chemo-radio-therapy in 49 patients – COC- Plovdiv, 2010-2020.

number	Efficiency and results	Indexes
		months %
1.	Mean time for declaring a recurrences in the process of treatment for hypopharyngeal carcinoma	10.3 (2.1 – 61.1)
2.	According to the recurrences :	
	-local-regional	- 100%
	-regional	- 23%
	-distant	- 19%
	-local	- 85%

Table 3: Cumulative survival in patients with hypopharyngeal carcinoma, being performed a primary operative treatment and chemo-radio-therapy.

number	Mean survival	1 year %	3 years %	Interval
1.	Mean survival in patients with performed operative treatment and chemo-radio-therapy	96%	79%	16.61 28.30
2.	Mean survival in patients with performed only chemo-radio-therapy	0	0	-

remains still with some of the worse and bad prognosis from all types of tumours in head and neck.

The treatment of the both types of the disease – primary one, and the recurrences,do play an important role over the survival period of time in patients. Our purpose was to determine the efficiency of the helpful procedures in recurrent cases, after the primary treatment been done.

We made a thorough follow –up of 49 patients, treated in COC-Plovdiv – 2010-2020. The mean time for diagnostic a recurrence from the beginning of the treatment is 10.3 months. The whole activities in reccurences was able in 45% of the patients. (table2)

According to the recurrences themselves, they are : local ones – in 85% of the cases, local-regional – in 100%, in all; regional – in 23 %, distant metastases – in 19 %.

We found out a certain cumulative survival – with non-activity tumour, as it follows : (table 3)

The results show, that patients with performed both operative and chemo-radio-therapy, in 96 % have1 year survival period of time, while 3 years may reach 79 %. We did not fell upon a person with 3 years survival period of time with performed only chemo-radio-therapy.

Conclusions:

1. Most of the patients with advanced hypopharyngeal carcinoma are to be seen in IV a clinical stage (69%), 21% - in IV b, 10% - in III clinical stage.

2. As a result of the performed neo-adju-

vant therapy, the whole summarised answer is in 66 % of the cases, among which – 60% - partial, 6% - full one, after the chemo-therapy with C-Platinum, Taxane, or with 5FU, also.

3. As a whole result of all that, surgical interventions have been performed in 30% of the patients, and organic- saved operations – in 57.5%.

4. The neo-adjuvant chemo-therapy could be a helpful procedure in patients with advanced hypopharyngeal carcinoma, especially for a next operative one.

5. We found out that the mean time for declaring a recurrence – from the very beginning, is 10.3 months.

6. The whole capacity of the helpful deeds in recurrences is to be seen in 45% of the explored by us people.

7. The mean 3 years survival period of time in patients with performed both – operative and chemo-therapy, is 79% of the cases.

8. We did not fell upon a person with 3 years survival after performing only a chemo-radio-therapy.

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Изисквания към авторите

Списание Българска медицина е издание на Българската академия на науките и изкуствата (БАНИ), Отделение наука, Научен център по медицина и здравеопазване. Излиза 4 книжки годишно. Списанието е достъпно електронно на сайта на БАНИ, раздел издания.

В списание „Българска медицина" се отпечатват оригинални статии, казуистични съобщения, обзори рецензии и съобщения за проведени или предстоящи научни форуми – симпозиуми, конгреси, или други в областта на клиничната и фундаментална медицина. Списанието излиза на английски език с подробни резюмета на английски и български език. Изключения се правят за обзорни статии по особено значими теми. Заглавието, авторските колективи и надписите и означенията на илюстрациите се отпечатват и на двата езика.

Материалите трябва да се представят в два еднакви екземпляра, на шрифт Times New Roman, размер 12, разстояние между редовете 1.5 линии. Обемът на всяка статия е до 10 страници, 12 страници за обзорните статии и 3-4 страници за казуистичните съобщения. Библиографията и илюстрациите са включени в този обем. За информация за научни прояви обемът е до 4 страници, за рецензии на книги – до 2 страници. В този обем не се включват резюметата на английски и български език, чийто обем трябва да бъде до 200 думи с 3-5 ключови думи. Резюметата трябва да отразяват конкретната работна хипотеза, използваните методи, получените резултати и заключение.

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Тези изисквания не важат за другите научни публикации (обзори, казуистика или съобщения и рецензии).

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За списание: автори, заглавие на статията, списание, година, том, страници от..до.

За книга: автори, заглавие на главата, В: заглавие на книгата, в скоби редактори, издателство, година, страници от…до.

Адрес за кореспонденция се дава в края на всяка статия и съдържа данните на кореспондиращия автор, включително адреса на електронната му поща. Всички ръкописи се изпращат с придружително писмо подписано от авторите, в което се отбелязва, че тя не е била предлагана на друго списание и не е отпечатвана у нас или в чужбина. Ръкописите не се връщат.

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Задължения на рецензентите: Рецензентите подпомагат редактора при вземане на решение, като те могат да подпомогнат автора за повишаване качеството на статията. Всички ръкописи, получени за рецензиране следва да се считат за поверителен материал и тяхното съдържание не следва да се разкрива пред никого, освен с разрешението на редактора. Рецензентите следва да се придържат към обективните стандарти за оценка. Лични нападки срещу авторите са неприемливи. Критичните забележки следва да бъдат подкрепени с аргументи.

Конфликт на интереси: Непубликувани материали не могат да бъдат използвани в собствени изследвания на редактора без изричното писмено съгласие на авторите. Авторите следва да обяват всички финансови или други съществени конфликти на интереси, които могат да окажат влияние върху интерпретацията на техните резултати. Всички източници на финансиране на проведените проучвания следва да бъдат обявени.

Етически съображения по отношение на самите изследвания: всички трудове, които отразяват клинични проучвания следва да имат подписано информирано съгласие от пациентите и получено разрешение за изследването от съответната Етична комисия в съответствие с Декларацията от Хелзинския комитет. Експерименталните проучвания следва да имат също разрешение от съответната Етична комисия и от Българската Агенция по безопасност на храните и лекарствата.

След положителна рецензия и одобрение на редколегията, авторите на статията дължат заплащане в размер 10 лв на страница на статията си, с оглед покриване на разноските по английската езикова редакция на текста и коректури.

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EXAMPLES:

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McLachan S, MF Prunel, B. Rappoport. Cell mediated humoral immunity. J. Clin. Endorcinol, Metab., 2011, 78(4): 1071-82.

References to a book chapter:

Delange F, Endematic Cretenism. In: The thyroid (Eds. L. Braveman and R. Utiger). Lippincot Co, Philadelhia, 2001, 942-955.

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