

MODERN VIEW ON EPIDERMAL DYSPLASIA CARCINOGENESIS

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Squamous cell carcinoma of the skin develops from the spectrum of facultative precancerous conditions, which in the course of malignant transformation through cancer stage *in situ* without early treatment fully transform into invasive squamous cell carcinoma. According to classical model of carcinogenesis, the transformation of actinic keratosis into squamous cell carcinoma of the skin occurs due to a mutation in one gene, more often a tumor suppressor, and undergoes a stage of development with lack of control of cell cycle. The *aim* of the research is to supplement current knowledge of genetic determination of pathogenetic mechanisms of epidermal dysplasia of the skin by studying the genetic determinant in the skin lesion of varying degrees of malignancy. *Materials and Methods:* We analyzed 85 skin biotates of patients with epidermal dysplasia of the skin (Gr 1 — 43 patients with actinic keratosis; Gr 2 — 21 patients with non-invasive squamous cell carcinoma of the skin; Gr 3 — 21 patients with invasive squamous cell carcinoma of the skin) by molecular genetic testing of gene polymorphisms: *TP53* (G13494A), *L-myc* (T3109G), *TNF-α* (G308A) in tumor tissue. The histological examination revealed the levels of dysplasia of the epidermis. *Results:* In case of the same disease duration in patients of Gr1/Gr3, *L-myc* (3109TT) is a genetic component of malignant transformation of epithelial skin cells ($p = 0.004$) and the development of invasive squamous cell carcinoma. Other variants of 3109TG and 3109GG genes do not have such prognostic value for the risk of skin cancer compared to 3109TT. Significant differences were found in the distribution of *TP53* (13494GA) when comparing Gr 1 with Gr 3 ($p = 0.014$) and Gr 2 with Gr 3 ($p = 0.038$). A significant increase in the distribution of 13494GA genotype was revealed in patients with invasive form of keratinocyte intraepidermal neoplasia. 13494A allele was more likely to be detected in patients of Gr 3 compared to Gr 2 ($p = 0.030$) that proves the association of this allele with the development of invasive malignancies of the skin. The association of 308GG genotype and *TNF-α* (308G) allele with the development of malignant skin lesions was found. Comparing the distribution of 308G allele in patients of Gr 1 and Gr 2, we found its significant increase in patients of Gr 1. Comparative analysis of gene polymorphism with tumor invasion level showed a significant difference only in 308GG genotype between patients with grade III of KIN (keratinocyte intraepidermal neoplasia) in Gr 2 and patients with KIN III of Gr 1 ($p = 0.007$), and 308GA between patients with KIN III of Gr 2 and KIN III of Gr 1 ($p = 0.027$). *Conclusions.* Our work has supplemented modern vision of genetic component in pathogenetic mechanism of the development of epidermal dysplasia of the skin. Thus, the association of *L-myc* (3109TT) with the development of malignant skin lesions of different invasiveness and the modifying effect of *TNF-α* (G308A) and *TP53* (G13494A) gene variants on pathological transformation in the focus of EDS depending on the level of epithelial dysplasia was revealed.

Key Words: epidermal dysplasia of the skin, squamous cell carcinoma of the skin, actinic keratosis, polymorphism of genes.

The effect of solar ultraviolet radiation (UVR) is considered to be a significant cause of cutaneous squamous cell carcinoma (cSCC) of the skin [1, 2]. The risk of cSCC is significantly higher among people with light skin who live in regions with high UVR levels and are exposed to the sun for many hours per day [1]. In addition, the aging of the population and a stronger recreational impact of UVR have significantly increased the incidence of cSCC [3]. Among the diseases that lead to an increased risk of cSCC are actinic keratosis (AK) and intraepidermal carcinoma (squamous cell carcinoma *in situ* — SCCis). AK, SCCis, cSCC refer to epidermal dysplasia of the skin (EDS).

It is estimated that 65% of cSCC foci occur at the site of the precursor — AK [4]. AK is associated with epidermal atypia and is thought to represent a continuum of progression from dysplastic keratinocytes to cSCC [5].

The prevalence of AK ranges from 18% of the UK population over 60 and reaches 64% of the population in Australia [6, 7]. AK is considered to be a risk factor for the development of cSCC, and is often present as multiple skin damage in the form of “field cancerization”.

The rate of AK progression to cSCC is estimated to be between 0.025% and 16% for single lesion per year [8, 9]. The typical patient has from 6 to 8 lesions; therefore, a patient with multiple AK has an annual risk of developing cSCC in the range of 0.15% to 80% [3, 8, 10, 11]. The exact rate of AK progression is unknown, however, a prospective study in the United States indicated a risk of progression for a single lesion of 2.57% over 4 years [4, 12]. This wide range of risk reflects the lack of accuracy of available knowledge about the progression of carcinoma in the epidermis.

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Abbreviations used: AK — actinic keratosis; cSCC — cutaneous squamous cell carcinoma; EDS — epidermal dysplasia of skin; FGFR2 — fibroblast growth factor receptor 2; HPV — human papillomavirus; KIN — keratinocyte intraepidermal neoplasia; MMP — matrix metalloproteinase; PDGF-C — platelet-derived growth factor C; SCCis — squamous cell carcinoma *in situ*; UVR — ultraviolet radiation.

The progression of AK to cSCC is also proved by genetic studies that report about AK karyotypic profile similar to cSCC but with a lower degree of complexity corresponding to an earlier stage of tumor development [13].

Despite the high prevalence of cSCC, the molecular mechanisms of tumor pathogenesis are poorly understood, especially the changes involved in the progression of AK to cSCC. Earlier studies have demonstrated significant karyotypic complexity of cSCC with frequent changes in chromosomes 3q, 8q, 20q, 3p, 4p, 9p, 13q, 17p, and 17q [13–18]. The latest level of exon sequencing of cSCC indicates a huge mutational burden in about 30,000 base pairs of the coding sequence [19]. This makes cSCC the most mutational human malignancy, complicating the definition of the “leading” molecular events underlying its development.

Current scientific evidence supports the important role of *TP53* and *NOTCH* tumor suppressor, but the contribution of additional genes and the pathways of malignant progression are not fully understood [19, 20].

It is believed that AK is a precancerous stage in the genesis of cSCC, and can characterize the processes related to the progression of skin carcinogenesis. Many previous studies on AK progression to cSCC have identified several genetically determined progression pathways of the two diseases [21–24].

It is known that, similar to other cancer cases, cSCC has genomic instability in keratinocytes as a result of UVR induced inactivation of p53, since approximately 58% of cSCCs contain UVB mutations such as CC → TT and C → T transitions [25–27].

UVR induces apoptosis in keratinocytes, but additional inactivation of *TP53* is provided by keratinocytes resistant to apoptosis and, as a consequence, uncontrolled proliferation, which is an early pathogenetic event in AK progression to SCCis and cSCC [5, 28–30].

Chronic exposure to UVR and epidermal cells damage lead to the activation of inflammatory pathways such as NFκB, COX-2 release, immunosuppression due to the changes in T-cell subsets as a result of cytokine imbalance [31, 32].

The loss of *NOTCH1* function as a result of the mutation is considered to be an early event in the progression of cSCC [33]. The Ras mutation, especially H-Ras, is also one of the key oncogenes in the development of cSCC [28, 34, 35]. However, the Ras mutation alone is not sufficient for the malignant transformation of keratinocytes [5]. In addition, c-myc, bcl-2, STAT-3, p63-FGFR2, ROS-induced PI3K/AKT-mTOR, Wnt/β-catenin, Shh/Gli1-3 and TGF-β-linked and PDGF-C pathways are other known signaling pathways involved in the progression of cSCC [5, 36].

In addition to UVR, there are other risk factors for the development of cSCC, such as chronic ulcers, immunosuppression, and HPV infection [37]. Moreover, chronic inflammation and activation of inflammatory

processes are associated with potential cSCC and AK progression to SCCis [5].

Although the prognosis of primary cSCC is favorable in most cases (5-year treatment efficiency of approximately 90%), the prognosis is poor for metastatic tumors [38]. The recurrence rate was 4.6%, the rate of involvement of lymph nodes was 3.7%, and death from tumor was 2.1% of primary cSCCs [39]. Early diagnosis and treatment of AK, SCCis, and primary cSCC lesions is the best strategy to prevent the progression of lesions to metastatic cSCC [5, 29, 40]. Regional lymph nodes in 85% of cases are the primary target organs for metastatic cSCC followed by metastases to the lung, liver, brain, and bones [40]. The size of lesion (> 2 cm), localization of lesion (lip, ear), deep tumor infiltration, immunosuppression, history of redundant irradiation, histopathological features, low differentiation, perineural invasion are some of the most important indicators of potential metastatic disease and recurrence of cSCC, which require routine observation and examination of patients [1, 29, 40]. Moreover, incomplete tumor resection is considered to be one of the major risk factors for cSCC recurrence [40].

Up to the present, only a few cSCC progression biomarkers have been identified. The phosphorylated form of STAT3 is thought to be a regulator of cell movement and is more markedly expressed in poorly differentiated cSCC than in well-differentiated tumors. In addition, p-STAT3 expression is associated with tumor invasion and metastases [41]. Another known biomarker for cSCC is molecule E-cadherin. Reduced expression of E-cadherin might be a sign of regional involvement of lymph nodes in patients with cSCC [42].

Ets-1 is a transcription factor that is involved in the regulation of various genes associated with angiogenesis and remodeling matrix, such as MMP [43, 44]. Ets-1, in turn, is expressed in low differentiated and metastatic cSCCs compared to well differentiated tumors and has been proposed as a marker for invasive tumors [45]. MMP-12 expression in macrophages has been identified as a prognostic marker for cSCC of the vulva [46]. MMP-13 (collagenase-3) is expressed in cSCC of the head and neck [47]. In cSCC of the skin, MMP-13 has been detected at the front of the epithelial tumor [48] and is associated with invasive capacity and tumor growth [49]. Epithelial expression of MMP-7, -12, and -13 has been identified as markers for distinguishing benign chronic wounds from cSCC arising from chronic ulcers [50]. MMP-19 is expressed in hyperproliferative keratinocytes but it disappears in invasive cSCC [50]. In addition, abundant MMP-12 expression was detected in cSCC and expression was found to correlate with tumor aggression [46].

In a very few studies, the effect of *L-myc* gene polymorphism on the progression of tumor of the head and neck was studied [51]. This family includes c-myc and N-myc and plays an important role in the regulation of cell proliferation and differentiation [52]. *L-myc* gene polymorphism is a typical genetic trait

responsible for the susceptibility of an individual to several cancers [53]. Initially, they were found in lung cancer cells, and then a number of studies proved their proto-oncogenic properties [54, 55]. In tumor cells, amplified forms of this protein are found that disrupt the general mechanisms of regulation peculiar to carcinogenesis [56]. The oncogenic potential of this family of proteins is associated mainly with its quantitative increase, which accompanies not only malignancy but also metastasis of tumor, its aggressive course [57].

Although the immune dysfunction in patients with cancer might be multifactorial, it can be modulated by the genetic background [58]. Tumor necrosis factor- α (TNF- α) is a member of the TNF/TNFR cytokine superfamily. In common with other family members, TNF- α is involved in maintenance and homeostasis of the immune system, inflammation and host defense [59]. However, in middle age and old age, TNF- α is involved in pathological processes such as chronic inflammation, autoimmunity, and malignant diseases [60]. The involvement of TNF- α in the inflammatory network contributes to all stages of the malignant process, and makes it possible to consider TNF- α as a target for cancer therapy [61].

While the overall survival of patients with cSCC is high and taking into account the high incidence, cSCC treatment represents a significant financial burden for the healthcare system. Therefore, the study of the genetic determinants of AK, SCCis and cSCC foci will provide a more complete understanding of the molecular pathways of their development, which in turn will substantiate the understanding of therapeutic approaches to the treatment of these diseases.

The objective of the research is to study genetic determinant in the formation of the lesions of EDS of different degree of malignancy.

MATERIALS AND METHODS

The research was performed at the State Scientific Institution “Scientific and Practical Center of Preventive and Clinical Medicine” of the State Administration jointly with the State Institution “Reference-Center for Molecular Diagnostic of Public Health Ministry of Ukraine” in 2016–2018.

Patient profile. In our study we used postoperative biopsy of 85 patients with EDS, among them 43 (50.6%) patients with AK, 21 (24.7%) patients with SCCis and 21 (24.7%) patients with cSCC. Gender-age characteristics of patients involved in the study is presented in Table 1.

Table 1. Gender-age characteristics of patients with epidermal dysplasia of the skin

Type of EDS	Gender	Number of patients by gender	%	Average age of patients $M \pm m$
AK (n = 43)	male	26	60.5	74.27 $\pm$ 3.12
	female	17	39.5	73.29 $\pm$ 4.08
SCCis (n = 21)	male	11	52.4	75.17 $\pm$ 3.21
	female	10	47.6	73.80 $\pm$ 2.84
cSCC (n = 21)	male	15	71.4	78.15 $\pm$ 2.77
	female	6	28.6	82.80 $\pm$ 3.63
Totally (n = 85)	male	52	61.2	76.06 $\pm$ 8.25
	female	33	38.8	74.76 $\pm$ 10.51

No statistically significant differences in the groups of patients with AK, SCCis and cSCC by gender or age were revealed ($p > 0.05$).

Biological samples characteristics. The first study group (Gr 1) involved biological preparations of patients with AK, the second group (Gr 2) included biological preparations of patients with SCCis, and the third group (Gr 3) involved biological preparations of patients with cSCC. The histological examination determined the level of epidermal dysplasia (KIN — keratinocyte intraepidermal neoplasia) according to the classification suggested by Cockerell and Wharton: KIN I — mild (is characterized by a typical basal and suprabasal cells in the form of increased nuclei, their hyperchromicity), KIN II — moderate (differs by abnormal proliferation of keratinocytes by two-thirds thickness of the epidermis), KIN III — severe (is manifested by proliferation of atypical keratinocytes in all layers of the epidermis), KIN IV — invasive form (atypical keratinocytes infiltration beyond the basement membrane) [62]. The distribution of biological samples of studied groups according to the level of epidermal dysplasia, tumor size and disease duration before diagnosis is presented in Table 2.

Table 2. Distribution of biological samples of studied groups according to the level of epidermal dysplasia, tumor size and disease duration

Parameters	Studied groups of biological samples		
	Gr 1	Gr 2	Gr 3
Total number of studied samples	43	21	21
disease duration (month)	8.5 $\pm$ 3.3	9.6 $\pm$ 2.7	11.7 $\pm$ 4.3
tumor diameter (cm)	0.7 $\pm$ 0.3	1.1 $\pm$ 0.2	1.3 $\pm$ 0.2
KIN I samples	17 (39.5%)	—	—
disease duration (month)	4.3 $\pm$ 2.7	—	—
tumor diameter (cm)	0.6 $\pm$ 0.3	—	—
KIN II samples	16 (37.2%)	—	—
disease duration (month)	7.3 $\pm$ 3.4	—	—
tumor diameter (cm)	0.6 $\pm$ 0.2	—	—
KIN III samples	10 (23.3%)	21 (100%)	12 (57.1%)
disease duration (month)	8.6 $\pm$ 1.6	9.6 $\pm$ 2.7	10.1 $\pm$ 3.1
tumor diameter (cm)	1.1 $\pm$ 0.3	1.1 $\pm$ 0.2	1.2 $\pm$ 0.2
KIN IV samples	—	—	9 (42.9%)
disease duration (month)	—	—	13.2 $\pm$ 4.7
tumor diameter (cm)	—	—	1.4 $\pm$ 0.2

The analysis of the disease duration and the diameter of tumor revealed that with the increase of the course of tumor, the size of the lesion also increased, but no statistically significant differences were found between the groups ($p > 0.05$).

Molecular genetic analysis. Polymorphic variants of G308A (rs1800629) in the promoter region of TNF- α [63], T3109G (rs3134613) of intron 2 of L-myc [64] and G13494A (rs1625895) of intron 6 of Tp53 in skin biotates in paraffin blocks were determined. After removing the paraffin, total DNA extraction was performed using the Quick-DNA™ Universal Kit (Zymo Research, USA). The studied gene fragments were amplified using commercial kit DreamTaq Green PCR Master Mix (Thermo Scientific, USA) and amplification primers (Metabion, Germany). Reaction products were analyzed using restriction fragment length polymorphism method in agarose gel.

Statistical analysis. Statistical data processing was performed using Microsoft Office Excel software to calculate the mean value ($M \pm m$) with the standard

deviation; the analysis was performed using Student's *t*-test and two-tailed Fisher's exact test.

RESULTS AND DISCUSSION

Genotype frequencies of *TNF-α* (G308A), *Tp53* (G13494A), *L-myc* (T3109G) in skin biotates after histological verification of the diagnosis of patients of three groups with different types of EDS were studied (Table 3). In patients of Gr 1 (AK), the frequency of genotype 3109TT on *L-myc* T3109G was significantly lower compared to patients of Gr 2 ($p = 0.034$) and Gr 3 ($p = 0.004$). Genotype distribution of this polymorphic variant did not differ in Gr 2 and Gr 3, although the distribution of genotype 3109TT was the highest in Gr 3 compared to Gr 1 and Gr 2. The comparison results show that in case of the same disease duration in patients of Gr 1–Gr 3, genotype 3109TT on *L-myc* gene is a genetic component of malignant transformation of epithelial cells of the skin and the formation of invasive form of cSCC. The obtained results are consistent with the results of experimental studies, from which we have known about the effect of *L-myc* gene expression on malignant cell transformation in the development of malignant neoplasms, which is accompanied by impaired apoptotic processes of varying degrees [65], possibly depending on the state of *TP53* gene and other modifier genes.

A number of researchers believe that regaining control of *L-myc* gene expression will be a successful step in the treatment of many tumors, since most tumor cells are dependent on its effects due to metabolic reprogramming, apoptosis disorders and toxic effects on the body [66]. In our study dealing directly with tumor site of the skin, other variants of 3109TG and 3109GG genes did not have such prognostic value for the risk of developing malignant invasive lesions compared to 3109TT. According to the results of the meta-analysis, various mutations and structural rearrangements of *L-myc* gene also determine the increasing risk of tumor progression [67], but the studied variants of 3109TG and 3109GG did not show this effect.

According to many studies, mutations and variants of *TP53* gene have been associated with aggressive forms of cancer and their unfavorable course, includ-

ing malignant neoplasms of the skin [68]. According to the results of individual studies, the polymorphism in intron 6 of *TP53* gene (*rs1625895*) influences the changes in p53 protein expression. The level of gene expression depending on the polymorphism determines the stability of template — RNA. For this polymorphism, associations with skin tumors have been proven, especially under the influence of occupational factors and UVR, and it has been proven that when combined with other genetic variants of *TP53*, the risk of lung cancer and other oncologic pathology is increased, especially with the effect of pleiotropic factors (smoking, etc.) [69].

These studies were performed in peripheral blood, and no genetic studies of known variants of *TP53* were conducted directly in the tumor site. In addition, population-based studies on the distribution of intron variant of *TP53* (G13494A) of examined patients are insufficient. According to our estimation, based on the analyzed sources, the specified variant of the gene in homozygous and heterozygous states can be determined in healthy individuals in no more than 20%, or not determined at all [68, 70]. In the examined patients, significant differences were found in the distribution of 13494GA genotype when comparing Gr 1 ($p = 0.014$) and Gr 3, as well as when comparing Gr 2 and Gr 3 ($p = 0.038$). The highest frequency of this genetic variant was in Gr 3 — 38.10% of the total number of patients. Additional analysis taking into account genotype variant and depending on the level of epidermal dysplasia (KIN) revealed a significant increase in the distribution of 13494GA genotype in patients with invasive skin cancer (cSCC) with KIN IV compared with KIN I in patients with AK ($p = 0.034$) (Table 4).

When assessing the frequencies of allelic variants, it was found (see Table 3) that 13494A allele was more likely to be detected in patients of Gr 3 compared to Gr 2 ($p = 0.030$), which is consistent with the data on association of this allele with the development of invasive malignancies of the skin. Sagne *et al.* [71] have reported that the heterozygous variant of intron polymorphism is associated with an increase in the risk of malignant skin lesions, and in our study the association of this variant with the development of the invasive form of cSCC has been established. The increase in the

Table 3. Polymorphism of *L-myc* T3109G, *Tp53* 6 G13494A and *TNF-α* G308A depending on the studied group

Gene	Variant of gene/allele	Gene polymorphism of the studied groups			Comparative characteristics of gene polymorphism of the studied groups by Fisher's exact test		
		Gr 1 (n = 43, %)	Gr 2 (n = 21, %)	Gr 3 (n = 21, %)	Gr 1/Gr 2	Gr 1/Gr 3	Gr 2/Gr 3
<i>TNF-α</i> G308A	308GG	30 (69.77)	20 (95.24)	16 (76.19)	0.045*	0.7680	0.184
	308GA	11 (25.58)	1 (4.76)	5 (23.81)	0.084	1.000	0.102
	308AA	2 (4.65)	0	0		0	0
	308A	71 (82.6)	41 (97.6)	37 (88.1)	0.020*	0.605	0.114
	308G	15 (17.4)	1 (2.4)	5 (11.9)		0.605	0.114
<i>Tp53</i> 6 G13494A	13494GG	31 (72.09)	18 (85.71)	11 (52.38)	0.348	0.162	0.025*
	13494GA	4 (9.30)	2 (9.52)	8 (38.10)	1.000	0.014*	0.038*
	13494AA	8 (18.60)	1 (4.76)	2 (9.52)	0.251	0.476	1.000
	13494A	66 (76.7)	38 (88.4)	30 (71.4)	0.091	0.522	0.030*
	13494G	20 (23.3)	4 (11.6)	12 (28.6)	0.091	0.522	
<i>L-myc</i> T3109G	3109TT	2 (4.65)	5 (23.81)	5 (23.81)	0.034*	0.004*	0.747
	3109TG	22 (51.16)	10 (47.62)	9 (42.86)	1.000	0.601	1.000
	3109GG	19 (44.19)	6 (28.57)	7 (33.33)	0.282	0.169	0.742
	3109T	26 (30.2)	20 (47.6)	19 (45.2)	0.077	0.116	0.831
	3109G	60 (69.8)	22 (52.4)	23 (54.8)	0.077	0.116	0.831

Note: * $p < 0.05$.

frequency of *13494AA* homozygous variant in patients of Gr 1 compared to Gr 2 and Gr 3 was not statistically significant ($p > 0.05$). The prevalence of *13494GG* genotype (wild-type gene) was not significantly different in patients of Gr1 compared to Gr 2 ($p = 0.348$), but when comparing patients of Gr 2 with patients of Gr 3 ($p = 0.025$), the differences in genotype distribution were significant, which suggest its protective effect (in case of the same disease duration) to the development of malignant invasion. In other words, *TP53* polymorphic variant show a clear modifying effect on the probability of malignant invasive cSCC.

The obtained results confirm the theory that gene activity influences the efficiency of apoptosis and is related to the preservation of genome stability [68]. Depending on interaction with other genes, both the level of gene expression and its functional deficiency can stop the apoptosis, stimulate mutation process in other genes and cause drug resistance [69, 70]. Biros *et al.* [72] and Sauka *et al.* [68] found that patients with lung cancer with *13494GA* heterozygous genotype on *TP53* gene have significantly reduced level of leukocyte apoptosis, which explains the potential pathogenetic mechanisms in the development of invasive forms of cSCC. A stepwise increase in the effect of *13494GA* heterozygous variant while reducing the protective effect of *13494GG* variant in patients with SCC is compared with cSCC might be a consequence of reducing the suppressive effect of *TP53* on the tumor process in the presence of *13494A* allele.

When analyzing the distribution of genotypes by *TNF-α* (*G308A*), it was determined that the frequency of *308GG* genotype distribution was significantly reduced in patients of Gr 1 compared with patients of Gr 2 ($p = 0.045$). When comparing the distribution of *308G* allele in patients of Gr 1 and Gr 2, we found

its significant increase in patients of Gr 1 ($p = 0.020$). Taking into account the fact that *TNF-α* gene polymorphism determines the level of cytokines and their pathological increase in the presence of the modified variant, its role in the development of carcinogenesis has been studied, but the issues of its direct participation in the course of oncological diseases and the formation of their different types have not been sufficiently clarified [73–75]. In view of the heterogeneous differences in variants of this gene among patients of three groups, we analyzed its dependence on KIN (Table 4).

According to the latest scientific data, epithelial tumors with the level of dysplasia KIN I and KIN III have the greatest tendency to transform into invasive form of squamous cell carcinoma of the skin [76]. In our study, Gr 1 included 27 samples (62.8%) with high invasive growth potential, and Gr 3 involved 12 samples (57.1%) prone to further invasive infiltration and 9 samples (42.9%) with invasive growth in the dermis (Table 5).

Comparative analysis of gene polymorphism with tumor invasion level (KIN) showed a significant difference only in *308GG* genotype between patients with KIN III of Gr 2 and patients with KIN III of Gr 1 ($p = 0.007$), and *308GA* between patients with KIN III of Gr 2 and KIN III of Gr 1 ($p = 0.027$), which in case of the same disease duration and depth of lesion indicates the development of SCC is in patients with *308GG* genotype, and in patients with *308GA* genotype it indicates the development of AK (Table 6).

Therefore, *TNF-α* gene variant has a modifying effect on the formation of EDS, and possibly on its subsequent course and risk of recurrence. The analysis of intergenic and gene-factor interactions in our group of patients is prospective for further analysis of pathogenetic mechanisms of EDS formation.

Table 4. Comparative characteristics of polymorphism of *L-myc T3109G*, *TP53 6 G13494A* and *TNFα G308A* depending on the level of epidermal dysplasia

Gene	Variant of gene	Groups of samples depending on the level of epidermal dysplasia				Fisher's exact test					
		KIN I	KIN II	KIN III	KIN IV	KIN I / KIN II	KIN I / KIN III	KIN I / KIN IV	KIN II / KIN III	KIN II / KIN IV	KIN III / KIN IV
		n = 17, %	n = 16, %	n = 43, %	n = 9, %						
<i>TNF-α G308A</i>	<i>308GG</i>	12 (70.6)	13 (81.3)	35 (81.4)	6 (66.7)	0.688	1.000	1.000	1.000	0.630	0.378
	<i>308GA</i>	4 (23.5)	3 (18.7)	7 (16.3)	3 (33.3)	1.000	0.488	0.661	1.000	0.630	0.349
	<i>308AA</i>	1 (5.9)	0	1 (2.3)	0	—	0.489	—	—	—	—
<i>TP53 6 G13494A</i>	<i>13494GG</i>	13 (76.5)	11 (68.8)	32 (74.4)	4 (44.4)	0.708	0.262	0.194	0.530	0.393	0.118
	<i>13494GA</i>	1 (5.9)	2 (12.5)	7 (16.3)	4 (44.4)	0.601	0.420	0.034*	1.000	0.142	0.081
	<i>13494AA</i>	3 (17.6)	3 (18.7)	4 (9.3)	1 (11.2)	1.000	0.393	1.000	0.393	1.000	1.000
<i>L-myc T3109G</i>	<i>3109TT</i>	1 (5.9)	1 (6.3)	7 (16.3)	3 (33.3)	1.000	0.420	0.104	0.427	0.116	0.349
	<i>3109TG</i>	7 (41.2)	9 (56.3)	22 (51.2)	3 (33.3)	0.494	0.573	1.000	0.373	0.411	0.124
	<i>3109GG</i>	9 (52.9)	6 (37.4)	14 (32.5)	3 (33.4)	0.491	0.238	0.429	0.763	1.000	1.000

Note: * $p < 0.05$.

Table 5. Polymorphism of *L-myc T3109G*, *TP53 6 G13494A* and *TNF-α G308A* depending on the studied group and the level of epidermal dysplasia

Gene	Variant of gene	Gr 1				Gr 2		Gr 3		
		AK				SCCis		cSCC		
		Totally	KIN I	KIN II	KIN III	KIN III		Totally	KIN III	KIN IV
		n = 43, %	n = 17, %	n = 16, %	n = 10, %	n = 21, %		n = 21, %	n = 12, %	n = 9, %
<i>TNF-α G308A</i>	<i>308GG</i>	30 (69.77)	12 (70.6)	13 (81.3)	5 (50)	20 (95.24)		16 (76.19)	10 (83.3)	6 (66.7)
	<i>308GA</i>	11 (25.58)	4 (23.5)	3 (18.7)	4 (40)	1 (4.76)		5 (23.81)	2 (16.7)	3 (33.3)
	<i>308AA</i>	2 (4.65)	1 (5.9)	0	1 (10)	0		0	0	0
<i>TP53 6 G13494A</i>	<i>13494GG</i>	31 (72.09)	13 (76.5)	11 (68.8)	7 (70)	18 (85.71)		11 (52.38)	7 (58.3)	4 (44.4)
	<i>13494GA</i>	4 (9.30)	1 (5.9)	2 (12.5)	1 (10)	2 (9.52)		8 (38.10)	4 (30)	4 (44.4)
	<i>13494AA</i>	8 (18.60)	3 (17.6)	3 (18.7)	2 (20)	1 (4.76)		2 (9.52)	1 (11.7)	1 (11.2)
<i>L-myc T3109G</i>	<i>3109TT</i>	2 (4.65)	1 (5.9)	1 (6.3)	0	5 (23.81)		7 (33.33)	2 (16.7)	3 (33.3)
	<i>3109TG</i>	22 (51.16)	7 (41.2)	9 (56.3)	6 (60)	10 (47.62)		9 (42.86)	6 (50)	3 (33.3)
	<i>3109GG</i>	19 (44.19)	9 (52.9)	6 (37.4)	4 (40)	6 (28.57)		5 (23.81)	4 (33.3)	3 (33.4)

Table 6. Comparative characteristics of polymorphism of *L-myc T3109G*, *TP53 6 G13494A* and *TNFα G308A* depending on the studied group of biological samples and the level of epidermal dysplasia

Gene	Variant of gene	Fisher's exact test						
		KIN I 1/ KIN II of Gr 1	KIN II/ KIN III of Gr 1	KIN I/ KIN III of Gr 1	KIN III of Gr 2/ KIN III of Gr 1	KIN III of Gr 2/ KIN III of Gr 3	KIN IV of Gr 2/ KIN IV of Gr 3	KIN III of Gr 3/ KIN IV of Gr 3
<i>TNF-α G308A</i>	308GG	0.697	0.189	0.234	0.007*	0.133	0.538	0.611
	308GA	1.000	0.369	0.414	0.027*	0.538	0.538	0.611
	308AA	—	—	1.000	—	—	—	—
<i>TP53 6 G13494A</i>	13494GG	0.708	1.000	1.000	0.358	0.106	0.106	0.669
	13494GA	1.000	1.000	1.000	1.000	0.159	0.159	0.673
	13494AA	1.000	1.000	1.000	0.237	1.000	1.000	1.000
<i>L-myc T3109G</i>	3109TT	1.000	—	—	—	1.000	1.000	0.611
	3109TG	0.285	1.000	0.440	0.704	1.000	1.000	0.660
	3109GG	0.731	1.000	0.695	0.685	1.000	1.000	1.000

Note: **p* < 0.05.

The analysis of scientific sources and the obtained results indicate the importance of the genetic component in the formation of skin lesions. Further study of the genetic component of squamous cell carcinoma of the skin will help to develop a personalized prevention and treatment strategy.

CONCLUSIONS

In conclusion, epidermal dysplasia of the skin is a multifactorial lesion of the skin. An important role in the mechanisms of its formation belongs to the genetic component — numerous genetic changes, the list of which is supplemented due to the results of our research. The association of *L-myc (3109TT)* with the development of malignant skin lesions (SCCs and cSCC) of different invasiveness and the modifying effect of *TNF-α (G308A)* and *TP53 (G13494A)* gene variants on pathological transformation in the focus of EDS depending on the level of epithelial dysplasia was revealed. The association of 308GG genotype and *TNF-α (308G)* allele with the development of malignant skin lesions was found; and it was revealed that with the increase of epithelial dysplasia level, the protective properties of *TNF-α (308GA)* in the tumor site are lost. The influence of *TP53 (13494GA)* on the course of the tumor process and pathological transformation of lesions was confirmed: association of 13494GA genotype and 13494A allele with the formation of malignant invasive lesion and 13494GG genotype with the formation of malignant non-invasive lesion of the skin.

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