REVIEW

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The molecular mosaic of the premalignant cutaneous lesions

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Abstract

In the last three decades, the premalignant cutaneous lesions have represented a milestone for the clinicians and the anatomopathologists given the increased risk of malignant transformation not only in the old but also in the young population. Recent research indicates the fact that, though multiple progresses were recorded in the diagnosis and treatment of the cutaneous squamocellular carcinomas, developed in more than 85% of the cases in premalignant lesions, however the prognosis and survival up to five years did not register significant improvements. For the achievement of the diagnosis with certainty, the histopathological examination, considered until recently the "golden standard", principally based on the TNM criterion, has an increased percentage of subjectivity and it is relatively unsure, being known the fact that two apparently identical tumors answer differently to the same therapy. The variability of the morphological aspects from simple dysplasia to *in situ* carcinomas and the cancers themselves impose the identification of some cellular and molecular markers typical to the premalignant and malignant cutaneous lesions. In this respect, the knowledge and characterization of the molecular mosaic allow the establishment of some clear criterion for an early diagnosis, corresponding monitoring and adequate treatment.

Keywords: squamocellular carcinoma, premalignant lesions, molecular markers.

Introduction

The phenotypical variability of the premalignant cutaneous lesions is induced by a mosaic of cellular and molecular interactions responsible, under normal conditions, for the achievement and maintenance of the tissue architecture and functionality.

The premalignant lesion, a term introduced in 1875 by the Romanian doctor Victor Babeş, is that lesion which, in the absence of any treatment, presents an increased risk of malignant transformation. In 2005, World Health Organization (WHO) classified the premalignant lesions in simple, moderate, severe and *in situ* cancers [1, 2].

The *cutaneous* premalignant lesions, whose prevalence varies between 0.76–5% in Asia and 13.4% in America, present cellular and molecular features whose description allows the identification of the mechanisms specific to the epidermal genesis of the cancer [3, 4]. The risk of malignant transformation of these lesions was reported to be between 6.6% and 36.4%, although a more recent meta-analysis, from 2011–2012, indicated a risk of 12.1%. The early diagnosis and the adequate treatment of the premalignant lesions prevent the development of the cutaneous cancers [2, 5]. Although ever since 1988, Bouquo emphasized the fact that approximately 80% of the cancer cases appear through the transformation of the premalignant lesions, the classification and establishing of the premalignant lesions, accompanied or not by dysplasia, present another series of unclear aspects which require a reconsideration [6, 7]. For example, until recently, it was considered that the squamocelullar epithelioma is a disorder of the old persons, but the incidence is growing also in the case of young persons, due to chronic sun exposure [8].

Although for dermatologists and anatomopathologists the diagnosis of the cutaneous neoplasia is relatively easy, however, the survival and the life quality of these patients did not significantly improve in the last three decades, even though the invasive and excisional treatment is applied. These aspects are due, on one hand, to the phenotype and molecular variability and diversity of these tumors, and, on the other hand, to the ignoring of the "multi-step" sequential process through which they develop [5, 9–12].

Worldwide, more than 50% of the neoplasms appear at the skin level [13]. The non-melanocytic cutaneous cancer, including the squamocellular carcinoma and the basal cell carcinoma, constitute the most frequent type of cancer diagnosed in the last decades among the Caucasian population, with an increased incidence beginning with 1960 of 3–8% each year [9, 12, 14–16]. It is 18–20 times more frequent than the cutaneous malignant melanoma, with more than one million cases estimated in 2005 [12, 17, 18]. In Europe, USA and Australia, this represents one of the most frequent oncological diagnoses [5, 16]. It is remarked an increase of incidence of the cutaneous squamocellular carcinoma not only in the areas with increased sun exposure, nearby the Equator, but also in those areas with a reduced exposure, such is the case of Finland, where the incidence increased by 4% in the last decades [5]. In 2014, this represented 20% of the total cutaneous cancers, being the most frequent metastatic cancer. In Egypt, the incidence of this epithelioma was estimated at 37.02% of the total cutaneous cancers [17].

Given the invasive, recurrent and metastases feature, it is associated with an increase not only of approximately 5% per year morbidity in Central Europe, but also with that of mortality, being responsible for the majority of the deaths due to cancer [5, 8, 17].

At the cutaneous level, the risk of emergence of squamocellular carcinoma is mainly influenced by the existence of the premalignant lesions such as the actinic keratosis and by the chronic exposure to sun, 80% of them being located in the photo-exposed areas. The *American Academy of Dermatology* estimates that 60% of the over-40 persons have actinic keratosis [19].

At the level of the *oral mucosa*, the concept according to which the development of the oral squamocellular cancer requires two stages involves an initial presence of the premalignant lesion [4]. The proportion of squamocellular carcinomas developed *de novo* or on the premalignant lesions is not yet established in percentage.

The most frequent premalignant oral lesions are leukoplakia, erythroplakia/erythroleukoplakia, submucous oral fibrosis and lichen planus [1]. Less frequent are tobacco keratosis lesions, leukoedema, discoid lupus erythematous and epidermolysis bullosa [2]. For these lesions, it was identified a rate of 17% for the progression and the malignant transformation, on a period of seven years from the time of the diagnosis. The highest rate of malignant transformation is identified in the case of the heterogeneous erythroplakia and erythroleukoplakia with dysplastic changes [1]. Some studies have shown that 16–62% of the diagnosed leukoplakia lesions present changes, which indicate a beginning of a malignant transformation squamocellular type [4].

Molecular markers

Studies regarding the prophylaxis of the premalignant lesions have, among their objectives, the identification of some biomarkers involved in the cellular and molecular mechanisms typical to the process of carcinogenesis [9, 18].

The cadherin/catenin complexes

Ever since 1988, it is known the fact that transmembrane glycol-proteins called cadherin, essential in the tissue integrity, have a major role in the cellular adhesion, being the basic components of the cellular coupling areas [7, 20].

The epithelial–mesenchymal transition, an important process of the embryogenesis, is present also in case of the transformation of the premalignant lesions in invasive carcinomas. During this process, the epithelial cells present morphological changes and a migratory/invasive capacity typical for mesenchymal cells, due to the disorganization of the adhesive bands and desmosomes, determined by the absence of the E-cadherin, as a result of some genetic/epigenetic mechanisms [21].

The E-cadherin is a transmembrane glycoprotein, which forms at the membrane level a stoichiometric complex 1:1:1 with β-catenin and α-catenin [22].

The E-cadherin function also as a tumor suppressor: the absence of the expression of this molecule in epithelial cells determines the dissociation of desmosomes, the tumor progression and a poor prognosis [23–25].

In 2005, using a 3D culture of an adenovirus as infection vector of the epithelial cells was proven that the E-cadherin expression decreases in the same time with the decrease of catenin, a fact that suggests the important role of these molecules in the transformation of the premalignant lesions in the squamocellular carcinoma [23].

The cutaneous squamocellular carcinoma presents changes of the Dsg2 and Dsg3 (type E-cadherin) expression, which were correlated by Harada and Scafer ever since 1996 with the hyperproliferation and extended hyperplasia of the keratinocytes [21]. Although the Dsg2 expression was associated with the invasiveness and metastases of the squamocellular carcinoma, contributing to the establishment of the malignant phenotype of the keratinocytes, however, a sporadic overexpression was identified also in the benign and premalignant papilloma [25].

With regard to the catenin, the *cutaneous* lesions of the squamocellular carcinoma, the β-catenin's expression is much more reduced or even absent compared to the premalignant lesions and it is associated with the dimensions of the tumor and degree of differentiation. The diffused reduction of the membrane expression of the β-catenin is correlated according to the data in the literature not only with a risk of malignant transformation, but also a reserved prognosis [26]. Although it was considered that only the γ-catenin can be used as predictor of the tumor progression, however, recent studies have emphasized the important involvement of the α- and β-catenin in the tumor invasion and metastases.

In the case of the *oral mucosa*, the development of the premalignant and malignant lesions is characterized by changes in Dsg3 expression, associated with the invasive and metastases phenotype [27].

In normal oral mucosa, E-cadherin is strongly expressed in basal and para-basal layers. In the oral premalignant lesions, the membrane expression of the E-cadherin is reduced in behalf the cytoplasmatic one and it is reversely correlated in proportion to the degree of dysplasia [27]. The progression of the dysplastic lesions, which lead to squamocellular carcinoma, is immunohistochemically characterized by the reduction of this marker, together with the overexpression of vimentin, as a mesenchymal marker [28].

The reduction or absence of the E-cadherin expression is correlated not only with a degree of dysplasia, but also with the risk of malignant transformation of the oral leukoplakia.

In low-risk leukoplakia malignant transformation, the expression pattern of the cadherin is similar to that encountered in the apparently normal mucosa but in the oral lichen planus lesions with increased risk of malignant transformation have not identified changes in E-cadherin expression.

Also, for the oral premalignant lesions, the reduction of the β- and γ-catenin expression represents an accurate indicator for malignant transformation. Thus, for the dysplastic leukoplakia compared to the non-dysplastic ones, it is noticed the diffused reduction of the β-catenin membrane expression and the increase of the nuclear expression aspect correlated not only with the degree of dysplasia, but also with the risk of malignant transformation [28].

Desmoplakin and plakophilin-1

Unlike the epithelial cells of the normal oral mucosa, which presents intensely positive immunoreactivity at the cytoplasmatic level for desmoplakin and plakophilin-1 at the membrane and nuclear level, the cells of the dysplastic epithelium present a low and diffused immunoreactivity of the desmoplakin at the cytoplasm level and a reduced immunoreactivity for the nuclear plakophilin-1. According to data from literature, the expression's quantification of these markers of cellular adhesiveness, especially of the desmoplakin, in the oral dysplastic lesions, can identify tissues with an increased risk of malignant transformation [29].

In the oral squamocellular carcinoma, the immunoreactivity of the desmoplakin is absent, and the expression of plakophilin-1 is absent at the membrane level, diffused in the cytoplasm and reduced in the nucleus.

Ezrin

Ezrin or cytovillin, a predominant protein of the apical pole of the epithelial cells, represents one of the new "hot spots" of the tumor metastases mechanisms, identified in the last years [30]. It belongs to the ERM (ezrin–radixin–moesin) complex and achieves the connection between the cytoskeleton and the cellular membrane. It links the F-actin to the cellular membrane, following phosphorylation, being necessary to many fundamental cell processes, or can activate the RhoA GTPase, thus inducing the cytoskeleton remodeling, an important process for the motility, proliferation, differentiation and migration of cells [31–33].

It interferes in the regulation of the activity of the adhesiveness molecules, being involved in the cell–cell and cell–matrix interaction, extremely important interactions in the tumor invasion and metastases. Thus, at the keratinocytic level, the increase of the E-cadherin and β-catenin can be correlated with the suppression of the ezrin [34]. Moreover, the ezrin's expression is positively correlated with the Ki-67 index, marker of tumor proliferation and severity [33].

The quantitative and qualitative changes of the ezrin expression present special importance in the pathogenesis of different keratinocytic tumors [35]. In normal epidermis, ezrin is located in basal layer cell's membrane, the intensity of this biomarker being reduced towards the upper-basal layers. Membrane expression in normal epithelial cells and benign lesions is prevalent than in malignant lesions, where the cytoplasmatic expression predominates, and the change of the ezrin's expression is correlated with the tumor progression [30, 34].

The results of Park *et al.* from 2010 have emphasized a higher increase of expression in the squamocellular carcinoma compared to the premalignant lesions [30, 36].

In the Bowen's disease, the actinic keratosis, keratoacanthoma and seborrheic keratosis, the ezrin is expressed at cellular membrane level, with the exception of the spinous layer and presents a reduced immunoreactivity compared to the squamocellular carcinoma lesions, where it is expressed at the cytoplasmic level [37].

In the case of the normal *oral mucosa*, ezrin is expressed at the membrane's level, while in case of the squamocellular carcinoma, it is present in cytoplasm. In metastases in lymph ganglion higher expression is not correlated with the histological type of the oral cancer [33, 38].

From the therapeutic point of view, the ezrin represents a target for the new anti-tumor therapies, such is the case of baicalein, a flavonoid with inhibitory action [34].

Cytokeratins

Besides the adhesive molecules, markers of epidermal differentiation disruption are also the cytokeratins, cytoplasmic proteins of the cytoskeleton [24]. The keratins have an important role in maintaining the cell and tissue integrity by forming, together with the desmosomic transmembrane protein complexes, a well-organized structure, resistant to the mechanical stress and lesions [21]. The increase of these proteins' expression is emphasized mainly as an initial reaction of the epithelial tissue to the action of noxious, chemical and physical stimuli [7]. If the pairs of keratin do not present adequate partners, these become instable and are susceptible to the degradation by the proteases [24].

In the layered squamous epithelium, such as the epidermis and epithelium of the oral mucosa, the cytokeratin K5–K4 pair is expressed in the proliferating basal layer, adjacent the basal membrane. The upper-basal cells are, normally, post-mitotic and through a differentiation program express different other pairs of keratins, such as K2e in the epidermis and K6, K16, K2p in the upper differentiated layers of the gingival mucosa and palate [7]. The epidermis and the gingival epithelial tissue express in the spinous layer K1, K2, K10 and K11, while in the upper-basal layers of the non-keratinized tissue, the mouth mucosa and soft palate, express K4/K13 [7, 39, 40].

At the *epidermal level*, the changes of the cytokeratins expression are met in different stages of the tumor progression [24]. Thus, in the incipient stages of the carcinogenesis, it was observed a decreased expression of K1 and K10 cytokeratins and K13 overexpression. In the following stages, changes of K8 cytokeratin expression were identified.

Despite all these, the change of K13 cytokeratin expression cannot differentiate the premalignant lesions with an increased risk from those with a reduced risk of malignant transformation [24]. This desideratum was achieved with the help of other immunohistochemical studies, which, analyzing the pattern expression of K14, K15 and K19 cytokeratins, were able to differentiate between the actinic keratosis and the Bowen's disease and squamocellular carcinoma, identifying in early stages the risk of malignant transformation for these lesions [41, 42].

In the cells of the basal layer of the normal *oral epithelium* are present K5/K14, while the upper layers are dominated by the presence of K4/K13 and K1/K10 keratins. The severity of the dysplastic lesions was correlated with the change of the expression of these keratin pairs.

Thus, K4/K13 and K1/K10 are absent or underexpressed, and K5/K14 are expressed in the parabasal

layers and the spinous layer, which reflect the hyperplasia of the basal cells, frequently met in dysplasia. Despite all this, Lindberg *et al.* described K19 as a marker characteristic of the dysplastic epithelium [4, 43]. The hyper-proliferating conditions are characterized also by the expression of K6 and K16 keratins, together with the basal cells' keratins K5, K14 and K17 [44]. In more than 50% of the dysplastic lesions, K8 and K18 were detected, undetectable keratins in normal epithelium [4].

Maspin

Maspin (mammary serine protease inhibitor) or B5 serpin, whose gene was identified in 1994 at the level of the 18q21.3-q23 chromosome, is a 42-kDa cytoplasmic protein which belongs to the superfamily of serpins and works as a tumor suppressor through the inhibition of the tumor angiogenesis, invasion and metastases and apoptosis stimulation [17, 37, 45, 46]. The maspin's expression is not universal, but specific to certain tissues and is variable for the same tissue with location in cytoplasm, nucleus or nucleo-cytoplasmic [17, 47]. This ubiquitous location of the maspin explains the implication in multiple biological and biochemical processes. Studies were undertaken in order to identify through which maspin exert its anti-metastatic effects. Thus, it is considered that regulates cellular invasive potential through the modification of the integrin's profile, after initially being indicated also the implication in the regulation of the p53 expression [45].

As inhibitor of the angiogenesis, it acts on some molecules like β-FGF and VEGF-type, significantly reducing the density of the micro-vascularity associated with the tumors. Research by Solomon *et al.* indicating the fact that the neoplasm in which the maspin is expressed not only cytoplasmic but also nuclear, present a decreased expression of VEGF and cyclooxygenase-2 (COX-2) compared to the neoplasm in which the maspin is expressed only cytoplasmic, thus suggesting the VEGF suppression by the maspin through a path mediated by COX-2 [45].

The maspin's expression is reversely proportioned to the risk of malignant transformation, being raised in normal cells and reduced or absent in the dysplastic and tumor cells [46]. The increase of the maspin expression inhibits the invasiveness through the reduction of MMP-2 expression and angiogenesis through the reduction of VEGF expression [47].

At the *cutaneous level*, maspin is synthesized by the normal epithelial cells of the upper-basal, spinous and granular layers, presenting a cytoplasmic and, sometimes, membrane pattern of the expression in the epidermis, hair follicles and mature sebaceous glands [17, 48]. It was identified the fact that its expression is associated with a squamous terminal differentiation. In some studies, the cytoplasmic position is encountered not only at the normal skin level, but also in numerous types of cutaneous cancers.

In the case of the cutaneous squamocellular carcinoma, the cytoplasmic position is associated with an increased risk of lymphatic metastases, and the nuclear expression is correlated with a good prognosis, being associated with a decreased rate of local and regional recurrence and with an increased rate of survival following the excision, in the old age patients [17, 47]. The tissue specificity of the maspin's expression is linked to the presence of a hormonal responsive element (HRE) from the promoter of the maspin gene, which reduces the transcription of this gene. It is considered that the activation of the androgenic receptors, ubiquitously distributed in the human body, can stimulate the HRE, blocking the transcription of maspin in the tumor's cell lines, at the cutaneous level. Likewise, it was observed that the expression of the protein and the specific mRNA at keratinocyte level is correlated with the aging and chronological age, and the powerful/moderate expression in the feminine sex is correlated with the positivity of estrogen and progesterone reaction's [17].

Maspin is more intensely expressed in the cutaneous squamocellular carcinoma (77.8–85.7% of the cases), compared to the oral one (58.9% of the cases) [17].

At the level of the *oral mucosa*, this marker represents an important predictor for transformation of the premalignant lesions in squamocellular carcinoma [49]. Oral malignant tumors are characterized by a reduced expression of the maspin [48, 50]. The results of the studies done by Yoshizawa *et al.* have shown that this reduction is negatively correlated with a degree of invasion translated through the TNM stage, lymph node metastases and degree of differentiation [37, 47–49]. These observations were later supported by numerous studies, which have emphasized that the loss of immunoreactivity for the maspin is associated with a bad prognosis of the oral squamocellular cancers, and the increase of this biomarker is associated with the stimulation of apoptosis and the improvement of survival [37, 45–49]. Thus, it represents an important prognosis factor, being a useful marker in the identification of the potential of tumor progression [47].

In the squamocellular carcinoma of the lip, the maspin is overexpressed not only at the level of the tumor's cell, but also at the level of the adjacent epithelium. In the actinic cheilitis, it is reversely proportioned to the degree of dysplasia, having an important role in the evaluation of the prognosis [46].

In the last five years, through therapeutic approaches of the cutaneous and oral squamocellular carcinoma, we can remind numerous components, which have as target maspin. Among these, it is counted also the curcumin, which inhibits the metastases and has effect of tumor suppressor through the stimulation of the maspin's expression dependent on the p53 protein. Similar effects are met also in the case of the extracts from the apple or the resveratrol, a polyphenolic antioxidant found in ground nuts, grapes and red wine [47].

Metalloproteinases

The changes in the expression of the metalloproteinases are met in different types of cancers, among which it is added also the cutaneous and oral squamocellular carcinoma [5].

At the *cutaneous* level, the MMP-1 or the collagenase-1 plays an important role in the proliferation of the tumor cells and the angiogenesis being correlated with a negative

prognosis. In the case of the dysplastic lesions with a significantly increased risk of becoming squamocellular carcinoma, higher levels of mRNA MMP-1 and MMP-9 were identified compared to the other metalloproteinases [28]. Immunohistochemical studies revealed elevated MMP-7 expression especially in the invasive edge of the cutaneous squamocellular tumors. MMP-7 activates heparin binding epidermal growth factor-like growth factor (HB-EGF) in cutaneous squamocellular cells. In functional studies, proliferation of cutaneous squamocellular cells was suppressed when the activation of HB-EGF by MMP-7 was inhibited [5]. In 2004, the research done by Pendas *et al.* suggests that the MMP-19 sub-expression reduces the susceptibility of the development for cutaneous cancer [49, 51].

In the case of the squamocellular carcinoma developed at the *oral mucosa* level, the overexpression of MMP-1, MMP-2 and MMP-9 is associated with the dedifferentiation, an increased risk of metastases and bad prognosis [50, 52]. Although there are relatively few studies of the MMPs expression at the level of the dysplastic oral lesions, showing that expression of MMP-1, but also MMP-2 and MMP-9 are reduced compared with squamocellular carcinoma lesions [51]. Likewise, oral dysplastic lesions that were detected by immunohistochemistry an overexpression of MMP-1, MMP-7, MMP-9 and MMP-10 are associated with an increased risk of malignant transformation [28, 52]. The MMP-2 expression, absent in the normal epithelial cells of the mucosa, is directly and proportionally correlated with the degree of dysplasia [53]. The MMP-8 has a dual role: it serves not only protumorigenic as well as antitumor in various stages of carcinogenesis. The protective role of MMP-8 was identified in the case of tongue squamocellular carcinoma. In 2011, Gialeli *et al.* mentioned that the MMP-13 expression is associated with the epithelial–mesenchymal transition (EMT), this being correlated by Stokes *et al.*, in 2012, with the invasive and metastasic phenotype of the squamocellular carcinoma located at the head and neck level [13].

Defensins

Alpha and beta human defensins, polypeptides belonging to the antimicrobial peptide family (AMP), are molecules expressed at the level of the phagocytes and epithelial cells. If in 1980, they were described also as components of the innate immune system, later were identified numerous other functions among which we remind the important role in the cellular proliferation. At the level of the cutaneous epithelium and of the oral mucosa, three subtypes of the *β*-defensins (hBD), noted hBD1-3, were emphasized, for which the expression is regulated by the action of some inflammatory cytokines, growth and infectious, bacterial or viral factors [54].

At the *epidermal* level, the hBD1 expression is negatively associated with the risk of malignant transformation of the actinic keratosis lesions, Bowen's disease and keratoacanthoma. In the case of these premalignant lesions, the immunohistochemical examination has emphasized a higher intensity of the hBD1 expression compared to the squamocellular epithelioma [55]. It is known the fact that the beta-HPV infection represents a predisposing factor for the development of the premalignant and malignant cutaneous lesions. The action of this virus can be inactivated especially for the hBD2, whose expression is negatively correlated with the risk of malignant transformation. Thus, the reduction of the hBD2 expression is associated with the increase of the susceptibility of the malignant transformation of the actinic keratosis lesions and of the Bowen's disease, associated with the human papillomavirus (HPV) infection [47].

Also, at the level of the *oral mucosa*'s epithelium, the defensins expression is correlated with the malignant transformation of the premalignant lesions, such are the leukoplakia ones. Thus, the squamocellular carcinoma, compared to the premalignant lesions, is characterized by a reduced expression of the hBD1 and an increased expression of the hBD3 [54, 56].

Conclusions

The present study brings at the forefront a present pathology for the global medical system and draws the attention to the importance of the identification and characterization of the entire specter of the biomarkers encountered in the premalignant cutaneous lesions. The description of the molecular mosaic of these lesions' pathology facilitate, on one side, the establishment of a correct and complete diagnosis in the initial stages of the malignant transformations and, on the other hand, imprints the approach of a targeted, specific and minimally invasive therapy.

Conflict of interests

The authors declare that they have no conflict of interests.

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