Study of some invasiveness markers as pathogenic factors in oral pseudoepitheliomatosus hyperplasia

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Abstract
Pseudoepitheliomatosus hyperplasia is a benign reactivated epithelial lesion secondary to another pathology, whose incidence is difficult to establish. There still exist controversies regarding the origin and pathogenesis of these lesions. For this purpose, we performed an immunohistochemical study upon 20 cases of oral pseudoepitheliomatosus hyperplasia associated with inflammatory and neoplastic conditions, investigating a series of markers with a possible pathogenic potential in developing this type of lesions. Thus, the immunoreactivity study for β-catenin showed the presence of a membrane reactivity in all the stratum spinosum and a predominantly cytoplasmatic reactivity, more rarely a nuclear one, in the cells of the basal stratum cells, especially in the epithelial apices that descend deeply in the chorion. Instead, in the case of vimentin, the reactivity was present only in the epithelial apices, especially in the peripheral cells, in comparison to the central ones, and especially in the cases where the epithelial apices descended deeply in the sublesional chorion. Moreover, we observed that the MMP9 reactivity in pseudoepitheliomatosus hyperplasia lesions was present in the cells at the epithelium–chorion interface and especially in the epithelial apices that descend deeply into the chorion, and also in the epithelial chorion and networks. The study for CXCR4 immunoreactivity showed a good reactivity in almost all layers of this hyperplastic lesion, with a maximum reactivity especially inside the epithelial apices that descend deeply into the chorion. Such an immunoprofile suggests the ability of the oral epithelial cells to undergo an epithelial mesenchymal transition process, thus acquiring mesenchymal characteristics through which it deeply migrates in the subadjacent chorion and contributes to the formation of epithelial apices in pseudoepitheliomatosus hyperplasia. Moreover, the invasive ability of these lesions is also given by the average quantity of matrix metalloproteinases present in the epithelium–chorion interface determined by the activation of CXCR4 receptors at this level.

Keywords: pseudoepitheliomatosus hyperplasia, invasiveness, oral mucosa, pathogeny.

Introduction
Pseudoepitheliomatosus hyperplasia is a benign reactivated epithelial lesion microscopically characterized by an irregular, prominent hyperplasia of the epithelium, with “tongue-like” epithelial projections in the adjacent dermis/chorion, an aspect often described as pseudo-invasion [1–3]. The mucous or tegumentary lesions seem to develop as a response to a great variety of infectious, neoplastic, inflammatory or traumatic stimuli, without being completely established the role played by these in its pathogeny [2, 3]. Being a lesion secondary developed to another pathology, the real incidence of this entity is difficult to estimate, being possible between 11 and 80 years old, affecting both sexes almost equally, most frequently being localized in the oral mucosa [3, 4].

At present, there are not yet clarified the molecular mechanisms that determine the etiopathogeny of pseudoepitheliomatosus hyperplasia [5], reason for which we proposed to investigate the pathogenic implications of several invasiveness markers in pseudoepitheliomatosus hyperplasia.

Materials and Methods
The study was performed on a group of 20 cases, the histopathological material being taken from the Laboratory of Pathological Anatomy within the Emergency County Hospital of Craiova, Romania, after the processing of surgical exeresis from the patients admitted to the Clinic of Oral and Maxillofacial Surgery within the same Hospital.

After the histopathological reevaluation of the cases, there was performed the immunohistochemical evaluation, using the double immunostaining method. At first, after deparaffinization and hydration, the serial sections with a 4 μm thickness were subjected to endogenous peroxidase blocking (with 3% hydrogen peroxide for 5 minutes), antigen demasking in the microwave (in pH 6 citrate, at 500 W for 20 minutes) and to non-specific sites blocking (with 3% bovine serum albumin – BSA). Then, in a first stage, we incubated the sections with the first antibodies (β-catenin and MMP9), an action performed at 4°C, in the fridge over night, and we used as a visualizing system the LSAB2-HRP (Horseradish peroxidase) kit (Dako, K0675), and the DAB (3,3’-Diaminobenzidine)
chromogen respectively, following the working instructions of the manufacturer. In the second part, after a previous blocking with Avidin–Biotin, the sections were incubated with the second primary antibody (vimentin and CXCR4) and we used as a visualizing system the LSAB2-AP kit (Dako, K0674) and the Vulcan Fast Red chromogen (Biocare Medical, FR805S), following the working instructions of the manufacturer. The characteristics of the used antibodies are presented in Table 1.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone / Manufacturer</th>
<th>Dilution</th>
<th>Antigen demasking</th>
<th>Positive control</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Catenin</td>
<td>β-Catenin-1 / Dako</td>
<td>1:100</td>
<td>Citrate, pH 6</td>
<td>Urothelium</td>
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<tr>
<td>Vimentin</td>
<td>SP20 / Thermo Fisher Scientific</td>
<td>1:200</td>
<td>Citrate, pH 6</td>
<td>Skin</td>
</tr>
<tr>
<td>MMP9</td>
<td>2C3 / Santa Cruz Biotechnology</td>
<td>1:50</td>
<td>Citrate, pH 6</td>
<td>Appendix</td>
</tr>
<tr>
<td>CXCR4</td>
<td>Polyclonal / Acris Antibodies</td>
<td>1:1000</td>
<td>Citrate, pH 6</td>
<td>Tonsil</td>
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</table>

The result of these reactions was the visualization of the targets with brown color (DAB), and red color, respectively (Vulcan Fast Red).

For the validation of reactions, we used positive external controls and negative external controls, by omitting the primary antibody.

A double reaction quantification was made only qualitatively, taking into consideration only the marking intensity categories, namely: 1 (poor), 2 (moderate), 3 (strong) for each of the two markers used. Also, we were interested in the cellular and tissular localization of the immunomarkings.

**Results**

The average age of the 20 patients included in the study was 46 years old, with a majority of men (men/women ratio 3:1) and, most frequently, the lesions developed on the dorsal side of the tongue (12 cases). The etiopathogenic conditions associated to the hyperplasia lesions were the following: candidosis (six cases), tuberculosis (four cases), oral squamous carcinoma (six cases) and granular cell tumor (four cases).

Histopathologically, in the investigated cases there were observed all the characteristic lesional aspects, namely the elongation of the epithelial apices that descend deeply in the chorion (Figure 1a), the presence of acanthosis, dyskeratosis, presence of “epithelial pearls” in the chorion (Figure 1b), a low degree of cellular/nuclear abnormalities and a small number of mitoses.

![Figure 1](image_url) — Pseudoepitheliomatous hyperplasia: (a) Elongated epithelial network and apices; (b) Epithelial pearls. Hematoxylin–Eosin (HE) staining, ×400.

**β-Catenin/vimentin double immunoreactivity investigation**

In the areas of normal oral mucosa, we observed the presence of β-catenin immunoreactivity, especially in the lower half of the stratum spinosum (Figure 2a). The reactivity progressively decreases in the upper layers of the stratum spinosum. A poor reactivity also exists in the basal stratum towards the stratum spinosum. The immunoreaction pattern was a membrane type one. In the areas of normal oral mucosa, we did not observe the presence of vimentin immunoreactivity in the epithelium, only in the subadjacent chorion (Figure 2a).

In the areas of pseudoepitheliomatous hyperplasia, the membrane pattern of the β-catenin expression was preserved in the acanthosis areas and inside the epithelial apices and networks (Figure 2b). The membrane marking was present up to the superficial strata. At the chorion–epithelium interface, especially in the areas with epithelial apices that descend deeply in the chorion, the marking becomes both cytoplasmic and nuclear (Figure 2c). This immunoprofile resembles the one found in the areas of carcinomatous proliferation in the cases of pseudoepitheliomatous hyperplasia associated to squamous carcinoma, where the intensity and the membrane pattern of the β-catenin immunomarking is clearly visible in the areas of well-differentiated squamous carcinoma, becoming a cytoplasmic and nuclear one in the invasion front at the periphery of carcinomatous proliferations, especially in the moderately and poorly differentiated forms.

In the pseudoepitheliomatous hyperplasia areas, vimentin immunoreactivity was observed only in the areas with epithelial apices and networks (Figure 2d). Here, the immunomarking was present especially in the cells of the epithelium–chorion interface and more rarely in the cells of the hyperplasia areas (Figure 2e). The immunoreaction pattern was a cytoplasmic one.

This immunoprofile is somehow similar to that
observed in the areas of carcinomatous proliferation from other cases of pseudoepitheliomatous hyperplasia associated to squamous carcinoma. In these cases, in the neoplastic islands, especially the moderately and poorly differentiated ones, there was a maximum vimentin reactivity at the periphery. Vimentin immunoreactivity was also observed in the sublesional chorion and in the tumoral stroma areas, the marker being present in the vascular endothelium cells, fibroblasts and associated inflammatory cells.

The study of possible co-localizations of β-catenin with vimentin showed us their existence almost exclusively in the epithelial apices, namely in the cytoplasm of the epithelial cells inside the apices, and, more rarely, in the epithelial cells of the epithelium–chorion interface, which were almost exclusively reactive to vimentin (Figure 2f).

Figure 2 – (a) Area of normal gingival mucosa – membrane reactivity for β-catenin, especially in the lower half of the stratum spinosum (×100); pseudoepitheliomatous hyperplasia, β-catenin (brown)/vimentin (red) IHC (immunohistochemistry) staining; (b) Membrane reactivity for β-catenin to the upper layers (×100); (c) Cytoplasmic immunomarking for β-catenin, including the epithelial apices (×200); (d) Cytoplasmic immunomarking for vimentin of the epithelial apices (×200); (e) Cytoplasmic immunomarking for vimentin of the epithelial apices, predominantly at the epithelium–chorion interface (×400); (f) β-Catenin/vimentin colocalization in the cells at the periphery of the epithelial apices, at the epithelium–chorion interface – detail (×400).
MMP9/CXCR4 double immunoreactivity study

In the areas with normal oral mucosa, the MMP9 immunomarking was generally a poorly visible one in some nuclei of the basal and parabasal stratum, and very rarely in the keratinocyte nuclei of the inferior layers of the stratum spinosum (Figure 3a). In the areas of normal oral mucosa, the CXCR4 reactivity was generally a low one, present in the cytoplasm of the stratum spinosum keratinocytes, especially in its superficial layers (Figure 3a).

In the areas of pseudoepitheliomatous hyperplasia, the MMP9 immunomarking was a poor one in the acanthosis and dyskeratosis areas. In the latter ones, the reactivity was higher in the basal cells, namely the cells at the epithelium–chorion interface (Figure 3b). The reactivity decreases progressively towards the interior of the epithelial apices and networks, being more visible in the superbasal cells and adjacent keratinocytes. The reaction pattern was a mainly nuclear one. A relatively similar pattern was also observed in the areas of carcinomatous proliferation in those cases of oral pseudoepitheliomatous hyperplasia associated to squamous carcinomas. Thus, the immunomarking progressively decreases from the periphery towards the center of carcinomatous islands (Figure 3c). The reaction pattern is both a nuclear and a cytoplasmic one.

Both in the sublesional chorion and in the areas of tumoral stroma, we highlighted the presence of a MMP9 reactivity of the vascular endothelial cells, fibroblasts and some of the inflammatory cells present here.

In the chorion of the hyperplastic lesions associated to the pathogenic inflammatory conditions, the MMP9 immunomarking was more highlighted, especially around the epithelial apices (Figure 3d).

In the areas of oral mucosa with pseudoepitheliomatous hyperplasia, the CXCR4 reactivity was observed more often, especially in the areas of acanthosis and dyskeratosis (Figure 3e) and in the epithelial apices, as well. The immunomarking was a cytoplasmic one, especially in the keratinocytes. The maximum of intensity was observed in the keratinocytes of the epithelial apices that descend deeply in the subadjacent chorion (Figure 3f). The cells in the basal stratum did not show any immunoreactivity for CXCR4.

A moderate to high reactivity was observed in the parabasal cells or in the keratinocytes found immediately above these, in the epithelial networks (Figure 3f). Here, as well as in the epithelial apices, there was observed

![Figure 3](image-url)
the CXCR4 co-expression (cytoplasmic pattern) with MMP9 (nuclear pattern) (Figure 3f).

The CXCR4 immunomarking in the epithelial apices of hyperplastic lesions was relatively similar in intensity to the ones in the areas of well-differentiated squamous carcinoma associated to these lesions. In the areas of carcinomatous proliferation areas, the immunomarking intensity gradually decreases towards the periphery, while the nuclear marking for MMP9 becomes more and more visible.

Moreover, in the chorion of the pseudoepitheliomatous hyperplasia lesions, as well as in the tumoral stroma areas, we observed a poor cytoplasmic reactivity for CXCR4 in the vascular endothelial cells, some inflammatory cells and fibroblasts (Figure 3f).

**Discussion**

At present, there are not clear the etiopathogeny and molecular mechanisms involved in the etiopathogeny of pseudoepitheliomatous hyperplasia.

The histopathological studies performed on HE-stained samples suggested the origin of these skin lesions in the interfollicular epithelium, pilous follicles or eccrine units [5]. The association of pseudoepitheliomatous hyperplasia to a series of etiopathogenic conditions suggests the involvement of various intracellular signaling ways in the pathogenesis of these lesions [5]. Thus, various studies suggested the intervention of various growth factors in the etiopathogeny of these lesions, namely EGF (epidermal growth factor) and TGF-α (transforming growth factor alpha) [6–8]. Other authors suggested the intervention of TNF-α and interferon-γ in the genesis of hyperplastic lesions associated to skin leishmaniasis, taking into consideration their expression in the papillary dermis subadjacent to hyperplastic lesions [9]. Another study conducted by Fu et al. highlighted the subexpression of the SCF (stem cell factor) and of its receptor c-kit in the skin lesions that associated pseudoepitheliomatous hyperplasia, thus suggesting their involvement in the etiopathogeny of hyperplastic lesions [10].

**β-Catenin/vimentin reactivity in oral pseudoepitheliomatous hyperplasia**

**β-Catenin** is a protein belonging to the Armadillo family, whose functions depend on its cellular localization, this having the possibility of interacting with other cellular proteins, both at membrane and cytoplasm or nuclear level [11]. In the cellular membrane, β-catenin forms with E-cadherin a complex that promotes intercellular adhesion, thus contributing to the formation and preservation of the squamous layered epithelium of oral mucosa, and, at the same time, it prevents the cell dissociation, so necessary for the cancer invasion and progression [12]. Also, there is a quantity of cytoplasmic β-catenin that acts as a transcription factor of the nucleus, on the Wnt canonic intracellular signaling way, thus leading to the transcription activation of a series of genes with various cellular functions [13]. The studies performed until now have shown that the structural and/or functional changes of β-catenin or the alteration of the molecules with which these interact may promote cancer progression, either by increasing the cellular mobility and invasion secondary to the loss of cellular adhesion functions, or by promoting the oncogene transcription connected to the Wnt canonic way dysregulation [14].

The loss of β-catenin membrane expression seems to be an early event of oral carcinogenesis, this fact being signaled in at least 50% of oral carcinomas [15], being related to the deterioration of the tumoral histological gradient [16] and to a higher lymphoganglionary dissemination rate [17]. In a percentage varying from 0% to 66.6%, premalignant lesions and oral carcinomas presented a cytoplasmic and/or nuclear expression of β-catenin, even when its membrane expression was preserved [15, 18, 19].

The nuclear expression of β-catenin was also related to the dysplasia presence and severity [15, 20], namely as a malignity sign and a poor prognosis in oral squamous carcinomas [15, 18]. Moreover, this abnormal β-catenin expression does not seem to take place in the normal oral epithelium [15]. In a more recent study, there was highlighted the fact that, in the dysplastic lesions of oral epithelium, the membrane expression of β-catenin is gradually lost in the basal and parabasal layers, simultaneously with an increase of its cytoplasmic accumulation at this level, together with the oral leukoplakia progression towards more severe dysplasia stages [21].
In the only literature study that investigated the β-catenin expression in pseudoepitheliomatous hyperplasia, there was highlighted a decrease of the E-cadherin expression at the same time with the increase of β-catenin expression in the epithelial–mesenchymal junction [22]. In our study, in the hyperplastic lesions, we observed an extended membrane reactivity in all the layers of stratum spinosum, and also the presence of a predominant cytoplasmic reactivity, more rarely a nuclear one, of the cells in the basal layer, especially in the epithelial apices that descend deeply in the chorion. In the cases associated to squamous carcinoma, in the carcinoma proliferative areas, we observed a decrease of the membrane reactivity for β-catenin (visible with a lower intensity, especially in the areas of well-differentiated carcinoma) and an increase of its cytoplasmic and nuclear reactivity (especially in the areas of moderately and poorly differentiated carcinoma, the nuclear pattern being a visible one, especially on the invasion front and the periphery of the carcinomatous areas).

Vimentin is a cytoskeletal protein of 57 kDa belonging to type III of proteic intermediary filaments, primarily expressed in the mesenchymal cells, but not in the adult epithelial cells [23]. A series of studies showed the fact that vimentin is also expressed in the migrating epithelial cells involved in embryogenesis, organogenesis, wound healing or tumoral invasion [24–26]. Moreover, there was shown that vimentin is a target of the signaling way of β-catenin/TCF (transcription factor), thus suggesting that this regulation of the epithelial cell function is involved in cell invasion and/or migration [27]. Among the functions of vimentin, there are also included the regulation of intercellular adhesions, subcellular organization and signal transduction from the cellular membrane to the nucleus. Moreover, there was suggested that the in vitro expression of vimentin constitutes a sign of differentiation [28, 29].

Tumor invasion and metastasis could not be possible without the process of epithelial–mesenchymal transition, epithelial malignant cells acquiring migration abilities due to this process precisely [30, 31]. During this process, there occurs a decrease of the E-cadherin expression, abnormal expression of E-cadherin complexes and de novo expression of vimentin [32, 33]. In the study performed by Zhou et al., there was observed the absence of vimentin expression in the oral mucosa, and a cytoplasmic expression in 25% of well-differentiated oral squamous carcinomas, 33.3% of moderately differentiated cases and 66.7% of poorly differentiated oral carcinomas, with no statistically significant differences of this expression and the degree of histological differentiation [34]. Moreover, the authors also observed the existence of higher reactivity in the cases of oral squamous carcinoma that also associated lymphoganglionary metastases, the tumoral reactivity being a higher one at the invasion front in comparison to the center of lesions. Sawant et al. showed that vimentin was also expressed in premalignant oral lesions (hyperplastic, dysplastic and submucous fibrosis), thus existing significant correlations between its expression level and the histopathological degree of these lesions [35]. The authors suggested that this expression plays an important part in the early events of oral tumorigenesis. On the other side, the same authors showed that in the samples of oral squamous carcinomas the vimentin expression level was significantly correlated with the tumor size, clinical stage, regional lymphohangionary metastases, local relapses and the survival of these patients [35]. Moreover, there was also highlighted the fact that the level of vimentin expression at the invasion front was statistically correlated with the development of lymphohangionary metastases and local relapses. Thus, highlighting this marker expression at the invasion front would constitute a prognosis factor, thus allowing the identification of the forms of oral squamous carcinoma with an aggressive potential and, implicitly, the use of an adequate treatment in order to increase the survival chances of these patients.

In the study performed by us, vimentin was expressed only in the epithelial apices of the pseudoepitheliomatous hyperplasia lesions, the immunomarking being more obvious in the peripheral cells, compared to the central ones. Moreover, the most intense reactions were observed in the apices that descended deeply in the sublesional chorion. On the samples that associated squamous carcinoma, the vimentin immunomarking was quite obvious in the carcinomatous proliferations, in comparison to the one in the epithelial apices of hyperplastic lesions.

The double β-catenin/vimentin reaction study allowed the highlighting of a co-localization only in the epithelial apices, namely in the cells inside them, where we observed an abnormal cytoplasmic expression for β-catenin and vimentin. Peripheral cells usually had reactivity only for vimentin, lacking a β-catenin reaction. Such a profile would explain the ability of the oral epithelial cells to acquire mesenchyme valence, deeply migrating into the subadjacent chorion and thus contributing to the formation of epithelial apices from pseudoepitheliomatous hyperplasia.

MMP9/CXCR4 reactivity in oral pseudoepitheliomatous hyperplasia

Matrix metaloproteinases are members of the superfamily of zinc-dependent endopeptidases that work as proteolytic enzymes, involved in the extracellular matrix remodeling as part of the process of normal tissue growth and differentiation [36, 37]. MMP9 is a gelatinase (type IV collagenase) with a main role in tissue remodeling during normal and pathological inflammatory processes [38]. Its main activity resides in regulating the intercellular matrix composition, cleaving the damaged collagen and collagen IV, the latter being a major component of the basal membranes.

Some studies indicate the fact that in the normal or hyperplastic oral mucosa, MMP9 expression is absent, and that this metalloproteinase is not expressed in the dysplastic mucosa either, only very poorly detected in the in situ carcinoma [39]. Other studies indicate a poor cytoplastic reactivity for MMP9 in the proliferation of basal cells in the basal stratum of the normal oral mucosa, and an intensely positive reaction of the basal stratum in dysplastic lesions [40]. Moreover, Jordan et al. showed that oral dysplastic lesions that progressed towards invasive cancers did not have a significantly high level of RNA expression as a messenger for MMP9 [41]. At the same time, it seems that the level of the MMP9 immunohistochemical expression level is correlated with
the dysplasia degree, this being a lot higher in high degree dysplastic lesions than in low degree ones, and significantly lower in comparison to the level of its expression in invasive squamous carcinomas [42].

On the other side, it is known the fact that a high level of metalloproteinases and basal membrane damaging are major events of the cancer invasion process [43]. Thus, high levels of metalloproteinases will facilitate the damaging of the basal membrane and extracellular matrix, thus creating tunnels that will allow cancer cells to migrate and metastasize along the blood and lymphatic vascular system [44]. The same process occurs in the case of head and neck squamous cancers, with numerous studies showing a MMP9 subexpression in tumor samples [45, 46] and a significant correlation of its expression levels with regional relapses and distance metastases [47–49]. Other studies showed the existence of certain associations between high levels of the MMP9 expression in neoplastic and stromal cells and the high aggressiveness of these cancers, resulting in a high mortality rate among patients with head and neck squamous cancers [50].

The only study that investigated the MMP9 expression in pseudop epitheliomatous hyperplasia indicated the absence of its reaction in the epithelium [51]. In our study, the MMP9 immunomarking was present in hyperplastic lesions with a predominantly nuclear pattern, especially in the cells at the epithelium–chorion interface and in the epithelial apices that descend deeply in the chorion. Moreover, the chorion of the epithelial apices and networks also presented an intense immunomarking for MMP9. Such an immunomarking would explain the infiltrative increase in the chorion depth of these hyperplastic lesions based on the MMP9 pole present at the chorion–epithelium interface, which would facilitate the migration of epithelial cells, especially in those lesions associated to inflammatory conditions.

CXCR4 is an α-chemokine receptor that belongs to the superfamily of G-protein coupled receptors (GPCRs) that selectively bind to the stromal-derived factor-1 cells (SDF-1) [52] and that is directly involved in a series of biological processes, among which organogenesis, hematopoiesis, immune response and vascularization [53–55]. Quite recently, there was shown that chemokines and their correspondent receptors are involved in the onset and progression of human cancers [56].

The CXCR4 expression is either low, or absent in many of the normal tissues, like the mammary gland and the ovary [57]. In the oral epithelium, the CXCR4 reactivity is generally quite low, thus being reported a positivity rate of 15.4% and with a lower intensity [58]. For premalignant oral lesions, there was reported a rate of the CXCR4 expression of 37.5%, with no significant differences compared to the expression rate in normal mucosa [58]. In oral dysplastic lesions, the immunomarking was predominantly a cytoplasmic one, but in some cells and nucleary it was evenly distributed in all its layers, with a poor to moderate reactivity also observed in the vascular endothelial cells of the sublesional chorion. Because in preneoplastic lesions there is a high expression rate for SDF-1 ([50] over 50% of cases) there was suggested that the SDF-1/CXCR4 axis may play a major part in the early events of oral malignant transformation, thus contributing to the progression of oral carcinogenesis [58]. At the same time, there was shown that in over 60% of primary squamous carcinomas there exists a CXCR4 expression, this expression being significantly associated to lymphoganglionary metastasis, invasion manner, tumor relapses and the prognosis of these patients [59].

In the studied pseudop epitheliomatous hyperplasia lesions, we highlighted the presence of a cytoplasmic reactivity for CXCR4, especially in the keratinocytes of the acanthosis, dyskeratosis areas and of epithelial apices. The maximum intensity was observed inside the epithelial apices that descend deeply in the sublesional chorion. The marking intensity at this level was almost identical to the one obtained in the carcinomatous proliferation areas of squamous carcinomas associated to the hyperplastic lesions. Moreover, at the epithelium–chorion interface of hyperplastic lesions and especially in the epithelial apices, we observed a progressive decrease of the CXCR4 marking intensity down to almost absent towards the interface, while the MMP9 immunomarking had a reverse behavior, namely a maximum intensity at the interface and almost absent towards the inside of epithelial apices. Such a phenotype would explain the pathogenesis of pseudop epitheliomatous hyperplasia lesions via the CXCR4 receptors inducing the MMP9 expression, and from here the epithelial front advancement towards the subjacent chorion.

As a matter of fact, some studies showed that CXCR4 is responsible for promoting the migration and invasion of oral squamous carcinomas, by inducing the expression of metalloproteinases 9 and 13 as a result of the activation of ERK (extracellular signal-regulated kinases) signaling way [50, 60, 61]. On the other side, the oral carcinomatous cells in which the CXCR4 expression was blocked proved to have a low invasiveness and a significantly lower growth rate [62]. All these data show us that CXCR4 may become a useful target in the treatment of patients suffering from oral cancer.

**Conclusions**

Our study highlighted the existence of a profile characterized by a cytoplasmic and nuclear positivity for β-catenin, and a cytoplasmic positivity for vimentin, respectively, in the basal stratum cells of the epithelial apices, a fact that could explain the ability of the oral epithelial cells to acquire mesenchyme valences, by deeply migrating in the subjacent chorion and, thus, contributing to the formation of epithelial apices in pseudop epitheliomatous hyperplasia. Moreover, the presence of a progressive decrease of CXCR4 reactivity towards the epithelium–chorion interface, and especially in the epithelial apices, may explain the pathogenesis of pseudop epitheliomatous hyperplasia lesions via CXCR4 receptors that may induce the MMP9 expression and, thus, the advancement of the epithelial apices in the subjacent chorion.

**Conflict of interests**

The authors declare that they have no conflict of interests.

**References**


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