Journal of Dentistry & Oral Disorders

Open Access

Austin Publishing Group

Research Article

Immunohistochemical Analysis of Ezrin Expression in Oral Premalignant Lesions

Temelie-Olinici D[1], Cotrutz CE[1]*, Hriţcu-Condurache OM[1]*, Dimitriu DC[1], Sin IA[2] and Gheucă-Solovăstru L[3]

[1]Department of Morpho-Functional Sciences II, "Grigore T. Popa" University of Medicine and Pharmacy Iasi, Romania

[2]Department of Cell and Molecular Biology, University of Medicine and Pharmacy Targu Mures, Romania

[3]Department of Dermatology, "Grigore T. Popa" University of Medicine and Pharmacy Iasi, Romania

***Corresponding author:** Carmen Elena Cotrutz, Department of Morpho-Functional Sciences II "Grigore T. Popa" University of Medicine and Pharmacy, Romania; Oana Mihaela Hriţcu-Condurache, Department of Morpho-Functional Sciences II "Grigore T. Popa" University of Medicine and Pharmacy, Romania

Received: April 27, 2020; **Accepted:** May 15, 2020; **Published:** May 22, 2020

Abstract

Objectives: Across the world, the incidence and prevalence of oral premalignant lesions have increased significantly. These lesions are the precursors to squamous cell carcinoma, one of the most aggressive types of cancer to be diagnosed over the past 30 years. Their phenotypical and molecular variability justifies a sitch from histopathological to molecular diagnosis in order to identify and describe the risk of malignancy. Ezrin, a molecule the ERM protein family (Ezrin-Radixin-Moezin), is one of the latest identified "hot spots" of tumor metastasis mechanisms.

Materials and Methods: Oral mucosal fragments were collected from forty-three clinically and histopathological diagnosed with oral premalignant lesions such as leukoplakia, erosive oral lichen planus and erosive actinic cheilitis have been processed through the immunohistochemical technique using Ezrin Polyclonal Antibody (Cell Signaling TechnologyR - BioZyme antibody). The immunohistochemical samples were examined using the Olympus BX40 photonic microscope with Olympus E330 photo camera attached.

Results: The results reveal an ezrin overexpression in the oral premalignant lesions analyzed, compared with the normal mucosa, indicative of an increased risk of malignant transformation.

Conclusion: Concurrently, our study highlights the importance of further research into the identification of specific markers allowing for targeted and individualized therapeutic strategies.

Keywords: Oral premalignant lesions; Ezrin expression; Squamous cell carcinomas; Malignant transformation; Immunohistochemical study

Introduction

Physiopathological processes in the oral mucosa are highly complex and impact the health of the entire body. The oral mucosa is where premalignant and malignant lesions may easily occur, due to its poor resistance to chemical, mechanical and traumatic irritations [1].

Oral lesions precursor to squamous cell carcinomas are becoming more and more common worldwide. These carcinomas are extremely aggressive in their complications both in situ as well as elsewhere in the body. Statistically, this also translates into a level of morbidity and mortality increasing with over 5% every year [2,3].

In contrast to other types of epithelial premalignant and malignant lesions, the absence of clinical manifestations which characterize most lesions occurring in the oral mucosa makes early diagnosis difficult and thus contributes to poorer prognosis and survival rates [4].

Most frequently, the development of oral squamous cell carcinoma is a multi-step process during which epithelial cells transform into preneoplastic cells and then tumor cells, gradually undergoing multiple morphological and molecular changes. Consequently, in over 50% of cases, this type of carcinoma occurs in premalignant lesions such as leukoplakia, erythro-plakia/erythroleukoplakia, oral submucous fibrosis, and oral lichen planus. Tobacco keratosis, leukoedema, leukoderma, discoid lupus erythematosus, and bullous epidermolysis are less common [5].

The rate of these lesions' malignant transformation is approximately 17% over a period of 7 years following diagnosis. The highest is in the case of heterogenous erythroplakia and erythroleukoplakia with dysplastic changes. However, some studies have found that 16-62% of leukoplakia lesions feature modifications indicative of squamous cell carcinoma at the time of diagnosis [1,6,7].

The clinical diagnosis is in most cases difficult because the subjective and objective signs are not specific and do not adequately reflect the degree to which epithelial and connective tissues are affected. In addition, patients do not seek dermatological and/or dental consultations until in advanced stages, which is unfortunate [8,9].

Although some researchers consider anatomopathological examination as the gold standard in evaluating the potential for malignant transformation, this method comes with its own limitations. For one, risk assessment is done mainly based on the presence/absence of dysplasia as suggested by certain structural and cytological changes [1,10-12].

Therefore, as far as epithelial dysplasia is concerned, histopathological diagnosis is to a large extent subjective and depends

Cotrutz et al. © All rights reserved

Citation: Temelie-Olinici D, Cotrutz CE, Hriţcu-Condurache OM, Dimitriu DC, Sin IA and Gheucă-Solovăstru L. Immunohistochemical Analysis of Ezrin Expression in Oral Premalignant Lesions. J Dent & Oral Disord. 2020; 6(3): 1134.

greatly on the anatomopathologist's level of expertise. There may be cases in which carcinomas develop freely in dysplastic lesions after having gone unidentified/undiagnosed in previous biopsies. The situation is further complicated by the fact that not all such lesions turn malignant and some may even regress. Also, the prognosis of premalignant and malignant lesions cannot be established with precision based only on their histopathology [7].

These current limitations justify the need for the identification of markers sensitive and specific enough for premalignant lesions precursor to oral squamous cell carcinoma, so that these lesions may be accurately and timely diagnosed, and their potential for malignant transformation adequately assessed. To this end, the study of molecular changes specific to oral premalignant lesions as early as their initial stages of development is an important step forward.

Recently, the ERM molecules (Ezrin-Radixin-Moesin) have been studied and evaluated in certain types of cancers. Ezrin has been found to be involved in pathways which regulate cell survival, proliferation, and migration, as well as in the regulation of cell-cell and cell-matrix adhesion. The suppression of these three proteins is also known now as a determining factor in cellular coupling disorganization [13-15].

Ezrin or cytovillin is a protein present in the plasmalemma of the apical pole of epithelial cells and it achieves the connection between the cytoskeleton and cellular membranes. It binds the F-actin to the cellular membrane, after phosphorylation, and is thus essential to many fundamental cellular processes. It also activates GTPases and RhoA leading to cytoskeletal remodeling, an important process in cell motility, proliferation, differentiation and migration [16-18].

In keratinocytes, the overexpression of E-cadherin and β-catenin may be associated with ezrin suppression, while ezrin expression correlates positively with the ki-67 index, a marker of tumor proliferation and severity [18-20].

A number of studies underline ezrin's role in tumor development and progression, thus suggesting its applicability as a biomarker useful in establishing the appropriate therapeutic approach in the case of head and neck carcinomas [21].

Some research has also found that ezrin expression is associated with the aggressive phenotype of prostate adenocarcinoma, breast cancer, astrocytoma, uveal melanoma, and soft tissue sarcoma. In such cases, on one hand, elevated levels of ezrin were detected in secondary tumor cells compared to primary ones, suggesting that ezrin plays an important role in metastasis, and, on the other hand, certain therapies were seen to influence the expression of this molecule [14].

By taking all of the above into consideration, and noticing the scarcity of published studies regarding ezrin expression in skin carcinomas (with only one study on skin premalignant lesions and none about oral ones), we sought to conduct this immunohistochemical analysis of the molecule in oral premalignant lesions such as leukoplakia, erosive oral lichen planus and erosive actinic cheilitis.

Material and Method

For the purposes of this research, samples of oral mucosa were collected from 43 patients by means of incisional/excisional biopsy with a scalpel blade. The patients were all non-smoker, non-drinker women aged between 45-60, diagnosed clinically and histopathologically with leukoplakia (28 cases, of which 20 with light-moderate dysplasia and 8 with moderate to severe dysplasia), erosive actinic cheilitis (10 cases), and erosive oral lichen planus (5 cases). Concurrently, oral mucosa normal in appearance was sampled from the same patients with the same technique, making up the control samples.

Prior to the surgical intervention, each patient gave her informed consent in accordance with the legislation in place. The anatomopathological diagnosis was given at the Anatomical Pathology and Morgue Service of the Railways Clinical Hospital Iași, and at the Anatomical Pathology Laboratory of the Emergency Military Hospital "Dr. I. Czihac" Iași. Some of the tissue samples were processed using the paraffin embedding technique, then sectioned and colored with hematoxylin and eosin in order to be diagnosed histopathologically.

Another batch was processed immunohistochemically using the NovoLinkTMMax Polymer Detection System kit produced by Biosystems Newcastle Ltd and Ezrin Polyclonal Antibody (Cell Signaling TechnologyR -BioZyme) as follows: fixation in neutral formaldehyde, dehydration in ethanol in incremental concentrations, clearing with xylol, embedding in paraffin, sectioning and placement on electrostatically charged slides, deparaffinization with xylene, ethanol hydration, tap water cleaning, application of Ezrin Polyclonal Antibody diluted 1/100 µl, washing with deionized water, endogenous peroxidase block with Peroxidase Block for 5 minutes, washing with PBS for 2x5 minutes, 30-min PostPrimary Block incubation, washing in PBS for 2x5 minutes, 30-min NovoLink Polymer incubation, washing in PBS for 2x5 minutes, application of DAB solution (50 µl -1ml), washing, countercoloring with hematoxylin, washing, dehydration, clarifying, mounting on slide and then examination with Olympus BX40 microscope and photography with Olympus E330 camera at the Discipline of Cellular and Molecular Biology, "Grigore T. Popa" University of Medicine and Pharmacy Iași, Romania.

Results

The analysis of oral mucosa samples processed via the standard technique for hematoxylin eosin coloration revealed signs indicative of the following diagnoses: leukoplakia with mild-moderate dysplasia (hyperplastic epithelial tissue with moderate, hyper and parakeratosis as well as marked acanthosis , the widening of the stratum granulosum and rare typical mycoses in the stratum basale, stroma with edema, vascular ectasia and polymorphous inflammatory infiltrate, as shown in Figure 2a; leukoplakia with moderate--severe dysplasia (epithelium with hyperkeratosis, marked hypergranulosis, moderate and focal acanthosis, focal acantholysis of stratum basale, stroma with edema, vascular ectasia and polymorphous inflammatory infiltrate, as shown in Figure 2b; erosive oral lichen planus (hydropic degeneration of stratum basale, acanthosis with formation of sharp papillary buds, discontinuous hypertrophy of stratum granulosum, hyperkeratosis, subepidermal blisters, band-like lymphohistiocytic inflammatory infiltrate at dermal level, Civatte bodies, as shown in Figure 2c; erosive actinic cheilitis (erythema, edema, ulceration, hyperkeratosis, and mild acanthosis, lymphohistiocytic infiltrate perivascularly and around the glandular ducts, as shown in Figure 2d.

The results of the immunohistochemical analysis aiming to

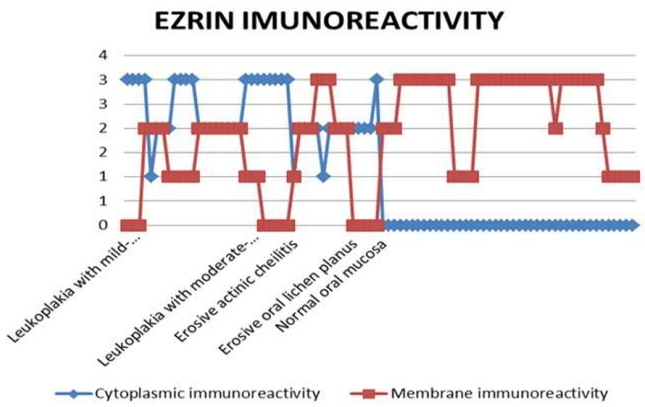

Figure 1: Ezrin Imunoreactivity.

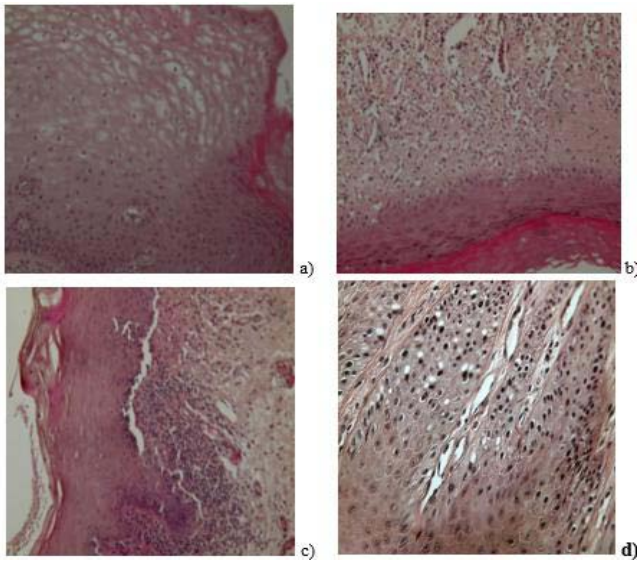

Figure 2: Oral mucosa. a) Leukoplakia with mild-moderate dysplasia. HE; 40x; b) Leukoplakia with mild-severe dysplasia. HE; 20x; c) Erosive oral lichen planus. HE; 20x; d) Erosive actinic cheilitis; HE; 40x.

identify ezrin expression, a little-studied molecule in the context of skin premalignant lesions and so far overlooked with regard to oral premalignant lesions, revealed a series of modifications in the case of tissue samples collected from premalignant lesions compared to those taken from normal oral mucosa.

Notably, the examination of normal oral mucosa samples processed via the above mentioned immunohistochemical technique indicated moderate positive perimembranous immunoreactivity to ezrin and lack of immunoreactivity to it cytoplasmically (Figures 1, 3).

By comparison, the examination of samples taken from leukoplakia-type lesions showed intense positive intracytoplasmic immunoreactivity even in the superficial stratum with parakeratosis, as well as perimembranous granular immunoreactivity, with a series of differences between the two types of leukoplakia encountered. Specifically, in the less severe forms of leukoplakia associated with mild dysplasia we found intense granular, perimembranous and

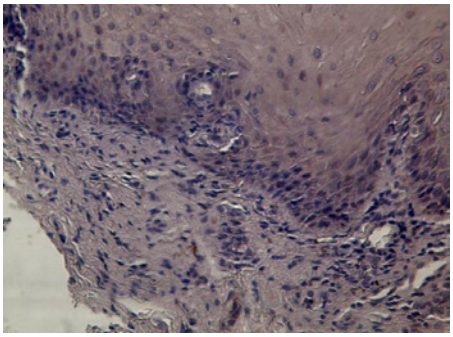

Figure 3: Normal oral mucosa; IHE; 40x.

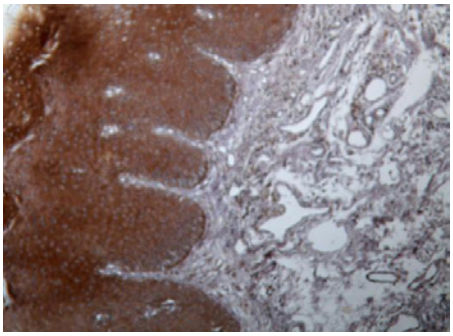

Figure 4: Leukoplakia with mild-moderate dysplasia; IHE; 40x.

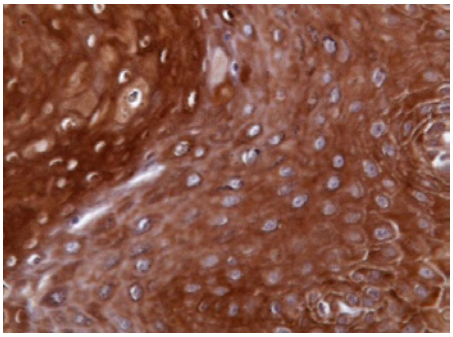

Figure 5: Leukoplakia with mild-moderate dysplasia IHE 40x.

intracytoplasmic immunopositivity up to the superficial parakeratotic layer (Figures 4,5), compared to a far more subdued reaction at membrane level, but intensely positive intracytoplasmically in severe forms of leukoplakia associated with severe dysplasia (Figures 1, 6).

In the case of erosive oral lichen planus, the tissue samples indicated punctiform cytoplasmic and drop-like perinuclear immunopositivity in stratum basale and the malpighian layer (Figures 1, 7).

Last but not least, the immunohistochemistry of erosive actinic cheilitis cases featured membranous and mild cytoplasmic immunopositivity to ezrin in stratum basale and the malpighian layer (Figures 1, 8).

Discussion

Our immunohistochemical study of ezrin is motivated by the fact that this molecule, through its influence on pathways and

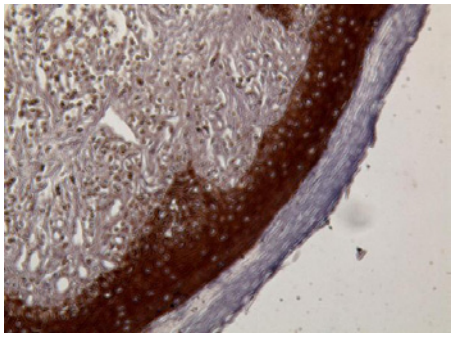

Figure 6: Leukoplakia with moderate-severe dysplasia. IHE; 40x.

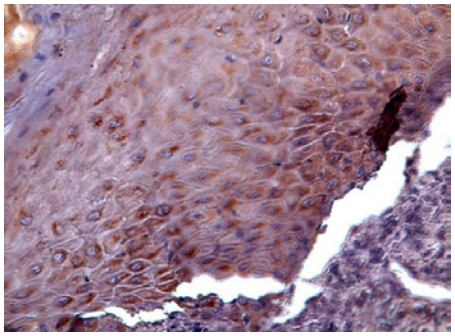

Figure 7: Erosive oral lichen planus. IHE; 40x.

mechanisms of malignant transformation, may be used as a biomarker for the early detection of such transformations in the oral mucosa. Ezrin's full potential in this regard is yet to be fully explored by research centers specializing in oral pathology and shared in the scientific literature.

The role that ezrin plays in intercellular and extracellular interactions, as well as in complex cellular adhesion mechanisms justifies the immunohistochemical assessment of its expression in the normal tissues and premalignant lesions of the oral mucosa.

Ezrin, raxidin, and moesin are tissue-specific, with ezrin being present mostly in epithelial cells, moesin in apical, luminal endothelial cells, and radixin in hepatocyte. Also, ezrin and radixin are found in epithelial cells limiting bile ducts and all three molecules feature in the epithelium of proximal renal tubules [22,23].

In parietal cells, ezrin is an anchor protein for the regulatory unit of protein kinase A (PKA) [23,24].

Although significant similarities have been identified in the amino acid sequencing of these proteins, their functions are quite diverse. For instance, ezrin and radixin are present in the apex of polarized cells and act as substrates for certain tyrosine kinases [23-25].

Ezrin features a redistribution during the development of cells with microvilli, and its dephosphorylation in serine/threonine residues is associated with microvilli disassembly during renal anoxia [22].

Ezrin occurs in two forms: active, which is physiologically located in the membrane, and inactive, found in the cytoplasm [24,26].

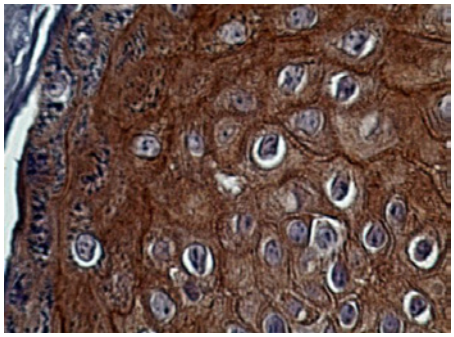

Figure 8: Erosive actinic cheilitis. IHE; 100x.

In the cytoplasm, ezrin, similar to the other ERM proteins, is expressed as dormant monomers with masked F-actin and plasmalemma binding sites. In addition, its configuration is closed due to intramolecular bonds between the N and C terminals, for the activation of which the bonds must be broken. Two factors are known to play a role in activation: PIP2 binding and the phosphorylation of C-terminal threonine residue [15,23-25].

In light of our results, which highlight the perimembranous expression of ezrin in normal oral mucosa and the lack of cytoplasmic immunoreactivity to this molecule (Figure 3), these aspects point to ezrin having a functional role in organizing cellular coupling and membrane features specific to each type of tissue. The intense cytoplasmic immunoreactivity in the case of erosive oral lichen planus and leukoplakia with moderate-severe dysplasia (Figures 6,7) suggests that epithelial cells undergo a modification of molecular phenotype, dormant monomers from the cytoplasm are activated, and phosphorylation reactions are triggered at this level. Such findings also indicate a clear tendency towards malignant transformation and an aggressive malignant phenotype, given reports in the literature about how cytoplasmic location in case of squamous cell carcinoma in the head and neck are associated with poor prognosis and reduced survival rates.

In the case of erosive actinic cheilitis, considered to be the mucous equivalent to cutaneous actinic keratoses, membranous immunopositivity is detected in all the layers, and a mild cytoplasmic reaction in stratum spinosum (Figure 8). This confirms theories in the literature according to which such lesions share etiopathogenic features indicative of similar mechanisms of carcinogenesis. These observations are supported also by the similarity and prevalence of ultrastructural changes found in lesions analyzed electronomicroscopically in a previous study by our team [27].

The mechanism responsible for the cytoplasmic location of ezrin as well as the way in which cytoplasmic ezrin contributes to the aggressive phenotype of squamous cell carcinoma is yet to be fully understood [28].

The results of molecular research suggest that numerous signal transduction pathways may be involved in the activation of ezrin as well as in its intermediary statuses [29].

According to published data, ezrin is directly involved in those mechanisms by which a series of cytoskeletal regulatory proteins are positioned near the actinic cytoskeleton or a series of receptors

in the area of signal molecules, thus playing a role in the molecular organization of plasmalemma and cortical cytoskeleton, as well as in the stability of adhesion junctions and the integrity of epithelia [30].

Recent studies have described ERM capacity to interact with transmembrane proteins, phospholipids, cytoplasmic proteins associated to the membrane and cytoskeleton, and to parti-cipate in numerous physiological processes, among which preserving cellular shape, motility, proliferation and development [31].

This grants ezrin a key role in organizing specific membrane areas ssential in intercellular signaling [32].

The active, functional part in ezrin may bind to the cytoplasmic end of many membranous proteins including CD44, CD43, and ICAM2. Interaction with the latter is also important in the activation of natural killer cells (NK) [29].

In this respect, the intense cytoplasmic immunopositivity to ezrin noted in this study in the case of erosive oral lichen planus and leukoplakia with moderate-severe dysplasia points to a possible interaction between ezrin and ICAM2 as molecular substrate for changes in the transduction mechanisms of certain extracellular signals characteristic to malignant transformation and, as such, constitutes another severity feature in these lesions.

It is known that cell migration and tumor invasiveness depend on the active remodeling of the actinic cytoskeleton. In their studies, Yo et al. demonstrated a correlation between ezrin and the risk of metastasis, and this hypothesis was also confirmed by the ezrin overexpression found in secondary tumor cells compared to primary ones [33,34].

Increased ezrin expression and activity in secondary tumors was investigated mostly in osteosarcoma and rhabdomyosarcoma. One outcome has been the understanding that ezrin expression differs depending on the stage of tumor development, and increases significantly in the context of tissue invasion and metastasis compared to its behavior in the primary tumor [35].

The metastasis regulatory role is also highlighted in an experimental study into osteosarcoma using mice, in which ezrin suppression correlated with a significant reduction in pulmonary metastases [36,37].

Moreover, a series of immunohistochemical studies aimed at understanding ezrin expression in different types of tumors revealed significant correlations between increased immunoreactivity to ezrin and the tumors histological grade. Also, in the case of pancreatic adenocarcinoma, increased ezrin expression correlated positively with metastasis potential [34,36,38-40].

With regard to melanoma cells, studies have shown that ezrin overexpression contributes to elevated levels of MAPK and stimulates their proliferation. MAPK signaling is known to regulate cell differentiation, proliferation, and survival. Inhibiting this pathway in melanoma cells leads to apoptosis and is thus a target for inhibitory pharmacological therapy [36,40].

In a recent study, Ilmoneu et al. were able to associate increased ezrin immunoreactivity with a higher mortality rate in patients with uveal and cutaneous melanomas. Thus, immunoreactivity correlates with the tumor proliferative index, which means that tumor cells in which immunoreactivity is absent also feature a low proliferative index [32].

In uveal and cutaneous melanomas immunoreactivity is diffuse, granular cytoplasmic. Two-thirds of uveal melanoma cases present increased ezrin immunoreactivity correlated significantly with microvascularization density, an indicator for tumor angiogenesis [36,40].

Similarly, in cutaneous melanoma, the prognosis for patients with intense positive ezrin immunoreactivity is poor compared to those with weak or absent positive immunoreactivity [36,40].

In light of the above, we may state that the membranous ezrin expression and mild cytoplasmic immunoreactivity detected in our study in the case of erosive actinic cheilitis indicates, on one hand, the premalignant status of these lesions, and, on the other hand, a less aggressive evolution compared to erosive oral lichen planus and leukoplakia with moderate-severe dysplasia.

It is possible, given how ezrin supports the stability of adhesion junctions in quasi-normal conditions, that the intense or moderate cytoplasmic immunoreactivity and the membranous immunoreactivity kept at a lower intensity level do not necessarily entail the complete unraveling of intercellular bonds. The disintegrated material substrate continues to maintain polarity under the influence of extracellular signals, to the reception of which ezrin continues to participate still undisturbed and unconverted to another type of proliferation and evolution. This appears to be a counterreaction to the inevitable progression towards malignant transformation.

Conclusion

Because the available literature lacks reports of ezrin expression in the oral mucosa of patients with premalignant lesions, the results of this study can only be assessed in relation to similar research on other types of carcinomas and cellular structures.

Given that ezrin overexpression has already been linked to aggressive tumor phenotypes in prostate adenocarcinoma, breast cancer, astrocytoma, uveal melanoma, soft tissue sarcoma, by extrapolation, our results may be seen as indicative of malignant evolution in the studied lesions.

In light of this and other data published in the literature pointing to ezrin expression as a potential biomarker of malignant transformation and prognosis in various types of tumors, we believe that it would prove useful to assess ezrin expression in dysplastic oral premalignant lesions while monitoring the clinical evolution of the lesions in question.

Concurrently, additional research is needed into unraveling the connections between ezrin expression and location, and the influence of various therapeutic strategies/approaches used in treating these lesions. Last but not least, ezrin intensity and location in oral premalignant lesions should be monitored in a study which also takes into consideration the immune status of the patients.

References

1. Nair DR, Pruthy R, Pawar U, Chaturvedi P. Oral cancer: Premalignant conditions and screening-an update. J Cancer Res Ther. 2012; 8: S57-66.

2. Abdou AG, Maraee AH, El-Sayed EM, Elnaidany NF. Immunohistochemical expression of ezrin in cutane-ous basal and squamous cell carcinomas. Ann Diagn Pathol. 201; 15: 394-401.

3. Abdou AG, Maraee AH, El-Monaem Shoeib MA, Abo Saida AM. Maspin expression in epithelial skin tumours: an immunohistochemical study. J Cutan Aesthet Surg. 2011; 4: 111-117.

4. Juretić M, Cerović R, Belušić-Gobić M, Brekalo Pršo I, Kqiku L, Špalj S, et al. Salivary levels of TNF-α and IL-6 in patients with oral premalignant and malignant lesions. Folia Biol (Praha) 2013; 59: 99-102.

5. Villa A, Gohel A. Oral potentially malignant disorders in a large dental population. J Appl Oral Sci. 2014; 22: 473-476.

6. Carnelio S, Rodrigues GS, Shenoy R, Fernandes D. A brief review of common oral pre-malignant lesions with emphasis on their management and cancer prevention. Indian J Surg. 2011; 73: 256-261.

7. Reibel J. Prognosis of oral premalignant lesions: significance of clinical, histopathological, and molecu-lar biological characteristics. Crit Rev Oral Biol Med. 2003; 14: 47-6.

8. Rojas IG, Martínez A, Brethauer U, Grez P, Yefi R, Luza S et al. Actinic cheilitis: epithelial expression of COX-2 and its association with mast cell tryptase and PAR-2. Oral Oncol. 2009; 45: 284-290.

9. de Oliveira Miranda AM, Miranda Ferrari T, Leite TC, Cunha KS, Dias EP. Actinic Cheilitis: Clinical Characteristics Observed in 75 Patients and a Summary of the Literature of This Often Neglected Premalignant Disorder. Int J Clin Med. 2014; 5: 1337-1344.

10. Messadi DV. Diagnostic aids for detection of oral precancerous conditions. Int J Oral Sci. 2013; 5: 59-65.

11. Dudás J, Fullár A, Bitsche M, Schartinger V, Kovalszky I, Sprinzl GM et al. Tumor-produced, active interleu-kin-1β regulates gene expression in carcinoma-associated fibroblasts. Exp Cell Res. 2011; 317: 2222-2229.

12. Shrestha B, Subedi S, Bayracharya D, Radhakrisnan R. Immunohistochemical expression of MMP-2 in an experimental progression model of oral cancer. JCMC. 2014; 4: 3-8.

13. Safi AF, Nickenig HJ, Rothamel D, Zirk M, Thiele O, Grandoch A, et al. Expression of ezrin in oral squamous cell carcinoma: Prognostic impact and clinicopathological correlations. J Craniomaxillofac Surg. 2015, 43: 1899-1905.

14. Mhawech-Fauceglia P, Dulguerov P, Beck A. Value of ezrin, maspin and nm23-H1 protein expressions in predicting outcome of patients with head and neck squamous-cell carcinoma treated with radical radiotherapy. J Clin Pathol. 2007; 60: 185-189.

15. Sun P, Cheng F, Jiang T, Liang SZ. The expression of ezrin protein in oral squamous cell carcinoma. Foreign Med Sci: Stomatol. 2013; 40: 6-9.

16. Gamei MM, Abd El-Naby NM, El Ashmawy AA, Shareef MM. Prognostic value of ezrin expression in common epithelial tumors: an immunohistochemical study. JMAU. 2014; 2: 67-76.

17. Garcia AS, Tjioe KC, Nonogaki S. Ezrin and podoplanin expressions are associated in invasion front of lip cancer. Head Neck Oncol. 2014; 6: 8.

18. Saito S, Yamamoto H, Mukaisho K, Sato S, Higo T, Hattori T, et al. Mechanisms underlying cancer progre-ssion caused by ezrin over expression in tongue squamous cell carcinoma. PLoS One. 2013; 8: e54881.

19. Bagheri M, Eghtedari M, Bagheri M. Expression of maspin and ezrin proteins in pe-riocular basal cellcar-cinoma. Dermatol Res Pract. 2014; 2014: 596564.

20. Wu B, Li J, Huang D. Baicalein mediates inhibition of migration and invasiveness of skin carcinoma through ezrin in A431 cells. BMC Cancer. 2011; 11: 527.

21. Li J, Wei K, Yu H. Prognostic value of ezrin in various cancers: a systematic review and updated metaa-nalysis. Sci Rep. 2015; 5: 17903.

22. Bretscher A, Reczek D, Berryman M. Ezrin: a protein requiring conformational activation to link microfilaments to the plasma membrane in the assembly of cell surface structures. J Cell Sci. 1997; 110: 3011-3018.

23. Eckert AW, Wickenhauser C, Salins PC. Clinical relevance of the tumor microen-vironment and immune escape of oral squamous cell carcinoma. J Transl Med. 2016; 14: 85.

24. Supp DM, Wilson-Landy K, Boyce ST. Human dermal microvascular endothelial cells form vascular analogs in cultured skin substitutes after grafting to a thymic mice. FASEB J. 2002; 16: 797-804.

25. Pujuguet P, Del Maestro L, Gautreau A. Ezrin regulates E-cadherin-dependent adherens junction assembly through Rac1 activation. Mol Biol Cell. 2003; 14: 2181-2191.

26. Rahman MA, Amin AR, Shin DM. Chemopreventive potential of natural compounds in head and neck cancer. Nutr Cancer. 2010; 62: 973-987.

27. Olinici D, Cotrutz CE, Mihali CV. The ultrastructural features of the premalignant oral lesions. Rom J Morphol Embryol. 2018; 59: 243-248.

28. Kanitakis J, Petruzzo P, Gazarian A. Premalignant and Malignant Skin Lesions in Two Recipients of Vascularized Composite Tissue Allografts (Face, Hands). Case Report Transplant. 2015; 2015: 1-4.

29. Soares CP, Zuanon JA, Teresa DB, Fregonezi PA, Neto CB, Oliveira MR, et al. Quantitative cell-cycle pro-tein expression in oral cancer assessed bycomputer-assisted system. Histol Histopathol. 2006; 21: 721-728.

30. Yadav A, Kumar B, Datta J, Teknos TN, Kumar P. IL-6 promotes head and neck tumor metastasis by indu-cing epithelial-mesenchymal transition via the JAK-STAT3-SNAIL signaling path-way. Mol Cancer Res. 2011; 9: 1658-1667.

31. Rajkumari S, Sathiyajeeva J, Kumar CS, Sunil PM, Thayumanavan B. Molecular predictors in the early diagnosis of oral cancer. J Clin Diagn Res. 2013; 7: 942-944.

32. Ilmonen S, Vaheri A, Asko-Seljavaara S, Carpen O. Ezrin in primary cutaneous melanoma. Mod Pa-thol. 2005; 18: 503-510.

33. Breitkreutz D, Koxholt I, Thiemann K, Nischt R. Skin Basement Membrane: The Foun-dation of Epidermal Integrity—BM Functions and Diverse Roles of Bridging Molecules Nidogen and Perlecan. International BioMed Research. 2013; 2013: 1-16.

34. Wood NH, Khammissa R, Meyerova R, Lemmerb J, Fellera L. Actinic Cheilitis: A Case Report and a Review of the Literature. Eur J Dent. 2011; 5: 101-106.

35. Prunier C, Masson-Genteuil G, Ugolin N, Sarrazy F, Sauvaigo S. Aging and photo-aging DNA repair pheno-type of skin cellsevidence towardan effect of chronic sunexposure. Mutat Res. 2012; 736: 48-55.

36. Roy A, Bhatnagar SP, Chatterjee M, Ghosh D, Mandal G, Mondal S, et al. Study of p53, PCNA, Ki-67 and Micronuclei Related to Genotoxic Damage for Risk Categorization in Pre-Malignant Oral Lesions. JCRT. 2014; 2: 10-15.

37. Pelengaris S, Littlewood T, Khan M, Elia G, Evan G. Reversible activation of c-Myc in skin: induction of a complex neoplastic phenotype by a single oncogenic lesion. Mol Cell. 1999; 3: 565-577.

38. Khalesi M, Whiteman DC, Tran B, Kimlin MG, Olsen CM, Neale RE. A metaanalysis of pigmentary charac-teristics, sun sensitivity, frecklingand melanocytic nevi and risk of basal cell carcinoma of the skin. Cancer Epidemiol 2013; 37: 534-543.

39. Kanitakis J, Petruzzo P, Gazarian A, Testelin S, Devauchelle B, Badet L, et al. Premalignant and Malignant Skin Lesions in Two Recipients of Vascularized Composite Tissue Allografts (Face, Hands). Case Rep Transplant. 2015; 2015: 1-4.

40. Clucas J, Valderrama F. ERM proteins in cancer progression. J Cell Sci. 2014; 127: 2667- 275.