ORIGINAL PAPER

RJME
Romanian Journal of Morphology & Embryology
http://www.rjme.ro/

E-cadherin and α-SMA expression in the epithelial–mesenchymal transition of salivary glands pleomorphic adenomas

AURELIA ENESCU[1)], ANCA ŞTEFANIA ENESCU[2)], CHAROULA FLOROU[3)], FLORIN PETRESCU[4)]

[1)]*Department of Emergency Medicine, University of Medicine and Pharmacy of Craiova, Romania*
[2)]*Research Center for Microscopic Morphology and Immunology, University of Medicine and Pharmacy of Craiova, Romania*
[3)]*PhD Student, Department of Histology, University of Medicine and Pharmacy of Craiova, Romania*
[4)]*Department of Internal Medicine, University of Medicine and Pharmacy of Craiova, Romania*

Abstract

The pleomorphic adenoma, known also as mixed tumor of epithelioma with reshuffling stroma, is the most frequent salivary gland tumor, representing 45–75% of the total salivary gland tumors. In our study, we have investigated the immunohistochemical expression of E-cadherin and alpha-smooth muscle actin (α-SMA) in 15 cases of pleomorphic adenoma of salivary glands. We noticed the constant presence of E-cadherin reactivity at the level of luminal cells that cover the proliferative structures of ductal-cystic type, which gradually disappears to the periphery. At the level of epithelial proliferative solid areas, the reactivity for E-cadherin was inconstant and heterogeneous, while α-SMA expression increased. This aspect indicated the involving of epithelial–mesenchymal transition process in the evolution of pleomorphic adenoma.

Keywords: E-cadherin, pleomorphic adenoma, epithelial–mesenchymal transition.

Introduction

The pleomorphic adenoma is the most common tumor of salivary gland, having an annual incidence of 3.5 to 100 000 inhabitants [1].

Although the etiology of this tumor is not known until now, it has been found that its incidence increases after 15–20 years of exposure to radiations [2]. According to the data in the literature, this type of tumor may develop at any age, having the maximum incidence during the 6th decade of life.

As for the localization of this tumor, the specialty literature shows that, although it may develop in any salivary gland, most frequently the pleomorphic adenoma crosses the superficial lobe tail of parotid gland (70–80%). The histogenesis process of pleomorphic adenoma of salivary gland continues to remain a controversial subject. Thus, while some authors suggest the origin of the two tumoral components (parenchyma and stroma) from different sources, mesenchymal and myo(epithelial) [3], others assert that the unicellular origin of this tumor, either from the epithelial cells [4, 5] or modified myo-epithelial cells [6] or mesenchymal [7].

For supporting the last theory, some authors bring as argument the existence of an epithelial–mesenchymal transition (EMT) process in pleomorphic adenoma, process through which the epithelial neoplastic cells would trans-differentiate into mesenchymal cells. Recent studies emphasized the involvement of this type of process in fibrosis [8] as well as in metastases of cancers [9].

In this work, we have analyzed the immunohisto-chemical expression of E-cadherin and α-SMA (alpha-smooth muscle actin) in pleomorphic adenomas of salivary glands and their role in the epithelial–mesenchymal transition process.

Materials and Methods

The study included a total of 15 cases diagnosed in the Laboratory of Pathology, Emergency County Hospital of Craiova, Romania, in 2010–2013, which were histo-logically reviewed. From the block paraffin were made serial sections, which were used for the immunohisto-chemical study.

The panel of antibodies used were represented by mouse monoclonal anti-human E-cadherin (clone NCH-38, dilution 1:50, Dako) and rabbit polyclonal anti-human α-SMA (dilution 1:100, Dako). For the simple reactions, after the antigen recovery (citrate buffer, pH 6), blocking endogenous peroxidase and blocking non-specific binding sites, sections were incubated overnight with the antibodies. The next day, sections were incubated with biotinylated secondary antibodies and reactions were amplified by using LSAB2-HRP system (code K0675, Dako) and developing was performed with DAB chro-mogen (code 3467, Dako). In order to determine the epithelial–mesenchymal transition have been used E-cadherin/α-SMA double reactions, following a sequential protocol, with LSAB2-HRP system (code K0675, Dako) and LSAB2-AP System (code K0674, Dako) for the reactions amplification and DAB (code 3467, Dako), respectively Vulcan Fast Red chromogen (code FR805S, Biocare Medical) used to observe the reactions.

For the validity of the reaction, we used external positive control represented by invasive ductal mammary carcinoma for E-cadherin and normal mammary gland

for α-SMA; also, we used negative external controls, by omitting the primary antibody.

The evaluation of immunohistochemical reactions was made independently by two investigators, and in cases there were inconsistencies, these were reinvestigated until it was reached a consensus.

The appreciation of the expression of markers was carried out qualitatively, the intensity of reactions being quantified according to scores: "-" – negative; "1" – poorly positive, "2" – moderate positive, "3" – intense positive. For the statistical analysis, there were used *chi*-square test, using SPSS 10 software.

The images were captured using the Nikon Eclipse 55i microscope, equipped with a video camera, and the processing and interpretation was made with the Image ProPlus AM97 imagery soft.

Results

The average age of the 15 investigated cases was of 43-year-old, the parotid gland being the most interested (85%). Our study emphasized a large range of development of the tumor between 17 and 84-year-old, the maximum incidence being registered in the 5th decade of life (35.55%) and the 6th decade (20%). Histopathologically, the typical pleomorphic adenoma was characterized by epithelial and myoepithelial neoplastic proliferations, taking the shape of a multitude of increase patterns to which are associated a series of reshaping of the stromal component, fact which leads to the carrying out of a real lesional polymorphism. Also, depending on the stromal/parenchyma component the majority of tumors presented a predominance of stromal component (46.6%), followed by tumors with balanced stromal/tumor parenchyma ratio (40%), and tumors with the predominance of epithelial component (13.4%).

E-cadherin expression was identified as membranous and cytoplasmic signal in all investigated cases. In case of α-SMA, the expression was noticed in the cytoplasm in all cases. Investigating the reactivity for E-cadherin in the 15 cases of pleomorphic adenoma, we noticed the constant presence of membranar reactivity at the level of luminal cells that cover the proliferative structures of ductal-cystic type, the intensity of reaction being moderate/intense (Figure 1).

The reactivity is also maintained at the level of abluminal cells in the neighborhood of luminal cells, but appears also at the cytoplasmatic level, gradually disappearing to the periphery of ductal-cystic proliferative structures (Figure 2).

At the level of epithelial proliferative solid areas, the reactivity for E-cadherin was inconstant and heterogeneous. Thus, in some solid areas, we have noticed the absence of reactivity for E-cadherin or this was present only at the level of ductal structures present in the composition of proliferative units (Figure 3).

In other solid proliferative areas, the reactivity for E-cadherin was present with focal, cytoplasmatic character, the intensity being poorly/moderate (Figure 4).

In addition, we have found the presence of a double reactivity for E-cadherin and α-SMA at the level of these epithelial proliferative units, as sign of an epithelial–myoepithelial transition process, the intensity of reaction for the both markers being moderate for E-cadherin and intense for α-SMA (Figure 5).

A moderate reactivity for E-cadherin and smooth muscle actin was also noticed at the level of areas of squamous metaplasia in the contents of solid epithelial proliferative units (Figure 6).

We have observed the absence of E-cadherin reactivity in myxoid stromal areas, in exchange the cells at this level presenting an intense/moderate cytoplasmatic positivity for α-SMA (Figure 7).

A similar reactivity was present at the level of chondroid or myxo-chondral areas, a part of lacunar cells being positive only for α-SMA. This suggests the transdifferentiation of myxoid stromal cells in cartilaginous-type cells (Figures 8 and 9). In this study, we found no statistically significant differences of E-cadherin and α-SMA expression in relation to clinical parameters and histopathological appearance (p<0.05). At the level of proliferative epithelial units, we found significant differences in the expression of analyzed markers, the increased expression in the periphery of α-SMA being associated with the decreased expression of E-cadherin.

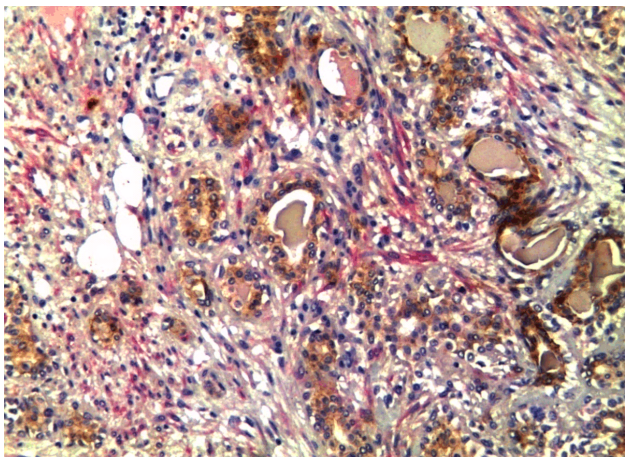

Figure 1 – ***Typical pleomorphic adenoma parenchyma/stroma balanced ration – tubulo-cystic proliferative area. Reactivity for E-cadherin (brown) predominant of luminal cells. E-cadherin/α-SMA immunostaining, ×100.***

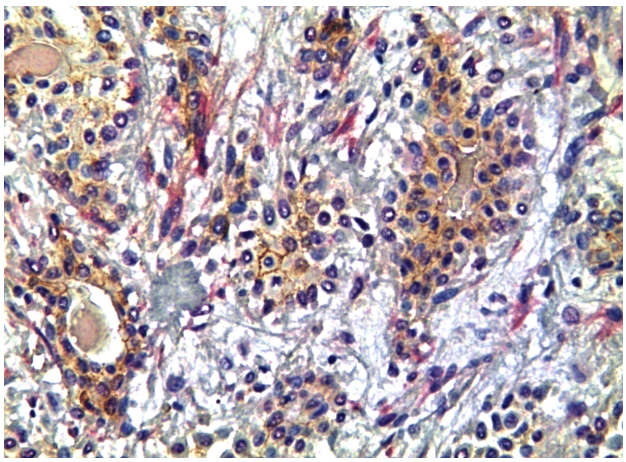

Figure 2 – ***Typical pleomorphic adenoma parenchyma/stroma balanced ration – tubulo-cystic proliferative area. Membranar reactivity for E-cadherin (brown) of luminal cells and cytoplasmatic reactivity of abluminal cells. E-cadherin/α-SMA immunostaining, ×100.***

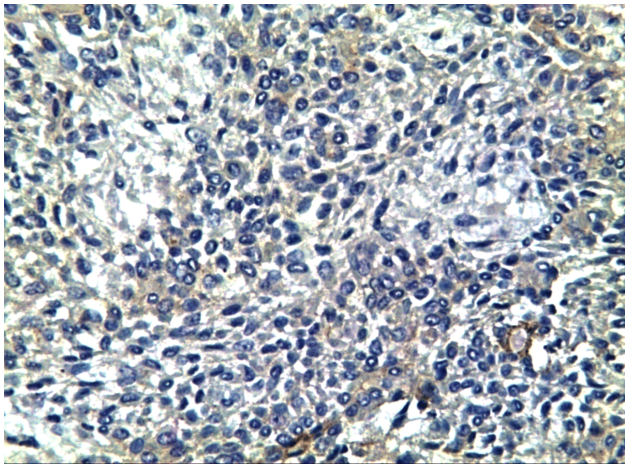

Figure 3 – ***Typical pleomorphic adenoma parenchyma/ stroma. Reactivity absent for E-cadherin of abluminal cells and reactivity present at the level of luminal cells. E-cadherin/α-SMA immunostaining, ×100.***

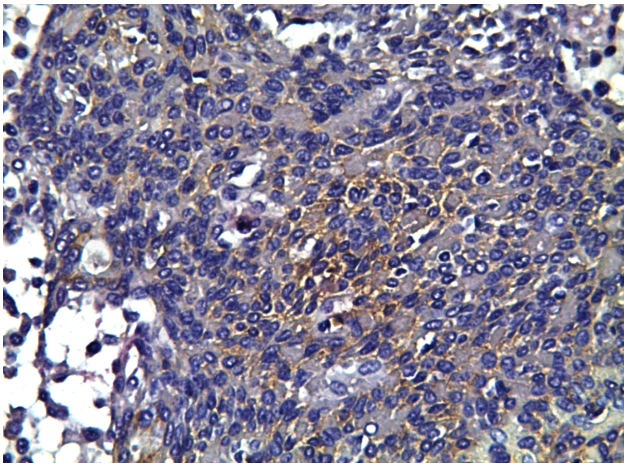

Figure 4 – ***Typical pleomorphic adenoma parenchyma/ stroma balanced ratio – solid proliferative areas. Reactivity present for E-cadherin of abluminal cells in the centre of proliferations. E-cadherin/α-SMA immunostaining, ×200.***

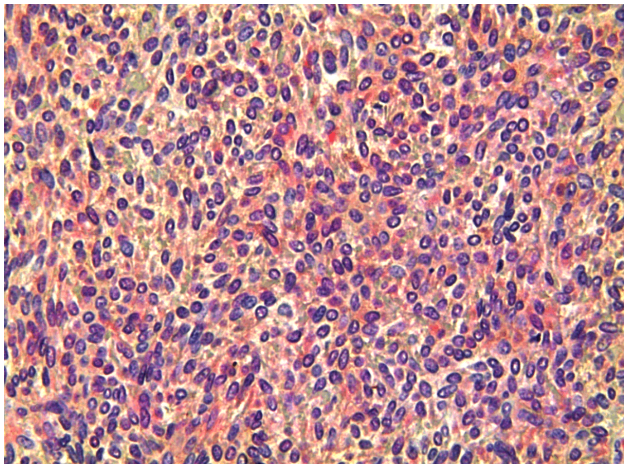

Figure 5 – ***Typical pleomorphic adenoma parenchyma/ stroma balanced ratio – solid proliferative areas. Reactivity present for E-cadherin (brown) and α-SMA (red) of abluminal cells in the centre of proliferations. E-cadherin/α-SMA immunostaining, ×100.***

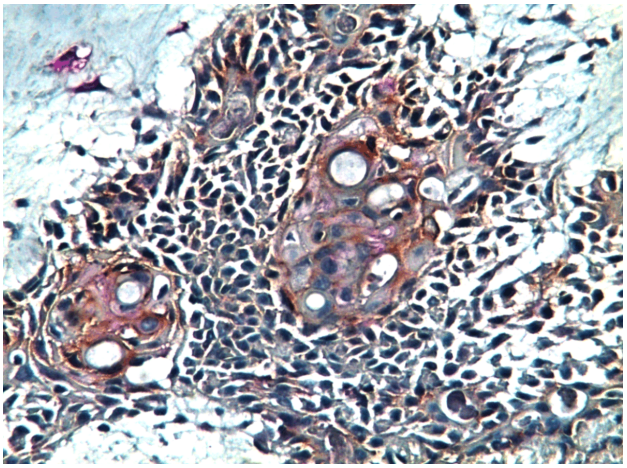

Figure 6 – ***Typical pleomorphic adenoma parenchyma/ stroma balanced ratio – solid proliferative areas. Reactivity present for E-cadherin and α-SMA of squamous metaplasia cells in the centre of proliferations. E-cadherin/α-SMA immunostaining, ×200.***

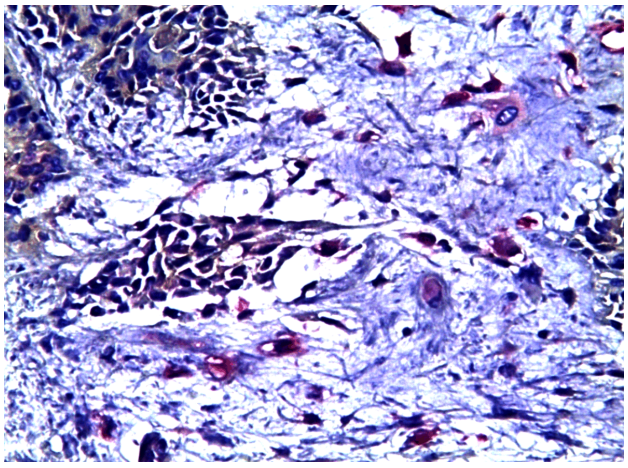

Figure 7 – ***Typical pleomorphic adenoma parenchyma/ stroma balanced ratio – solid proliferative areas and myxoid stromal areas. Reactivity for E-cadherin absent at the level of cells in the myxoid areas, but the reactivity is present for α-SMA (red) from the part of cells with plasmacytoid and stellated morphology. E-cadherin/α-SMA immunostaining, ×200.***

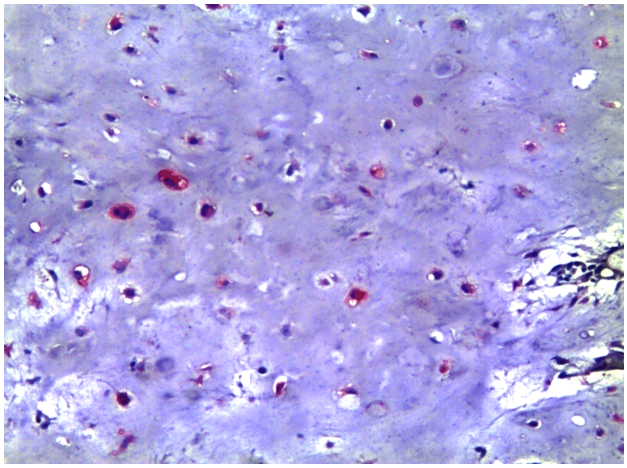

Figure 8 – ***Pleomorphic adenoma with predominance of stroma – chondroid areas. Reactivity present only for α-SMA (red) from the part of lacunar cells. E-cadherin/α-SMA immunostaining, ×200.***

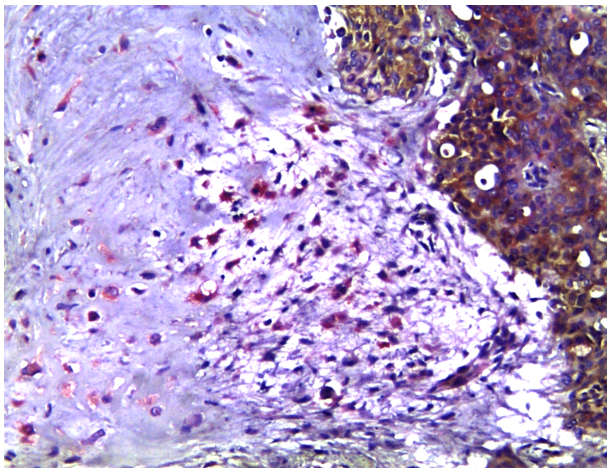

Figure 9 – ***Pleomorphic adenoma with predominance of stroma – chondroid–myxoid areas. Reactivity present only for α-SMA (red) from the part of lacunar cells. E-cadherin/α-SMA immunostaining, ×200.***

Discussion

The pleomorphic adenoma is characterized by a high morphological plasticity, fact which seems to be due especially to the myoepithelial component [10]. Initially, to these cells was given a contractile function, but more recently it has been proved are involved in the embryonary development, the extracelullar matrix synthesis, paracrine modeling and signaling [11]. In addition, it has been noticed the fact that in mammary cancer the myoepithelial cells inhibit angiogenesis, the proliferation and invasion of tumoral cells [12]. It could seem that during the neoplastic transformation the myoepithelial cells would change the immunophenotype, losing its reactivity for some markers and acquiring the expression of other markers [13], fact which would be at the basis of different morphological and cytological aspects specific for this type of salivary gland tumors [14]. In the specialty literature, there is a multitude of studies regarding the immunohistochemical aspects of myoepithelial cells in pleomorphic adenoma of salivary gland, using a great diversity of mono- and polyclonal antibodies of cytokeratin type, muscle specific proteins, S-100, vimentin and other markers [15]. All these studies indicated a variable immunoreactivity of the myoepithelial cells of the salivary gland pleomorphic adenoma, and this is the reason cannot talk about a specific strict marker to identify them.

Cadherins are a family of transmembranary calcium-dependent glycoproteins, which mediate the intercellular contacts, being involved in the morphogenesis and the maintenance of tissue structures, but also during the growing processes and cellular differentiation [16]. E-cadherin mediates intercellular adhesion at the level of epithelia and is expressed in all the cells of stratified squamous epitheliums except for keratinized cells [17].

Reductions of expressivity of E-cadherin were signaled in human carcinomas developed at the level of the esophagus, mammary gland, female genital system, colon [18], oral mucous and gums [19], the lost of its expression being associated with an abnormal stratification [20]. At the level of salivary glands, E-cadherin is expressed by acinar and ductal cells, the maximum of reactivity being signaled at the level of basal pole of striated ductal cells [21]. In pleomorphic adenoma, it is noticed the reduction or the absence of E-cadherin expression in the cells at the periphery of ductal and solid proliferative units and in the cells with clear stromal differentiation [21]. In our study, ductal luminal cells were positive for E-cadherin showing their epithelial origins. For E-cadherin, except for luminal cells, we have notices a reactivity both membranary, but especially cytoplasmatic at the level of some abluminal cells and of some myoepithelial cells, cuboidal or plasmacytoid, in the contents of solid proliferative areas. Also, in our study in the periphery of proliferative epithelial units, the expression of E-cadherin decreased, while α-SMA expression increased.

As a component of the epithelial–mesenchymal transition process from the tumorigenesis is indicated the cadherin "switch" consisting in reduction of the E-cadherin expression and increased of N-cadherin signal [22]. Brieger *et al.* suggests that cadherin-11 supraexpression in recurrent pleomorphic adenomas, similar to N-cadherin from another tumoral entities, would promote dissociation one by one of the cell from neoplastic solid proliferative island looking like "shoot gun seeding" and multifocal growing from this recurrent tumors. One concepts regarding the histogenesis of pleomorphic adenoma is the epithelial–mesenchymal transition (EMT) process, whereby during the development of this type of tumor of salivary gland would be a complete transition from a purely epithelial phenotype to an intermediate mixed epithelial–mesenchymal type, so that in the final to result a purely mesenchymal one [3, 6, 13].

Conclusions

Investigating the reactivity for E-cadherin in pleomorphic adenoma, we noticed the presence of membranar reactivity at the level of luminal cells covering the proliferative structures of ductal-cystic type. The reactivity gradually disappears to the periphery of ductal-cystic proliferative structures. At the level of epithelial proliferative solid areas, the reactivity for E-cadherin was inconstant and heterogeneous, while α-SMA expression increased. This aspect indicated the involving of epithelial–mesenchymal transition process in the evolution of pleomorphic adenoma.

Acknowledgments

Dr. Anca Ştefania Enescu was supported by the Project "Excellence program for multidisciplinary doctoral and postdoctoral research in chronic disease", Grant No. POSDRU/159/1.5/S/133377 partially supported by the Sectoral Operational Programme Human Resources Development 2007–2013, financed from the European Social Fund.

Author contribution

All authors have contributed equally to the present work.

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Corresponding author
Anca Ştefania Enescu, MD, PhD, Emergency County Hospital of Craiova, 1 Tabaci Street, 200642 Craiova, Romania; Phone +40727–416 181, e-mail: ancaenescus@yahoo.com

Received: April 9, 2014

Accepted: December 24, 2014