MINISTRY of EDUCATION and RESEARCH
University of Medicine and Pharmacy of Craiova
DOCTORAL SCHOOL

DOCTORAL THESIS

- abstract -

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STUDY OF THE EPITHELIAL MESENCHYMAL TRANSITION PROCESS IN ADENOID CYSTIC CARCINOMA OF THE SALIVARY GLAND

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CONTENT

KEY WORDS: adenoid cystic carcinoma, epidemiology, salivary glands, pathogeny, histopathology, immunohistochemistry, invasiveness, prognosis, epithelial-mesenchymal transition.

1. INTRODUCTION

Adenoid cystic carcinoma is a rare tumor with an annual incidence global population of 3-4.5 cases per million inhabitants and is responsible for 10- 12% of all salivary glandular tumors, respectively for about 22% of total salivary cancers. Although adenoid cystic carcinoma is a rare tumor, it has an indolent and variable behavior, being extremely aggressive locally and with a great potential for recurrence and metastasis along the nerve threads and by lymphovascular dissemination. Based on such behavior, many authors have included this type of salivary glandular tumor among neoplasms of high degree, the most reserved prognosis being present in the case of metastatic disease.

In this sense, we set out to investigate the particularities of the process epithelial-mesenchymal transition (TEM) in adenoid cystic carcinomas diagnosed and treated during the last decade in the SCJU in Craiova, trying to outline a particular morpho-clinical profile of the most aggressive forms. At the same time, we aimed to investigate immunophenotypic alterations particular underlying the TEM process in these tumors with highlighting a specific immunohistochemical profile of the inducing transcription factors of TEM during the pathogenesis of investigated adenoid cystic carcinomas. The lot of study included 32 patients diagnosed and treated for adenoid carcinomas cystic in the Oral & Maxillofacial Surgery Clinic between 2010 - 2019.

A descriptive epidemiological investigation was initially performed on this group continued with a descriptive histopathological investigation attempting to outline a clinical-morphological profile particular to the aggressive tumor forms investigated. The research continued with an immunohistochemical analytical study part aimed at highlighting some particular immunophenotypic alterations that stand at the basis of the TEM process, respectively the loss of the expression of specific markers epithelial differentiation and gaining the expression of markers of differentiation mesenchymal. On the other hand, the immunohistochemical is aiming to contour a particular immunoprofile, of process-inducing transcription factors TEM in adenoid cystic carcinomas investigated.

2. THE CURRENT STATE OF KNOWLEDGE

In **subchapter 2.1** on **the epidemiology** of salivary gland cancers the latest information on the overall incidence is briefly presented related to sex, age groups, ethnicity, geographical distribution and topography tumor, respectively data on the survival and mortality rate of patients with salivary gland tumors, particularizing for carcinoma cystic adenoid.

In **subchapter 2.2** on **the pathogenesis** of salivary gland cancers was made a review of the main risk factors, after which they were presented the latest data on the histogenesis of salivary gland tumors, emphasizing the role of stem cells in the histogenesis of tumors salivary gland. Finally, we presented the key aspects of molecular pathogenesis of salivary gland tumors, highlighting genetic alterations with potential oncogenes involved in the pathogenesis of salivary adenoid cystic carcinomas.

Subchapter 2.3 presents general data on the pathogenic implications of **the epithelial-mesenchymal transition** process in oncogenesis of salivary tumors, detailing the particular aspects of this process in the progression of adenoid cystic carcinomas of the salivary gland.

3. PERSONAL CONTRIBUTION

3.1. WORKING HYPOTHESIS AND GENERAL OBJECTIVES

The working hypothesis were:

- The existence of an epidemiological and morphological profile especially for the patients with adenoid cystic carcinomas diagnosed and treated the last decade in SCJU Craiova;
- The presence of a particular immunophenotypic alteration which are at the basis of the TEM epithelial-mesenchymal transition process, process that is supposed to play an important part in the local invasion and in the dissemination at a distance of such salivary cancers;
- The existence of a specific immunohistochemical profile of the transcription factors involved in the TEM process of the pathogeny of the adenoid cystic carcinoma investigated.

A retrospective study was performed to explore the first working hypothesis descriptive, spread over a period of 10 years, between 2010 and 2019, on a batch of 32 of patients diagnosed and treated for adenoid cystic carcinomas. First a **descriptive epidemiological investigation** was performed followed by a descriptive histopathological study.

An analytical study was performed to investigate the second hypothesis **immunohistochemical**, qualitative and semiquantitative using an enzymological method with chromogenic detection, respectively the streptavidin-biotin complex method (LSAB), and as markers, antibodies were used to highlight morphological changes suffered by cancer cells during the process TEM occurring in the progression of adenoid cystic carcinomas.

In the case of the third hypothesis, an analytical study was also used **immunohistochemical**, qualitative and semiquantitative study using the same method as chromogenic detection, but other markers, addressed in principle to the factors transcriptional agents considered to play a major role in the development of the TEM process.

3.2. METHODOLOGY

3.2.2 Material and Method

A retrospective study included a number of 32 hospitalized patients and operated in the Oral and Maxillofacial Surgery Clinic of the Clinical Hospital Emergency County of Craiova, in the period 2010-2019. For the **clinical and epidemiological study,** the clinical observation sheets and protocols were studied operators, and the variables of interest followed were: age and sex of patients, location of tumors (major salivary glands: parotid, sublingual, submandibular, minor salivary glands: palatine, labial, buccal, lingual, etc.), reasons for hospitalization (swelling / facial asymmetry, pain, ulceration, etc.), the relative period of clinical latency until the time of diagnosis.

Diagnostic blades were used **for histopathological study** and the corresponding paraffin blocks obtained from histopathological processing a surgical excision pieces of the patients included in the study. The variables of interests were: tumor development pattern (solid, tubular and cribriform), loco-regional invasiveness, the presence of perineural invasion, of metastases, histopathological stage (pTNM) and resection margin status.

Paraffin blocks were used **for the immunohistochemical study** correspondents from which preparations were made that were processed immunohistochemically in the laboratory of the pathological anatomy discipline of UMF Craiova. The study was performed by an enzymological method with chromogenic detection, respectively the method of the labeled streptavidin-biotin complex (LSAB), using the Dako kit, and the antibodies used are reproduced in the table below:

Antibodies	Clone / Producer, Nr. catalogue	Diluțion	Antigenic unmasking	Positive external control
cytokeratin AE1/AE3	Cocktail of two monoclonal antibodies produced in mice: AE1 și AE3 / Novocastra, PA0909	Diluted ready to work	Citrate solution0.1 M, pH 6	skin
E-cadherin	Monoclonal mouse-36B5/ Novocastra, PA0387	Diluted ready to work	Citrate solution 0.1 M, pH 6	skin
Vimentin	Monoclonal mouse- V9/ Novocastra, PA0640	Diluted ready to work	Citrate solution0.1 M, pH 6	skin
Fibronectin	Polyclonal rabbit / Dako, A024502-2	1:400	proteinase K	kidneys
N-cadherin	Monoclonal mouse-IAR06/ Novocastra, NCL-L-N-Cad	1:50	Citrate solution0.1 M, pH 6	testicles
P-cadherin	Polyclonal rabbit / Sigma-Aldrich, HPA001767	1:100	Citrate solution0.1 M, pH 6	placenta
Twist	Polyclonal rabbit/ Abcam, ab50581	1:300	Citrate solution0.1 M, pH 6	colon
Snail	Polyclonal rabbit / Novus Biologicals, NBP1-80022	1:100	Citrate solution0.1 M, pH 6	kidneys
Slug	Polyclonal rabbit/ Abcam, ab27568	1:150	citratesolution0.1 M, pH 6	placenta
ZEB1	Monoclonal mouse-CL0151 / Sigma Aldrich, AMAb90510	1:100	Citrate solution00.1 M, pH 6	prostate adenocarcinoma

3.3. RESULTS

3.3.1 Clinical-epidemiological study results

The average age of development of this type of salivary cancer was 58.31 years, and the median was 57.5 ± 12.55 years. The age limits between which they were developed the investigated cystic salivary adenoid carcinomas were 31 years old and 91 years, respectively, with a peak incidence in the VI (34.37%) and VII decades (28.12%) of life. The gender distribution of the case series indicated the prevalence of tumors in males with 19 cases, representing 59.37% of the total cases. The average age of male patients was 59.95 years, while the median was 58 ± 13.89 years. The sex ratio was 1:46 in favor male. The topographic distribution of casuistry showed us the prevalence cases developed in the parotids with 9 cases (28.12%), followed by localization at the level of the palate with 6 cases (19.2%) and oropharynx with 5 cases (15.62%)

Regarding the clinical aspects, these tumors most often presented in the form of swellings that have slowly deformed over time, the respective regions. This clinical appearance was dominant, being present in 25 cases (78.12% of casuistry) both in the location of the major salivary glands and from the level of the intraoral salivary glands. As the most common symptomatology the sick in our case complained of pain, instituted insidiously, slowly in time, at various time intervals, in the various locations in which they have developed tumors. This symptom was reported in 23 cases and approximately 91%, respectively. of cases, in 19 cases succeeding the glandular swelling (59.37%), and in 4 cases associated with tumor ulceration (12.5%). The latency period of the symptomatology ranged from 4 months in the case of a 63-year-old patient with oropharyngeal localization, to 18 months, as in the case of a parotid tumor developed in a 31-year-old man.

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3.3.2 The results of the histopathological study

The macroscopic appearance of the tumors after their surgical excision indicated the presence of nodular masses, relatively well delimited, but not encapsulated, with dimensions ranging from 1.2 cm (in the location of the upper lip) to 11 cm (in the parotid gland) in maximum diameter.

The histopathological investigation highlighted the prevalence of solid variants of salivary adenoid cystic carcinoma, reported in 15 cases, representing 46.88% from the investigated cases. In second place in terms of incidence was the tubular variant, diagnosed in 11 cases, representing 34.37% of all cases studied. On the last place was the cribriform variant, found in 6 cases, respectively 18.75% of the case. From a cytological point of view this type of

tumor, regardless of histopathological subtype was predominantly composed of small neoplastic cells with poorly defined margins, little cytoplasm and clear and with angular, tachycromatic nuclei. The tumor stroma varied quantitatively from one case to another and for the most part it had a fibrous appearance, often hyaline and less frequently the appearance was of the myxoid type.

A characteristic histopathological aspect of perineural invasion was observed in 22 cases, respectively in 68.75% of investigated cases. Vascular invasion was present in only two cases, 6 • both observed in patients with solid tumor type, one in localization oropharyngeal, and the other developed in the right sublingual gland. Lymph node dissemination was recorded in only 6 cases, representing 18.75% of the cases investigated. Examination of the status of the resection margins highlighted that 13 cases presented at least one of the margins of resection invaded (40.62%), most often being the margin of resection deep (10 cases). Analysis of histopathological stages (pTNM) of patients included in the study group showed the prevalence of case studies for stage II-pTNM with 23 cases (71.87%), followed by stage III- pTNM with 6 cases (18.75%), stage IV-pTNM with 2 cases (6.25%) and stage I-pTNM with only one case (3.12%). Statistically, significant differences were present between the mean age of patients with tumors developed in the major salivary glands (54.31 ± 13.85 years) compared to the mean age of those with tumors originating in minor salivary glands (62.31 ± 9.97 years), test "t" - Student being t (30) = - 1.87, p = 0.035 (Figure 3.29). We also found significant differences between mean age of patients with perineural invasion (54.00 ± 13.80 years) compared to those who did not have perineural invasion (60.72 ± 11.46 years), the test ”T,, - Student being of t (30) = - 1.65, p = 0.048.

3.3.3 Results of the immunohistochemical study

Regarding the reactivity of tumor tissue for AE1 / AE3 were recorded positive cytoplasmic reactions, but with a degree of heterogeneity dictated especially of the histological subtype. The average value of the immunoreactivity score (IRS) for the whole case was 8.5 ± 3.255. Maximum reactivity was recorded in the solid subtype (IRS = 9 ± 1,667). In this variant the pattern reactivity was generally diffuse and intense, but focal in the center of the islands and tumor intensity of immunolabeling was weaker. At the level of tumor tissue, the reactivity for E-cadherin was heterogeneous, with both cytoplasmic and membrane pattern. Overall tumor reactivity (IRS = 8 ± 1,934) was slightly lower than that for cytokeratin AE1 / AE3. Differences in reactivity were also observed depending on the function of the histopathological subtype. The tubular variant

showed a maximum of IRS reactivity = 9 ± 1,566, the reaction pattern being both membrane and cytoplasmic.

In tumor tissue, immunoreactivity score values for vimentin were close to those obtained for E-cadherin, respectively IRS = 8 ± 3,614. The highest values were obtained in the solid variant IRS = 9 ± 1,569, followed by the cribriform IRS = 7 ± 1.966 and the tubular one with IRS = 3 ± 1,549. In the solid version the reactivity was heterogeneous, intensely reactive areas alternating with areas with low or even no reactivity. Reactivity to fibronectin was present in tumor tissue especially at the matrix between neoplastic proliferations and inside pseudocystic structures and lumens of neoplastic ducts (Figure 3.60). As intensity, the highest reactivity was observed in the tubular variant tumor at the level of the material present in the lumens of tubular proliferations neoplastic, followed by the cribriform variant at the level of the material present in inside the screen-shaped spaces and on the last place was the solid version, in which reactivity was present in the matrix between neoplastic proliferations. Tumor reactivity for **N-cadherin** was observed only in 11 cases, which represented 35% of the total cases investigated. Analyze Semi-quantitative immunolabeling showed the following average scores: 4 ± 1,214 for the solid version, 3 ± 0.577 for the tubular version and 2.5 ± 0.707 for the cribriform variant. Regardless of the histopathological subtype, the reactivity for N-cadherin was much more intense in cases with perineural invasion and invasion lymphovascular, the reactivity being more evident in the invasion front and in neoplastic cells at the periphery of tumor proliferations. In tumor tissue the highest reactivity to **P-cadherin** was present in the tubular variant (with mean IRS value = 12 ± 1,544), followed by cribriform variant (IRS = 7 ± 1,505) and finally solid variant with the lowest reactivity (IRS = 4 ± 1843). Regardless of the histopathological subtype the reactivity pattern was both membrane and cytoplasmic, with small differences between the tumor content and the invasion front. Statistically, we observed a strong correlation between the immunohistochemical scores of E-cadherin and vimentin ($r = 0.999$, $p = 0.027$), which suggests the existence of a tendency in invasive cases to decrease reactivity for E-cadherin with a concomitant increase in vimentin expression.

Of all the TEM-inducing transcription factors we studied, **Twis**t was by far the most intensely expressed in tumor tissue, having a score immunoreactivity average over all investigated cases of 6,875 ± 1,979. Reactivity was much more evident in tumors developed at the level minor intraoral salivary glands. Regardless of the lesion topography, the variant solid adenoid cystic carcinoma was the most reactive for Twist, with mixed subcellular pattern, cytoplasmic and

nuclear, predominantly nuclear. In tumor tissue, **Snail** reactivity was observed in 20 cases (62.5% from case studies), the mean immunoreactivity score being 2.9 ± 2.716. The reactivity itself was heterogeneous, with positive areas alternating with areas negative regardless of the histopathological variant of adenoid cystic carcinoma or of tumor topography. Regarding tumor reactivity for **Slug**, it was present in 25 of the cases, which represented 78.12% of the case under investigation. In total, an average score of immunoreactivity of 5.28 ± 3,215, with no differences in tumor topography. The predominant subcellular pattern was cytoplasmic regardless of the histopathological variant of the tumors. In all cases investigated we did not record reactivity for **ZEB1** in the tumor parenchyma, regardless of histopathological variant, tumor topography or pTNM stage. However, the tumor stroma was positive in all cases investigated, **ZEB1** being especially positive in the associated stromal fibroblasts and endothelial cells of blood vessels in and around the periphery of neoplastic proliferations.

Statistically, there was a moderate correlation between all scores immunoreactivity of these TEM-inducing transcriptional markers (Twist / Snail- $r = 0.580$, $p < 0.001$; Twist / Slug- $r = 0.614$, $p < 0.001$; Twist / ZEB1- $r = 0.619$, $p < 0.001$; Snail / Slug- $r = 0.557$, $p < 0.001$; Snail / ZEB1- $r = 0.676$, $p < 0.001$; Slug / ZEB1- $r = 0.687$, $p < 0.001$).

4. DISCUSSIONS

It is a rare tumor with an overall annual incidence of 3-4.5 cases per million inhabitants [1], being responsible for 10-12% of all tumors salivary glands [2] and for about 22% of all cancers, respectively salivary [3]. The literature cites about this type of salivary tumor that it would most often develop in the fifth and sixth decades of life, although they were reported cases in all age groups [4]. Most authors indicate age 52 years as the average age of patients with such carcinomas [5, 6].

Data from the literature indicate for this type of salivary tumor the origin of intercalary ducts, consisting of ductal-like cells and modified myoepithelial-like cells [7]. Adenoid cystic carcinomas usually contain a combination of one or three growth patterns (solid, tubular and cribriform), but usually one is dominant [1]. Most authors indicated the variant cribriform as the most common histopathological subtype encountered (44.1-70.7%), followed by the solid variant (20.6-29.3%) [5, 6].

The perineural invasion seems to be a common morphological aspect, even in cases diagnosed in the early stages [8, 9]. This together with distant metastases have been seen as unfavorable prognostic factors [10].

Most authors reported a higher incidence of T2 tumors (60% up to in 74% of cases diagnosed with adenoid cystic carcinomas) with average dimensions of about 3.1-4.3cm (with limits ranging from 1-7cm) [1, 11]. Into the regarding lymph node metastasis, data from the literature reported values of 17-19% for tumors developed in the major salivary glands [12]. Regarding the status of the resection margins, the data in the literature record the existence of residual tumors in about 32.6-65.2% of all cases [5]. Into the case of local recurrences, the literature reported values ranging from 10.3-74% [6, 11, 13, 14].

Regarding the rate of metastasis in adenoid cystic carcinomas, the literature reports values ranging from 17.2% to 68.5% [6, 14- 17]. The five year survival rate of patients with adenoid carcinomas cysts that did not develop recurrences is cited at values ranging from 62.9% [18] up to 100% [5], while for patients with relapses the values decrease to 56.2% [18] and 66% [5], respectively.

The process of metastasis of the adenoid cystic carcinomas appears to be regulated by the expression and modification of the extracellular matrix, which in turn are responsible for promoting the TEM process in tumor cells [19].

In 2007, Meer and Altini investigated the cytokeratin profile at the level tumors of the salivary glands and observed that in the adenoid cystic carcinomas

neoplastic cells were intensely and diffusely positive for **AE1 / AE3** mode similar to the reactivity of the residual parenchyma of the major salivary glands or minor [20]. Some studies have shown that **E-cadherin** expression was lower in adenoid cystic carcinomas compared to the parenchyma residual salivary glandular, and this fact was made responsible for the invasion nerves, lymph node dissemination, development of regional recurrences and distant metastasis [21].

Caselitz et al. highlighted reactivity for **vimenti**n in neoplastic cells in adenoid cystic carcinomas, this being present in all histopathological variants, but with a higher intensity at the level of the neoplastic cells in the outer layers compared to those in the inner layers of neoplastic proliferations [22]. Most researchers showed a positive reaction to **fibronectin** in all the investigated adenoid cystic carcinomas, with a prevalent filamentous pattern but also irregular both in the tumor matrix and inside the pseudocysts [2. 3].

Zhao et al. reported a positivity rate of 68.1% in carcinoma cystic adenoid, reactivity that did not vary with the histopathological subtype, but was higher in cases associated with perineural invasion [24]. So far there have not been conducted studies to examine the reactivity of **P-cadherin** in both normal salivary glandular parenchyma as well as in human salivary tumors. In studies performed on tumor models, including oral squamous cell carcinoma has been shown to P-cadherin acts as a tumor suppressor, as its absence is associated with a much more aggressive phenotype of cancer cells [25]. All this data presented above come to suggest the key role played by the TEM process in the acquisition of an invasive and metastatic potential by adenoid carcinomas salivary cystic [26].

Shen et al. showed that **Twist**'s expression was significantly higher in adenoid cystic carcinoma compared to pleomorphic adenoma and the normal salivary glandular tissue, suggesting the involvement of this marker in development and the progression of this salivary cancer [27]. In addition, the prognostic role was observed of this marker as a result of its increased expression in those cases that have associated distant dissemination compared to non-metastatic cases, suggesting the involvement of this protein in the metastasis of adenoid carcinomas cystic by promoting the TEM process [27].

Jiang et al. have reported a positivity rate for **Snail** of 58.68% of adenoid carcinomas investigated salivary cystic, with a mixed nuclear and cytoplasmic pattern [28]. The authors found that there are differences in reactivity for Snail and prognosis in various histopathological variants of adenoid carcinoma cystic and that high levels of its expression were significantly correlated with perineural

invasion, loco-regional recurrences and distant metastasis [28]. Tang et al. (2010) reported that 71.9% of the investigated salivary adenoid cystic carcinomas were reactive for the **Slug,** and its expression correlated significantly with tumor topography, histopathological variants, perineural invasion, loco-regional recurrences and distant metastasis [29].

Yao et al. (2017) showed that in the oral squamous cell carcinomas **ZEB1** was overexpressed while **E-cadherin** expression was inhibited and that this immunohistochemically profile correlated with the lymph node metastasis, grading tumor, such patients having a poor prognosis [30].

All these studies highlight the involvement of transcription factors in induction of an active TEM process in salivary adenoid cystic carcinomas, process that appears to be largely responsible for perineural and lymphovascular invasion, respectively by their distant metastasis.

5. CONCLUSIONS

Our study highlighted the prevalence of adenoid cystic carcinomas in males (59.37%), the vast majority of cases developing in the VI (34.37%) and VII (28.12%) decades of life, the average age of the patients included in the study being 58.31 ± 12.55 years.

The topographic distribution of the case study indicated the more frequent interest of parotid glands (28.12%), followed by localization at the level of the palate (19.2%) and oropharynx (15.62%), respectively. Statistically, the trend was noticeable, adenoid cystic carcinomas originating in the minor salivary glands to develop in older people, at least 10 years older than those which have developed tumors in the major salivary glands.

Histopathologically, the solid variant predominated (46.88%), followed by the tubular variant (34.37%) and on the last place the cribriform variant (18.75%). A particular histopathological aspect was that of perineural invasion present in 68.75% of cases, 18.18% of them also showing intraneural infiltration. Statistically it was observed the tendency for patients with tumors which have developed perineural invasion to be slightly younger, at least 6 years older compared to those who did not have invasion perineural.

The vast majority of cases were diagnosed in stage II-pTNM (71.87%), followed by stage III-pTNM (18.75%). The histopathological study highlights the much more aggressive nature of solid tumors that have developed much more frequently perineural invasion and lymphovascular dissemination, being diagnosed also in much more advanced stages of p-TNM compared to the others histopathological variants.

Immunolabeling with cytokeratin AE1 / AE3 suggests that on the one hand the solid variant would be the most aggressive, and on the other hand that at the invasion front the neoplastic cells lose some of their epithelial differentiation. Regardless the histopathological subtype the neoplastic cells from the periphery of proliferations, especially those in the invasion front, as well as the cells in the surrounding proliferations or infiltrates nerve threads showed a reduced reactivity to E- cadherin, and the pattern changed from membrane predominantly to cytoplasmic one.

The vimentin immunophenotype that we found suggests the existence of a TEM process much more prominent in the solid variant of adenoid cystic carcinoma, the mesenchymal phenotype being more evident in neoplastic cells at the front of invasion and those that surround or infiltrate the nerve threads. The

fibronectin tumor immunoprofile established by us certifies this marker as being a key factor in the process of invasiveness of these tumors.

The reactivity to N-cadherin though was limited to 35% of the investigated cases suggested the existence of a trial TEM more evident in the solid variant and especially in the peripheral cells from the front of invasion and respectively those who infiltrate perineural. As a premiere our study highlighted that the reactivity to P-cadherin was present especially in the tubular and cribriform variants, the most reactive being the luminal neoplastic cells, as well as those that delimited the spaces sieve.

It is therefore outlined, at least in the invasive cases, especially in the solid variant and especially in the neoplastic cells of the invasion front, respectively in cells that infiltrate perineural and invasive lymphovascular ones an immunoprofile type AE1-AE3 (-) / E-cadherin (-) / P-cadherin (-) / fibronectin (-) / vimentin (+) / N-cadherin (+). Such an immunoprofile suggests the existence of a process active TEM in the progression of the adenoid cystic carcinoma, its solid variant being the most aggressive subtype. In addition, regardless of the histopathological variant this immunoprofile identifies the invasion front, respectively the perineural infiltrating cells and those that invade lymphovasculary as being the site of predilection of the TEM process in such salivary cancers.

Twist was the most intensely expressed of all transcription factors studied, all investigated cases being positive, and the highest reactivity has been reported in the adenoid cystic carcinomas developed in the minor salivary glands and especially in the solid variant of these tumors. The reactivity for Snail was recorded in 62.5% of the investigated cases, the pattern being a heterogeneous one, positive areas alternating with negative areas regardless of histopathological variants and tumor topography. Slug was reactive in 78.12% of the cases, its cytoplasmic reactivity being higher in the solid version, at the invasion front and in cases that have associated perineural invasion and lymph node dissemination. Reactivity to ZEB1 was not present in the tumor parenchyma level, but it was identified at the stromal level in all the investigated cases and appears to be higher in the invasion front and in those cases that associated perineural invasion and lymph node dissemination.

So all the four transcription factors are involved in the TEM processes from this type of salivary cancer and underlie its aggressiveness, identifying the solid variant developed especially in the minor salivary glands as the most aggressive type of cystic salivary adenoid carcinoma. So these markers may be useful as

prognostic markers of this type of salivary cancer. In addition, based on the results given above it can be concluded that some of these markers could be used as molecular targets of adjuvant therapies that would allow to increase the survival rate in the case of such patients.

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ANNEXE 1

LIST OF PUBLICATIONS

1. **Belulescu IC**, Margaritescu C, Dumitrescu CI, Dăguci L, Munteanu C, Margaritescu OC. Adenoid Cystic Carcinoma of Salivary Gland: A Ten-Year Single Institute Experience. Curr Health Sci J. 2020 Jan-Mar;46(1):56-65. doi: 10.12865/CHSJ.46.01.08. Epub 2020 Mar 31. ISSN 2067-0656. PMID: 32637166. PMCID: PMC7323724.

2. **Iulia Cristiana Belulescu**, Claudiu Margaritescu, Cristiana Iulia Dumitrescu, Maria Cristina Munteanu, Otilia Clara Margaritescu Immunophenotypical alterations with impact on the epithelial-mesenchymal transition (EMT) process in salivary gland adenoid cystic carcinomas. Rom J Morphol Embryol. 2020, 61(1):175–187. ISSN (print) 1220–0522, ISSN (online) 2066–8279. doi: 10.47162/RJME.61.1.20. PMID: 32747909. PMCID: PMC7728137.

3. **Iulia Cristiana Belulescu**, Claudiu Margaritescu, Cristiana Iulia Dumitrescu, Maria Cristina Munteanu, Luminita Daguci, Otilia Clara Margaritescu, Marius Matei. The immunophenotype of epithelial to mesenchymal transition inducing transcription factors in salivary gland adenoid cystic carcinomas. Rom J Morphol Embryol. 2020, 61(3):In Press. ISSN (print) 1220–0522, ISSN (online) 2066–8279 doi: 10.47162/RJME.61.3.y