The role of pleomorphic adenoma stroma on its neoplastic progression: state of art

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Abstract:
The tumor development is linked to its stroma. The study of tumor stromal cells enables the understanding of the neoplastic progression. The present narrative literature review aimed to describe tumor stromal cells, such as endothelial and inflammatory cells, that may induce the neoplastic progression of pleomorphic adenomas. The Latin American and Caribbean Center on Health Sciences Information (BIREME) and PubMed electronic databases were searched for scientific articles on this subject published in Portuguese and English from 2007 to 2017. The search focused on information about the clinical, imaging, and anatomopathological characteristics of pleomorphic adenomas and its neoplastic progression. After analyzing abstracts and reading the cataloged manuscripts, 44 articles were selected. It was shown that the tumor stroma is important for neoplastic progression by providing elastic properties to the tumor and by enabling its nutrition. Thus, it is relevant to study the cellular and molecular mechanisms that occur in extracellular matrix in order to understand the biological behavior of pleomorphic adenoma.

Keywords: Extracellular Matrix; Neoplasm; Pleomorphic Adenoma

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INTRODUCTION

Salivary glands tumors comprise a heterogeneous group of neoplasms in the maxillofacial region with complex morphological characteristics and variable clinical behavior. These peculiarities may hinder the diagnosis and selection of an adequate clinical approach by dental surgeons. Thus, a detailed medical history; correlation between clinical, imaging, and morphological findings; and the anatomopathological analysis of the lesion are critical for a reliable diagnosis.

Regarding the epidemiological data, tumors of the salivary glands account for approximately 3% to 10% of head and neck tumors. Of these, 54% to 79% are benign, while 21% to 46% are malignant. The parotid gland is the anatomical site most affected by these lesions (63.9%), followed by the minor salivary (26.2%), submandibular (9.6%), and sublingual (9.3%) glands. Although the parotid is the most frequent site of onset of salivary gland tumors, the incidence of malignant neoplasms in this gland is less (1.5% to 32%) when compared to the incidence rates of the sublingual (70% to 90%), submandibular (45%), and minor salivary (50%) glands. When the tumor affects the minor salivary glands, it usually develops in the region of the hard palate, followed by the upper lip, tongue, floor of the mouth, and retromolar space.

Among benign neoplasms located in the parotid, the most frequent is pleomorphic adenoma, which accounts for approximately 53% of all reported tumors, followed by Warthin’s tumor (7.7%), oncocyto (1.9%), and basal cell adenoma (1.4%). In general, these tumors grow silently and the increased volume of the anatomical region affected is, most often, the only observed clinical manifestation. The mucoepidermoid carcinoma (9.6%) stands out among the malignant neoplasms of salivary glands, followed by acinar cell adenocarcinoma (8.6%), cystic adenoid carcinoma (3.3%), mixed malignant tumor (3.2%), and squamous cell carcinoma (2.1%)1. Such tumors generally show variable symptoms, including pain, facial paralysis, and skin ulceration. The rates of local, regional, and remote recurrence of malignant tumors are 40%, 15%, and 11%, respectively, and are related to a worse prognosis.

Studies on tumor progression have reported cytogenetic evidence on the transformation of pleomorphic adenomas into ex-pleomorphic adenoma carcinomas. El-Naggar et al.6 suggested that some genes could be related to this malignant transformation. In their study, the authors comparatively analyzed the 8q, 12q, and 17p genes in the DNA of 26 neoplasms and 13 pleomorphic adenomas. They proposed that these chromosomes could be involved in the malignant transformation of the pleomorphic adenoma because the onset of adenomas with the potential development of transformation into carcinomas was concomitant to successive changes in the chromosomal arms of 8q and /or 12q. Furthermore, they observed many mutations in the 17p chromosome arm prior to the malignant transformation and progression of pleomorphic adenomas.

The present narrative literature review highlights the clinical and histopathological characteristics of pleomorphic adenoma, the most prevalent salivary gland tumor in the oral cavity. Furthermore, this review analyzes how the extracellular matrix of the tumor stroma may contribute to the development and progression of this neoplasm.

MATERIAL AND METHODS

This was a narrative literature review study. The Latin American and Caribbean Center on Health Sciences Information (BIREME) and PubMed electronic databases were searched for scientific articles on the subject and manual searches were performed for citations in the studies identified in the above databases. The following health sciences descriptors (DeCS) were used to perform these searches: “tumor stroma”, “neoplastic progression”, “pleomorphic adenoma”, and “carcinoma ex-pleomorphic adenoma”. The Boolean expressions “E” or “AND” were used for the associations of words, thus allowing for combinations of descriptors. Six combinations were used with the following descriptors: “Tumoral stroma and neoplastic progression”, “Tumoral stroma and pleomorphic adenoma”, “Neoplastic progression and carcinoma ex-pleomorphic adenoma”. The Boolean expressions “E” or “AND” were used for the associations of words, thus allowing for combinations of descriptors. Six combinations were used with the following descriptors: “Tumoral stroma and neoplastic progression”, “Tumoral stroma and pleomorphic adenoma”, “Tumoral stroma and e carcinoma ex-pleomorphic adenoma”, “Neoplastic progression and pleomorphic adenoma”, “Neoplastic progression and carcinoma ex-pleomorphic adenoma”, and “Pleomorphic adenoma and carcinoma ex-pleomorphic adenoma”.

Two authors individually analyzed the abstracts of the articles to assess which studies were relevant to the subject of this review. Based on the inclusion criteria, full-length articles in Portuguese and English available online and published from 2007 to 2017 that reported the clinical, imaging and anatomopathological characteristics of pleomorphic adenomas and their neoplastic progression were selected. Some relevant articles published before this period were also included.
between the acinar and ductal cells and the basement membrane and are characterized by an eosinophilic, hyaline, and homogeneous cytoplasm, most often with an eccentric nucleus. They may have an epithelioid, polygonal or fusiform pattern.

The myoepithelial cells of the salivary gland presumably have a function limited to contractile action, helping in the excretion of the glandular contents. They reportedly also participate in extracellular matrix synthesis, particularly that of the basal membrane, and also play a key role in tumor suppression. This tumor suppressor role was attributed to myoepithelial cells because they accumulate large quantities of extracellular matrix, and thus promote the onset of a mechanical barrier against tumor development. Their potential to secrete high levels of tissue inhibitors of metalloproteinases (TIMPs) is linked to their tumor suppressor role.

Tumor dissemination starts with the extracellular matrix degradation by matrix metalloproteinases (MMPs). In addition to these enzymes, neoangiogenesis enables tumor growth. The vascular plexus facilitates oxygen transport to neoplastic cells. Thus, in the absence of vascularization, tumor cells fail to spread, and cell death occurs due to hypoxia.

3. Relevance of the stroma to tumor development

The tumor stroma plays a key role in the development of neoplasms. A large quantity of connective tissue and extracellular matrix (ECM) and numerous mesenchymal cells such as fibroblasts and adipocytes, blood and lymphatic vessels, nerves, and inflammatory and immune cells have also been observed in the tumor stroma. The ECM consists of fibrous proteins and glycoproteins. The fibrous proteins include collagen and elastin, whereas the glycoproteins include fibronectin, proteoglycans, and laminin.

The tumor stroma is important for neoplastic progression because its composition may vary; for example, increased collagen biosynthesis may render the tumor elastic. Furthermore, the tumor stroma enables neoplasm nutrition through the formation of blood vessels. The tumor stroma comprises fibroblasts, which are responsible for producing the collagen present in the extracellular matrix. Fibroblasts play a key role in tumors because they are the precursors of myofibroblasts, which are cells of mesenchymal origin found in physiological and pathological conditions. For example, under physiological conditions, they can...
modulate tissue healing\textsuperscript{18}, whereas they can promote tumor progression under pathological conditions\textsuperscript{19}.

In addition to fibroblasts, other cell types may act as precursors for myofibroblasts. For example, smooth muscle cells, pericytes, endothelial cells, adipocytes, and myoepithelial cells can be transdifferentiated into myofibroblasts. Recent studies have also shown that tissue-resident mesenchymal stem cells can also differentiate into myofibroblasts\textsuperscript{20}.

In the tumor stroma, myofibroblasts may contribute to the deposition of collagen fibers. The increased presence of these fibers renders the stroma desmoplastic\textsuperscript{20}. Clinically, this reaction of the stroma is characterized by the hardening and retraction of several malignant tumors. This tumor retraction is attributed to the contractile forces generated by myofibroblasts\textsuperscript{19,20}.

The collagen matrix of the tumor stroma primarily contains type I and III collagen. Type III collagen is most often observed in newly-formed stroma, whereas type I collagen is most evident in the fibrous zones of carcinomas and is more related to tumor progression and metastasis\textsuperscript{21}. Picrosirius is a staining enables the differentiation of collagen fibers, particularly between types I and II\textsuperscript{1,22-24}.

4. Neoangiogenesis and inflammation of the pleomorphic adenoma stroma

Neoangiogenesis is defined as the formation of new blood vessels from pre-existing vessels. It occurs under both physiological and pathological conditions such as embryogenesis, tissue healing, tumor growth, and metastasis\textsuperscript{12,25}.

The main physiological stimulus of neoangiogenesis is hypoxia. This occurs due to the activation of pro-angiogenic factors such as vascular endothelial growth factor (VEGF), which binds to receptors on endothelial cells, smooth muscle cells, and pericytes. This ligand-receptor interaction activates the cells and promotes the budding of new capillaries from pre-existing vessels\textsuperscript{26}.

VEGF is one of the main neoangiogenesis-inducing growth factors. Although it may directly stimulate endothelial cells and fibroblasts to form new vessels, the underlying molecular mechanism of its stimulation remains unknown\textsuperscript{29}. Although the function of VEGF in neoangiogenesis is well established in the literature, little is known about its involvement in tumors of the salivary glands, particularly in pleomorphic adenomas.

Swelam et al.\textsuperscript{27} studied VEGF immunostaining in 20 pleomorphic adenomas and seven normal submandibular glands. In normal salivary glands, VEGF staining was observed in the serous acini and epithelial ducts, whereas staining occurred in the ductal formations and solid areas of pleomorphic adenomas and was stronger in these areas than that in tumor nests. The myxoid areas were also strongly stained by VEGF. Thus, the authors concluded that pleomorphic adenoma is a poorly vascularized tumor because areas with hypoxia were clearly identified in these tumors by VEGF labeling.

The vessels formed in angiogenesis are loosely delimited by solitary cells termed pericytes, a cell group derived from mesenchymal smooth muscle cell lines that play a key role in the tumor context because they may contribute to neoplastic progression\textsuperscript{29}. Pericytes affect the stability of blood vessels by activating or producing molecules that promote the differentiation or quiescence of endothelial cells. Thus, pericytes contribute to neoplastic progression when they induce the differentiation of endothelial cells, allowing the formation of new vessels, which favors the nutritional contribution to neoplastic cells. In addition, studies have also shown that pericytes may be myofibroblast precursors because pericyte are stem cells capable of differentiating into a number of different cell lines\textsuperscript{28,29}.

Although some studies have suggested that pericytes and myofibroblasts may be strategic targets for the treatment of malignant neoplasms\textsuperscript{1,23,24}, studies that clarify the molecular mechanisms whereby they can affect the biological behavior of malignant tumors are still necessary.

In spite of inflammatory cells, they comprise macrophages or tumor-associated macrophages (TAMs), mast cells, neutrophils, T and B lymphocytes, natural killer (NK) cells, and invariant T lymphocytes (NKT cells). Although each inflammatory cell is important in the tumor context, TAMs are the most prominent cells in the regulation of tumorigenesis\textsuperscript{30}.

TAMs may be activated by the classical or alternative pathways. When activated by the classical pathway, they differentiate into M-1 macrophages, whereas they differentiate into M-2 macrophages when activated by the alternative pathway. M-1 macrophages are usually found in the tumor microenvironment of early lesions and are able to eliminate tumor cells by producing reactive oxygen and nitrogen species (ROS and RNS, respectively). Furthermore, M-1 macrophages secrete large quantities of interleukin [IL]-12 and
activate CD8+ T lymphocytes, which are responsible for promoting an efficient antitumor response. To maintain homeostasis or through the mechanisms of tumor cell evasion, macrophages activated by the alternative pathway of the immune system differentiate into M-2 macrophages. These macrophages, in turn, secrete IL-10, which negatively regulates the immune response to tumors by impairing the activation of cytotoxic cells and by favoring an immunosuppressive environment that promotes tumor progression. In addition, M-2 macrophages also produce growth factors such as VEGF, which contribute to tumor maintenance and facilitate the development of metastases.

Although some studies have suggested that M-2 macrophages may contribute to tumor survival, studies that may clarify the molecular mechanisms by which they are able to influence the biological behavior of the tumor are still required. Similarly, although the literature has shown the importance of macrophages for tumor development, studies about the participation of these cells in tumors of salivary glands, especially pleomorphic adenomas, are needed to further understand its immunopathogenesis.

5. Profiles of the genes involved in the neoplastic progression of pleomorphic adenomas

Pleomorphic adenoma has well-defined clinical and histological characteristics. However, the effect of molecular factors related to its malignant transformation is still not fully elucidated. Thus, cytogenetic studies are necessary to assess the biological behavior of this neoplasm.

Mitelman et al. constructed a database of chromosomal changes in cancer. Analysis of 224 pleomorphic adenomas revealed that 30% of these tumors had a normal karyotype, whereas 70% had some type of chromosomal alteration. The most commonly observed changes were balanced, inverted, and unbalanced chromosomal translocations. The balanced chromosome translocations included the gene regions t(3;8)(p21;q12), t(8;9)(q12;p22), and t(5;8)(p13;q12), whereas the inversions involved the 12p12-p13 and 12q15-q24 gene fragments. The unbalanced changes were characterized by the 8p23 and 8q12 regions.

The genes involved in balanced chromosomal translocations in pleomorphic adenomas are PLAG1 and HMGIC. PLAG1 is the most important for the tumor context of the pleomorphic adenoma and is frequently altered in cytogenetic studies of this type of lesion. Knowing that changes in PLAG1 in pleomorphic adenomas result from recurrent, balanced chromosomal translocations, Voz et al. reported that the most frequent chromosomal translocations in the onset of altered PLAG1 expression involved the t(3;8)(p21;q12) and t(5;8)(p13;q12) regions.

Altered PLAG1 expression apparently contributes to the onset of pleomorphic adenomas. Asp et al. and Kandasamy et al. performed cytogenetic and molecular assays, reporting that the heterogeneity of rearrangements in the PLAG1 gene contributes to the development of pleomorphic adenomas. The recurrence of these lesions may be caused by the increased number of genetic mutations.

Some factors may significantly affect the malignant transformation of tumors. Deguchi et al. assessed the expression of c-myc, ras, p21, and p53 in pleomorphic adenomas and carcinoma ex-pleomorphic adenomas. Although the expression of these proteins has been observed in pleomorphic adenomas, these oncoproteins were overexpressed in carcinoma ex-pleomorphic adenomas. Their study showed the importance of these oncoproteins for the prognosis of pleomorphic adenomas, particularly regarding malignant transformation. According to Felix et al. the activation of the oncogenes p53 and c-erbB-2 was implicated in the malignant transformation of pleomorphic adenomas. They observed that p53 was involved in the neoplastic progression of this tumor, whereas c-erbB-2 participated in the development of a more aggressive phenotype.

CONCLUSION

The current knowledge regarding the role of pleomorphic adenomas stroma in the neoplastic progression is increasing. However, studies that point out the molecular profile of these tumors are still necessary in order to elucidate their biological behavior.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES


