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REVIEW ARTICLE

Immunohistochemical biomarkers in ameloblastomas

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ABSTRACT

Ameloblastoma is an aggressive odontogenic tumour, which is locally invasive and highly recurrent. Studies show that ameloblastoma is a benign odontogenic neoplasia, being relatively rare and occasionally presenting behaviour of malignant lesions. In addition to these particularities, the histological diagnosis of ameloblastoma can be challenging when the tumour shows high rates of mitosis, absence of nuclear pleomorphism, basilar hyperplasia and neural invasion. In order to help in the diagnosis, prognosis and treatment of this neoplasia, some immunohistochemical markers were shown to be associated with tumoural epithelium. The identification of these markers as well as of their association with clinical signs can be useful to elaborate more efficient treatment strategies and to control this pathology, including improvement of the quality of life of patients affected by this neoplasia. This article aims to review some markers associated with specific molecular pathways, bone remodelling, cell proliferation, apoptosis, cell signalling and tumour suppression.

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Introduction

Odontogenic tumours are lesions resulting from epithelium, ectomesenchymal and/or mesenchymal elements, which participate in the formation of the tooth structure. According to the World Health Organisation (WHO), these tumours are exclusively found in the maxillofacial region and they can occur in any age group.[1]

Ameloblastoma is a benign epithelial odontogenic tumour resulting from the cellular components of the enamel organ. According to the literature, ameloblastoma is the second most common odontogenic tumour, presenting a very extensive clinical and histological diversity and accounting for 1–2% of all maxillary cysts and tumours. According to authors, ameloblastoma can occur at any age between 0 and 98 years old being more prevalent in the fourth decade of life, with a slight variation depending of the study.[1–4]

The clinical presentation of the ameloblastomas is variable, but they are commonly associated with non-painful mandibular displacement due to their slow growth. Pain and paresthesia are rare symptoms and in general the dental elements associated with these tumours can be impacted or displaced.[1,5]

According to the clinical and radiographic aspects, these tumours can be classified into three main types: solid/multicystic, unicystic and peripheral. Usually, the solid/mulitcystic type is characterized by a locally aggressive behaviour with a high risk of recurrence if not removed adequately. This variant can be subdivided histologically as follicular, plexiform, acanthomatous, desmoplastic, granular cell, and basal cell subtypes, with the first two being the most common.[1,6]

Although the pathogenesis is still unknown, studies on the mechanism of cell proliferation using tumour markers have been conducted to establish the tumour nature and prognosis. These molecules are substances present in or produced by a tumour and can identify cellular and intracellular changes, several mutations and abnormal signalling pathways.[6–10] Epithelial–mesenchymal transition (EMT) is a process which is associated with a loss of intercellular adhesion, acquired mesenchymal characteristics, and increased motility by epithelial cells, playing an essential role in tumour invasiveness. The participation of this mechanism in ameloblastoma aggressiveness is still poorly understood, with few studies showing the high expression of Twist, a key protein involved in cancer metastasis.[8,9]

The present review aims to determine immunohistochemical markers, which can be useful for the prognosis of ameloblastomas by summarizing the results associated with tumour growth, progression and apoptosis.

Clonality

The clonal origin is an important parameter involved with neoplasias. Tumours resulting from the mitotic division of somatic cells are defined as clonal. The clonal nature of the

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odontogenic tumours can be determined by phenotypic assays because of the lack of defined genetic markers. Authors have found that as well as the majority of the odontogenic tumours, ameloblastomas have a monoclonal pattern, which suggests that there is an association with changes in only one clone of odontogenic cell.[11] This finding also suggests that molecular change is the first event of the tumour development.

Cell proliferation markers

The nuclear antigen Ki67 is a cell proliferation marker widely used in the study of tumour behaviour and recurrence of normal and neoplastic tissues. It is one of the most reliable proteins of cell proliferation as it is both present in all active phases of the cell cycle (GI, S, G2 and mitosis) and absent in quiescent cells. Olimid et al. studied 22 patients diagnosed with ameloblastoma and found that the Ki67 protein was present in all cases, being more concentrated in the follicular variant and less concentrated in the cell-granular variant.[12]

The positivity for Ki67 antigen is mainly located in follicular-type cells of the peripheral ameloblastoma as well as in plexiform areas and basal-type cells of unicystic ameloblastoma. Studies found a strongly positive presence of this marker in the follicular subtypes and recurrent lesions.[13–15]

In addition, some authors have demonstrated higher rates of this marker in tumours with microsatellite changes, with such cell proliferation possibly being associated with these changes.[16]

CD10

In order to find answers for the aggressive nature of some types of ameloblastoma, Iezzi et al. assessed the presence of CD10 in stromal cells of these tumours.[17] This protein plays an important role in both cell growth and differentiation, thus being used as indicator of dysplasia and prognosis of several malignant diseases (e.g. epidermoid carcinoma, kidney cell carcinoma, melanoma, acute lymphoblastic leukaemia, among others). By analysing this molecule in 45 ameloblastoma variants, positivity for CD10 was found in stromal cells in only 22.7% of the cases of unicystic and peripheral types. On the other hand, the solid/multicystic variant showed positivity in 60.9% of the cases. Therefore, this marker may be strongly associated with the potential of growth and local invasion of ameloblastomas.[17]

Apoptosis

Caspase is a family of intracellular proteases, which can be activated either intrinsically or extrinsically, besides inducing to apoptosis. Caspase-3 is particularly an important enzyme associated with cell death as its activation results in modification of the cellular DNA. Immunohistochemical analyses of the expression of caspase-3 showed that 61% of the ameloblastomas had diffuse positivity in the central area of tumour islands.[18]

Fas and Fas ligand (FasL) belong to the tumour necrosis factor (TNF) family and act as death receptors and initiate apoptosis (programmed cell death) through extrinsic pathway. Fas, FasL and caspase-3 were described in ameloblastomas.[19]

In addition to these markers, Bcl-2 is an anti-apoptotic protein, which is expressed in 50% of the cases of ameloblastomas.[18] Due to the presence of this marker in the ameloblastomas, many authors emphasize the existence of two cell types in these tumours, namely, anti-apoptotic cells in the (basal) peripheral layer and pro-apoptotic cells in the central portions of the tumour islands.[20,21]

An interesting aspect regarding apoptotic markers is that, differently from Ki67, they were not distinguished in primary and recurrent ameloblastomas, thus proving not to be a predictive marker of recurrence.[18]

The expression of the protein p16 associated with ameloblastomas was studied elsewhere. This protein acts by inhibiting the normal cycle of cell proliferation, mostly involving ameloblastomas, and therefore its presence can influence the biological behaviour of these tumours and their infiltrative nature.[12,22,23]

In the subtypes of ameloblastomas with low rates of recurrence, there is a great expression of p16 in central tumour cells, thus indicating that this epithelium was controlled by this oncoprotein. In the more aggressive subtypes, on the other hand, there is a similar presence of p16 in both central and peripheral cells, which suggests that the localization of this protein can express the infiltrative nature of this tumour.[23]

In addition to p16, the gene p53 is also associated with mutations in malignant disorders. Although the lack of allelism in suppressor tumour genes has been demonstrated in ameloblastomas, mutations in gene p53 were not shown to be associated with the pathogenesis of the neoplasia.[24,25]

The Akt pathway is an intermediate signaller, which can play a role in reducing the apoptosis through activation by phosphatidylinositol 3-kinase (PI3K) inhibitors, which also promote an increase in cell proliferation in odontogenic tumours. On the other hand, the protein PTEN acts as an inhibitor through Akt, causing apoptosis of the cells. A study by Kumamoto et al. showed strong reactivity for both Akt and PI3K in ameloblastomas, with the latter being found in all variants although its expression is significantly higher in the plexiform variant.[26]

The reduction in the PTEN expression was also observed, but its association with transformations of the epithelium have not been investigated.[26]

Finally, the proteins p63 and p73 have also been investigated in ameloblastomas. These proteins are members of the p53 family and play an oncogenic role in the regulation of epithelial cell proliferation and differentiation. Authors found a positive expression of these proteins with statistically significant difference in such tumours and suggest that these markers play a role in differentiation and proliferation of odontogenic epithelial cells.[27,28]

Although genetic alterations are relevant in the pathogenesis of neoplasias, the epigenetics is crucial in the expression of its genome. Changes in the methylation of the P21 gene of the ameloblastoma were found when compared to those of tooth follicles. However, further studies are needed to

demonstrate that the transcriptional activity of this gene is regulated by methylation.[29]

Ameloblastin and other matrix proteins

Ameloblastin is an important dental protein expressed during internal differentiation of the odontogenic epithelium, but this protein is not expressed in these tumours, thus suggesting that tumour cells do not reach functional maturation in the secretory phase of the ameloblasts. However, the amelogenin, which is associated with differentiated ameloblasts, was found in epithelial cells of the ameloblastoma. Due to such results, authors have suggested that the deficiency of ameloblastin may be one of the causes of tumourigenesis and that its presence can play a protective role in the development of ameloblastoma.[29]

Bone remodelling factors

The recent advances in bone biology have identified regulatory molecules of the bone remodelling, which are crucial for formation, differentiation and activity of osteoclasts. Among these regulatory molecules, there is the TNF, a super-family consisting of 19 ligands and 29 receptors whose main function is to stimulate osteoclastogenesis. All members of this super-family exhibit pro-inflammatory activity through activation of the transcriptional nuclear factor NF-Kb (RANK). The RANK is present on the osteoblast surface and is activated by the RANKL (nuclear factor NF-kB ligand), a member of the TNF family, which is crucial for the formation of osteoclasts.[30] The osteoprotegerin (OPG) is a soluble glycoprotein capable of mediating bone remodelling, since it binds to the RANKL receptor and interrupts the RANKL/RANK interactions expressed by bone tissue (osteoblasts and mesenchymal cells), immune cells (T and B cells) and vessels (endothelial and vascular smooth muscle cells).[30]

The system RANK/RANKL/OPG, with its functional balance, is crucial for homeostasis of the bone remodelling. In order to find answers for this osteoclastic activity, authors have investigated the presence of several markers in ameloblastomas such as PTH, ODF (osteoclast differentiation factor), RANKL, OCIF (osteoclastogenesis inhibiting factor) and OPG (osteoprotegerin). As a result, these molecules were found to be very prevalent and there is evidence that ameloblastomas secrete RANKL and TNF-α, causing bone destruction and promoting tumour expansion.[30,31]

The analysis of the distribution of OPG, RANKL and RANK in solid/multicystic and unicystic ameloblastomas shows higher levels in the first subtype. In a study, authors have found higher numbers of RANK and RANKL-positive cells in the stroma of solid/multicystic ameloblastomas when compared with unicystic ameloblastomas, dentigerous cysts and dental follicles. According to them, these results account for a larger bone/tooth resorption activity in solid/multicystic ameloblastomas when compared with unicystic ameloblastomas.[30]

In another study, authors have found that RANK and OPG are widely expressed in almost all recurrent solid/multicystic ameloblastomas, associated with a marked loss of RANKL expression. They argued that a plausible explanation for the disparity it might be that the tumours investigated represents an indolent/dormant subset of tumours distinct from their primary counterparts. The low/non-expression of RANKL in these tumours might imply an altered local bone metabolism with low resorptive activities.[32]

Matrix metalloproteinases

The biological behaviour of ameloblastomas has also been associated with the presence of matrix metalloproteinases (MMPs). These MMPs are proteolytic enzymes produced by epithelial and mesenchymal cells, which degrade the extracellular matrix and promote bone resorption under pathological conditions. Among the several types of MMPs, those of types 2 and 9 are involved in the process of tumour invasion due to their great ability to degrade the collagen. Studies have demonstrated an increased expression of these proteins in all cases of ameloblastoma when compared to odontogenic keratocystic tumours and odontogenic cysts. Among the several histological types and subtypes of ameloblastoma, it was found that these proteins have an increased expression in the plexiform (solid), follicular (solid) and mural (unicystic) variants. These findings suggest that the presence of these proteins can contribute to the biological behaviour of ameloblastomas as well as to the increase in their aggressiveness.[33,34] Studies have also demonstrated that MMP2, TIMP2 and MMP14 are highly expressed in primary tumours of greater size and in cases of recurrent multicystic ameloblastoma.[35]

Intermediate signalling molecules

There are several signalling pathways activated by cell processes, which vary in terms of proliferation metabolism. The Ras/MAPK signalling pathway is activated outside the cell, controlling cellular processes such as proliferation and differentiation by means of phosphorylation cascades. Fibroblast growth factor receptor 2 (FGFR2) is one of the several receptors that activate RAS.[36–41]

Once activated, these contribute to the activation of RAF, RAF1 and BRAF. Particularly, BRAF is a protein that may be associated with several malignant neoplasias after undergoing mutation, including melanoma, hairy cell leukemia and colorectal cancer. Ninety percent of the BRAF mutations involve the replacement of a glutamine by a valine (V600E).[37–41]

MAPK signalling was recently reported to be associated with ameloblastomas and other odontogenic tumours with ameloblastic morphology.[38–42] BRAF V600E mutation occurs in somatic cells and demonstrates to play a prominent role in the pathogenesis of ameloblastoma.[41]

Brown et al. observed that BRAF V600E mutation is associated with in a younger population and not commonly found in maxilla cases. Kurppa et al. analysed 24 samples of ameloblastomas for BRAF V600E mutation by deep sequencing and immunohistochemistry. Interestingly, they found BRAF

mutation in 63% of the cases.[39] Besides that, a high concordance was observed between immunohistochemistry and molecular detection of BRAF V600E mutation, demonstrating that both techniques may be useful in the diagnosis of ameloblastomas.[41]

In addition, BRAF mutation can also be a predictor of recurrence free survival. A study with a multivariate analysis that included BRAF status and treatment procedure showed that they were independent and statistically significant predictors of recurrence. This finding reinforces that BRAF V600E can be also used as a prognostic marker.[41]

Other signalling molecules

Syndecan-1 (CD138)

This is an extracellular transmembrane receptor, which binds to growth factors and matrix molecules, thus initiating numerous signalling cascades, including the Ras-MAPK pathways. This receptor also promotes adhesion to the extracellular matrix and delays the migration of cancer cells, thus being used as prognostic marker of various malignancies.[36]

Al-Otaibi et al. found a decreased expression of syndecan-1 in variants of follicular, plexiform, cell-granular and acanthomatous ameloblastomas. The unicystic type of ameloblastoma showed an increased expression of syndecan-1 when compared to the solid variant, but no statistically significant difference was reported. The decreased expression of this marker can be associated with the invasiveness of ameloblastomas, and further studies are needed to determine target therapies involving this marker.[43]

Wnt signalling

The Wnt signalling pathway involves the Wnt binding to its receptor, thus stabilizing the cytoplasmic beta-catenin. This interaction speeds up the expression of genes related to cell proliferation and cellular cycle. The accumulation of beta-catenin was demonstrated in ameloblastomas and associated with oncogenesis and cell proliferation.[44]

Calretinin (CALB2) is a protein with diffuse expression and intense activity in the internal epithelium of the enamel organ and secretion of ameloblasts during odontogenesis. This protein is highly expressed by ameloblastomas and can play an important transitional role in the remnants of the dental lamina in the ameloblastomas.[45]

Cytokeratins

Cytokeratins (CK) has also been classically studied on ameloblastomas, since they present high selectivity depending on the linage and cell differentiation. CK are intracellular intermediary filaments which are expressed depending on both type and cell differentiation, involving 20 different types in the human epithelium. Ameloblastomas can present a heterogeneous group of these proteins depending on their level of cell differentiation. The presence of CK 5, 6, 8, 13, 14, 16, 18 and 19 was observed in the tumour epithelium, with CK14 being predominant and CKs 6, 15, 16 and 19 useful for differentiating histological subtypes and for indicating rapid growth.[46–48]

In order to analyse the aggressiveness of ameloblastomas, authors have compared the expression of immunohistochemical markers with that of ameloblastic carcinoma. There were no differences in the expression of CK7, 14 and 19. However, the positivity for CK18, MMPs 2 and 9, and Ki67 showed statistically significant differences, which may indicate a distinct factor of aggressiveness between ameloblastomas and their malignant variant.[48]

Kumamoto et al. also described a study of epithelial markers comparing malignant neoplasias to ameloblastomas. These authors found a diffuse expression of CK19 in the cases of spinal-cell carcinoma, basal-cell carcinoma, adamantinomas, ameloblastic carcinoma and ameloblastomas. These data suggest a similarity in the expression of this protein for both malignant odontogenic and non-odontogenic neoplasias.[49]

New perspectives

Recent studies showing frequent mutations in the MAPK pathway, specially the BRAFV600E, have opened new perspectives on the treatment possibilities of the ameloblastoma.[39–42,50–54] Target therapy for BRAFV600E-mutated melanomas presents good results in clinical trials and some inhibitors, such as the vemurafenib, are approved by the U.S. Food and Drug Administration (FDA).[51] Two recent works showed that BRAFV600E-mutated ameloblastic cells are sensitive to vemurafenib *in vitro*.[40,41] In recently published case reports, patients with multiple, recurrent, metastatic and BRAFV600E-mutated ameloblastomas were treated with dabrafenib and trametinib, a combination with BRAF and MEK inhibitor respectively, responding dramatically to the initial therapy.[52–54]

The association between clinicopathologic features of the ameloblastoma and molecular markers are still poorly understood and more studies regarding this aspect are necessary. Investigations on specific cellular pathways, such as MAPK – frequently altered in ameloblastomas, could lead to the establishment of a good marker to guide to different and more effective treatment options compared to the traditional surgery, improving the effectiveness of the treatment and quality of life of the patients.

Conclusions

It is of extreme importance to better understand the expression of biomarkers associated with ameloblastoma for the establishment of treatment propositions, as the biological behaviour of this neoplasia is not well established yet. Current evidences indicate the different roles of these molecules and may also provide directions for clinical treatment of these recurrent tumours. The recent description of mutations associated with ameloblastomas opens the possibility of choosing target therapies, thus changing the current paradigm in which surgery is the only proposed therapy.

Disclosure statement

Authors have no conflict of interest to declare.

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