Review

Molecular review of odontogenic myxoma

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SUMMARY

Odontogenic myxoma (OM) is a benign odontogenic neoplasm that tends to recur due to bone infiltration. This review focuses on the molecular aspects of the OM. The following topics are discussed: clonal nature, matrix metalloproteinases, apoptosis and cell proliferation, genetic alterations, and other markers. Translational studies are necessary to identify the prognostic markers of this lesion, and also, molecular biology studies may help to identify the etiologic factors and to develop more effective and less aggressive approaches, other than surgery, to the treatment of this infiltrating odontogenic tumor.

Introduction

Odontogenic myxoma (OM) is an intraosseous neoplasm that comprises 3–20% of all odontogenic tumors and is characterized by stellate and spindle-shaped cells embedded in an abundant myxoid or mucoid extracellular matrix.1 It presents most commonly during the second or third decade of life. This tumor may lead to an extensive bone expansion and usually infiltrates the adjacent bone.2–5

The histogenesis of OM is most likely related to the odontogenic ectomesenchyme of a developing tooth or undifferentiated mesenchymal cells in the periodontal ligament and there are many theories that support the odontogenic origin of the OM.6 These theories are supported by the histological similarity between OM and pulpal ectomesenchyme, proximity to the tooth-bearing areas of the jaws, periodic association with missing or unerupted teeth, occasional presence of inactive odontogenic epithelium, and its uncommon occurrence in other parts of the skeleton.6 Sivakumar et al.7 investigated a panel of antibodies to characterize and distinguish the nature of the cells in odontogenic myxoma and suggested that OM is a tumor of a dual fibroblastic-histiocytic origin and the cells comprising odontogenic myxoma are of myofibroblastic origin.7

An abundant myxoid extracellular matrix (ECM) has been recognized in various reactive and neoplastic lesions, including OM.8 The ECM is composed of glycosaminoglycans, proteoglycans, collagens, fibronectin, and tenasin.4,9,10 The specific composition and the relative amount of each component contribute to the edematous appearance of the myxoid ECM, facilitating diffusion of metabolites and the formation of networks permissive for cell growth and migration.8,11 Further studies comparing reactive lesions with benign and malignant neoplasms will help to elucidate which specific component of ECM in OM is associated with its invasive behavior.

There is no consensus regarding the mechanisms of invasiveness of OM and the theories include from expression of matrix metalloproteinases (MMPs) to the expression of antiapoptotic proteins, alterations in receptor activator of nuclear factor kappa B ligand (RANKL), its receptor RANK, and osteoprotegerin (OPG) system, and genetic alterations.4,9,10

In this paper, we discuss the main molecular aspects of the OM, highlighting the genetic alterations described so far, as well as the published data about apoptosis and cell proliferation, MMPs, and other markers that may take part in its pathogenesis.

Clonal nature

An issue of controversy is the clonal/polyclonal origin of neoplasias. We recently published some results of odontogenic tumors clonality assessment based on the HUMARA assay.12 We were only
able to include two samples of odontogenic myxomas. While one revealed monotypy at the HUMARA, the other was polyclonal. Depending on the size of the patch, this monotypy may represent a true monoclonality. Taking together the fact that the origin of the OM is not clearly defined and the size of the odontogenic patch is not determined and one sample was polyclonal, the clonal nature of such a lesion remains to be established.

**MMPs**

MMPs are a group of enzymes involved in regulating and remodeling the ECM and play an important role in the regulation of cell communication and other processes such as cell surface receptors, cytokines, adhesion molecules, and growth factors. MMPs hydrolyze components of ECM and are synthesized by cells of the connective tissue such as fibroblasts, osteoblasts, and also by odontoblasts. MMP-2 and -9 degrade type IV collagen, the major component of the basal membrane, and due to this fact are associated with the invasive phenotype of some lesions. In addition, as MMPs seem important in odontogenesis and considering that in theory OM are derived from the tooth-forming ectomesenchyme, MMPs have been extensively investigated in these lesions.

Randall and Hall reported the expression of MMP-1, -2, -3, and -9 during tooth development. They observed, during the bud stage, that the MMPs were expressed within both epithelial and mesenchymal cells. During the cap stage, differential expression was observed highlighting the expression of MMP-3 within the enamel knot. MMP-9 immunoreactivity was observed in both, the inner epithelium of the enamel organ and pre-odontoblasts, with weak staining in dental papilla, suggesting a role of MMP-9 in odontogenesis, acting especially on the degradation of the basement membrane at the dental papilla/enamel organ interface. During the early bell stage, the four MMPs were expressed in the inner enamel epithelium, basement membrane, and pre-odontoblasts, but the dental papilla presented a weak immunooexpression. In the late bell stage, the expression of MMPs was negligible in the inner enamel epithelium, the pre-ameloblasts were weakly positive, and, in the dental papilla, the pre-odontoblasts were positive for the MMPs studied.

Some years later, the role of MMPs in tooth development was evaluated using a culture of embryonic mouse tooth germs. The study suggested that MMP-2 plays a role in basement membrane degradation; thus, allowing physical communication between odontoblasts and ameloblasts. At a later stage of development, its expression in dental tissues was restricted to differentiated odontoblasts. MMP-9, on the other hand, was intensely and transiently expressed in the early dental mesenchyme surrounding the invaginating tooth bud, but its expression diminished at the later stages.

Using immunohistochemistry, Bast et al. showed that 90% of OM cells (n = 26) stained positively for MMP-2 compared with positivity of 10% in myxomatous dental follicular tissue and myxoid dental pulp used as controls. In this same publication, specimen and control tissues were negative for MMP-3 and -9. Moreover, attempting to improve the understanding of the OM local invasiveness capacity, Miyagi et al. investigated MMP-2 and -9 in tumor samples as well as in cell line derived from OM. Contrasting the results of Bast et al., the authors demonstrated MMP-2 and -9 expression in all cells of an OM cell culture, using immunofluorescence. However, this data should be analyzed with caution, once it is based on a cell line originating from an OM and the experiments were conducted between the 8th and 10th passages. As the tumor cells interact with their environment, it is possible that some original characteristics are lost or changed once these cells are cultured and they no longer have contact with the environment. They also analyzed the expression and activity of MMP-2 and -9 in OM with zymographic analysis and demonstrated that the active and latent MMP-9 were present in the tumoral cells, but the MMP-2 was only found latent in these cells, suggesting that MMP-9 is important to the invasive behavior of OM.

Analysis of the immunohistochemical expression of MMP-1 in OM revealed a lower expression (66.7%) compared with that observed in the ECM of dental papillae (75%) but this difference was not significant (p > 0.05), suggesting a possible role of this enzyme in the pathogenesis of OM. In parallel to the functions proposed for this protease in degenerative processes and malignant tumors, in OM the MMP-1 may act by remodeling the extracellular matrix and by facilitating the dissemination of the tumor through neighboring bone. This suggestion is supported by the observation of an association between this MMP and adhesion molecule receptors, such as integrins, as reported previously.

The role of MMP in the invasion process of OM is still a matter of debate. It was demonstrated that the OM expressed more MMP-1 than MMP-2 and more MMP-2 than MMP-9; however, these authors did not prove a different expression pattern of these MMPs in OM when compared to tooth germs. As previously described by Randall and Hall, the MMPs-1, -2, -3, and -9 are expressed during normal tooth development, exhibiting an important role in remodeling of ECM and in regulation of the shape of tooth germ. As the majority of MMPs described in tooth development in different stages are also described as positive in OM, it was not possible, until now, to associate their temporospatial expression to the development of OM. In addition, most of the present data is only based on immunohistochemical results, which may lead to different results depending on factors such as sample fixation, processing, and different antibodies clones. Functional studies using cell culture are necessary for the better understanding of the role of MMPs in OM growth. MMPs modulation can be an interesting target in future OM molecular therapy.

**Apoptosis and cell proliferation**

Bast et al. demonstrated an increased immunoreexpression of Bcl-2 and Bcl-x, antiapoptotic proteins, in OM (n = 26) compared to the dental follicle and dental pulp. While an average of 6.5% and 10.4% of tumor cells were positive for Bcl-2 and Bcl-x, respectively, a positivity of about 1% in normal dental pulp tissues was found for both proteins. In addition, Bak and Bax, proapoptotic proteins, were not detected in tumor cells. As they found a low proliferation activity in the same tumor samples, they pointed to the production of antiapoptotic proteins as a possible mechanism of tumor progression.

However, a study of a large series of cases (n = 62) found less than 1% of Bcl-2 and MIB-1 (Ki-67) positive cells in OM. Negative staining for Bcl-2 was reported in another study including 12 samples, but MIB-1 (Ki-67) stained about 4% of the 'stromal' cells, leading the authors to conclude that 'OM mainly grows due to the proliferative activity of the stromal cells'. As the 2 largest series of studies with OM including 62 and 26 samples, respectively, demonstrated an overall low proliferation activity in the neoplasia, it seems that the proliferative activity of tumor cells is not the major factor responsible for tumor growth. The apoptotic/antiapoptotic proteins were investigated only based on immunohistochemistry experiments, and the overall findings do not entirely support the idea that the overexpression of antiapoptotic proteins is one of the growth mechanisms of OM. New studies relying on more accurate/sensitive techniques are needed to elucidate the real role of the apoptotic/antiapoptotic proteins on the OM pathogenesis.

**Genetic alterations**

Mutations that inhibit the GTPase activity of a subunit of the stimulating G protein (Gsa) have been demonstrated in the
myocardium of patients with McCune-Albright syndrome. Boson et al. investigated the presence of Gs alpha gene mutations in 23 OMs and, although there are histopathological similarities shared by cardiac and jaw myxomas, no Gs alpha mutations could be demonstrated in any of the tumors analyzed by these authors.

Cyclic adenosine monophosphate (cAMP)-dependent protein kinase A (PKA, EC 2.7.1.37) is the major mediator of the cAMP effects in eukaryotic cells. In its inactive state, the PKA holoenzyme is a tetramer comprised of a dimer of two regulatory subunits bound to two catalytic subunits. When cellular cAMP concentrations are elevated, two molecules of cAMP bind each of the regulatory subunits, which leads to dissociation of the tetramer into regulatory dimers and two free catalytic subunits. The free catalytic subunits represent active serine threonine kinases that regulate downstream effector enzymes, ion channels, and transcription of specific genes that mediate cell growth, proliferation, and differentiation. In human, four different regulatory (Ria, Rib, Rla, and Rlb) and four catalytic subunits (Ca,Cb,Cg, and PRKX) have been identified thus far.

PRKAR1A is the gene encoding RI-α. This protein is a relatively abundant regulatory subunit type IA of cAMP-dependent PKA, the central hub of all cAMP-related signaling. PRKAR1A-inactivating mutation was identified as the founding mutation of the syndrome known as Carney complex. This is a multiple endocrine neoplasia syndrome associated with various types of myxomas such as cardiac and skin myxomas. OM is not related with Carney syndrome; however, cardiac myxoma and those of the skin and other locations are not substantially different histologically from OM. The same spindle-shaped cells appear in an abundant stroma of myxoid material. Our research group in collaboration with other group screened 17 odontogenic myxomas from patients without Carney complex for PRKAR1A mutations and PRKAR1A protein expression by immunohistochemistry. We found 53% of the samples with decreased immunostaining for RI-α protein product and 2 samples harboring previously undescribed mutations of the PRKAR1A gene. One mutation (c.725C > A) led to a nonconservative amino acid substitution in a highly conserved area of the gene; the other was a single base-pair deletion that led to a frameshift (del774C) and a stop codon, 11 amino acids downstream of the mutation site; both tumors were heterozygous for the respective mutations. Why a decrease in RI-α immunostaining in the remaining tumors in the absence of any coding sequence PRKAR1A mutations still needs to be explained. Tumor development-related gene silencing through methylation or a related mechanism may be the underlying explanation.

PKA signaling is essential in embryonic meso- and ectodermal differentiation. RI-α has ubiquitous distribution and is primarily involved in the control of proliferation and neoplastic transformation. It also plays an important role in the transition from the G1 to S phase of the cell cycle. RI-α-deficient mice display a severe phenotype, with gross developmental defects in mesodermal morphogenesis and early embryonic lethality due to incomplete development of the primitive heart tube. However, there are conflicting data in the literature about PRKAR1A’s role in human neoplasia, cancer cell lines, and animal models. In contrast with the Carney complex that arises from loss of PRKAR1A, the expression of RI-α is frequently increased in several cancers. Functional studies are necessary to delineate the role of PRKAR1A gene in OM development and/or growth.

HMG2A, a member of a group of proteins related to tumor formation through transcriptional activation of specific genes, was studied by Sato et al. HMG2A is a member of the high-mobility group A proteins and was formerly referred to as HMG1-C. These proteins are small non-histone, chromatin-associated proteins that function as activators or repressors of gene expression through transcription factors, being involved in fibroadenomas of the breast, salivary gland and endometrial hyperplasia, pituitary adenomas, enhanced adipogenesis, and others. HMG2A may suppress the cell cycle arrest at G1 that is mediated by p16 and p19ARF, and also senescence. The HMG2A protein is widely expressed during embryogenesis, but it is undetectable in most normal adult human tissues. The overexpression of the protein is a constant feature of malignant tumors, whereas rearrangements of the HMG2A gene are features of benign mesenchymal tumors. Sato et al. suggested that HMG2A rearrangement and HMG2A protein overexpression might be associated with the tumorigenesis of OM.

Other markers

Osteoclast formation, differentiation, and activity are regulated by RANK/RANKL/OPG system. Ligation of RANKL to RANK results in the fusion, differentiation, and activation of osteoclasts, while OPG inhibits this interaction. The majority of the mesenchymal cells in OM exhibit a tendency to demonstrate a higher content of RANKL than OPG. Translational studies are necessary to demonstrate if RANKL/OPG index is has impact on OM recurrence or behavior.

Nestin is one of the intermediate filaments constituting the cytoskeleton, a marker of neural stem cells or progenitor cells, also related to tooth development and repair of dentine. Its expression has been reported in pathological conditions, such as central and peripheral nervous system tumors. It seems reasonable that some odontogenic tumors express nestin because odontogenic ectomesenchymal tissue is derived from neural crest. Referring to OM, Fujita et al. reported four of nine cases positive for nestin and a variable expression among various odontogenic tumors, probably related to the origin and the different stages of differentiation of each group of tumor investigated. The results of these authors imply that there are two possible histogenesis of OM affecting the jaw: the dental papilla cells of tooth germ and mesenchymal cells such as fibroblasts or myofibroblasts other than odontogenic ectomesenchyme.

Conclusion

OMs are extensively described as case report studies. However, larger studies investigating biomarkers related to OM development and growth are necessary. Despite efforts to delineate molecular aspects, the invasive behavior of these lesions has not been explained. These advances are imperative in order to establish new approaches in the therapy of this ectomesenchymal odontogenic tumor.

Conflict of interest statement

None declared.

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