Molecular investigation of ameloblastic fibroma: how far have we gone?

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Abstract
The ameloblastic fibroma (AF) is a tumour composed of odontogenic ectomesenchyme resembling the dental papilla with epithelial strands and nests similar to the dental lamina and enamel organ, but with no dental hard tissues¹. The AF occurs more often in the first two decades of life, and the mandible is more frequently affected than the maxilla, with a predilection for the posterior area². The tumour presents as a painless swelling with a well-defined, uni- or multicocular radiolucent finding, usually exhibiting a radio-opaque boundary in the affected region². Surgical excision or curettage with the removal of affected teeth is the treatment of choice and malignant transformation of the mesenchymal counterpart may occur, thereby inducing the transformation of AF into ameloblastic fibrosarcoma (AFS)¹,³–⁵.

If there is dentin or enamel formation, the lesion is classified as ameloblastic fibrodentinoma (AFD) or ameloblastic fibro-odontoma (AFO), respectively¹. The AF, AFD and AFO comprise a group of lesions called mixed odontogenic tumours and, they histologically resemble various stages of tooth formation. Whether these tumours should be classified as distinct entities or represent different stages in the maturation of the same entity is a matter of debate. Some authors suggest that it is difficult to distinguish the true tumour and a developing odontoma at one stage exhibiting the same histologic appearances of an AF²,⁶. Chen et al reported some findings that do not support the concept of AF developing into AFD or eventually complex odonto-roma⁶. They also highlighted that AFs are commonly seen in adults past the tooth-developing age (>22 years), and they present a tendency to recur and have the potential of malignant transformation, which together reinforce the neoplastic nature of the lesion. Chen et al identified 123 reported cases of AF² and according to them, majority of AFs can be considered to be true neoplasms, but some, especially those that occur in childhood, could represent the primitive stage of a developing odontoma².

Because of its rarity, studies on AF pathogenesis and molecular mechanisms are scarce. For obvious reasons, most of the molecular investigations on odontogenic tumours include lesions that are more frequently diagnosed, such as ameloblastoma and the odontogenic keratocyst (keratocystic odontogenic tumour)⁸–¹⁰.

This review focuses on the pathogenesis and the main molecular aspects of the origin and behaviour of AF, including investigations of its malignant counterpart.

Introduction
The ameloblastic fibroma is an uncommon odontogenic tumour that may present an aggressive behaviour and may have the potential for malignant transformation. Despite all the efforts to clarify the pathogenesis of odontogenic tumours, the origin of the ameloblastic fibroma is still uncertain. This review focuses on the molecular pathogenesis of the ameloblastic fibroma.

Methods and discussion
Immunohistochemical findings
Most of the studies involving AF are based on the immunohistochemical detection of proteins involved in the cell cycle, apoptosis and epithelial–mesenchymal interactions⁴–¹¹. In addition, several of these studies rely on a small number of cases in view of the rarity of the lesion. Although there is no consensus on a definitive useful marker, the deregulation of cell cycle proteins seems to be important in AF aetiopathogenesis.

Immunohistochemical identification of diverse cytokeratins (CKs) is considered to be a useful tool to characterise the cells and as an attempt to explain the histogenesis of some lesions²,³. Five AFs were investigated and all of them expressed CK 7, 13 and 14, similar to the immunophenotype of the dental lamina⁴. The authors suggested that the clinical behaviour of AF is determined by the influence of the ectomesenchymal tissue in the epithelial cells.

AF cell proliferation index is a matter of debate. While one study concluded that both epithelial and mesenchymal components have similar cell proliferation activity¹², another group reported a higher proliferation index of the epithelial component of AF when compared with the mesenchymal counterpart¹¹. The epithelial component of recurrent AF has shown a higher proliferation index (based on Ki-67 immunoexpression) than the epithelium of lesions that did not recur. The same results were found within AFS samples⁴,¹¹. Such results support the idea that the cell proliferation activity of AF is linked to tumour aggressiveness. The immunohistochemical analysis of Ki-67 may be helpful in understanding the biological mechanisms

References
related to the possible transformation of AF into AFS.11,16

Positive Ki-67, p53 and proliferating cell nuclear antigen (PCNA) are considered to be useful biomarkers of malignant transformation of AF into AFS.11,13,15, as AFS shows higher positivity of these markers. When the ectomesenchymal components of both lesions (AF and AFS) are compared, it is predictable that the malignant cells present a higher index of proliferation. Conversely, murine double minute-2 (MDM-2), a negative regulator of p53, showed a negative expression in the benign and sarcomatous component of AFS.16 The immunohistochemical expression of the p53 protein exclusively in the mesenchymal tissue of AFS is indicative of malignant transformation and alterations in TP53 might play a role in the pathogenesis of this tumour.15

The aggressiveness of the tumour is also related to the imbalance between bone resorption and bone apposition. The RANK/RANKL/OPG is a system comprised of the following proteins: receptor activator of nuclear factor kappa B ligand (RANKL), its receptor RANK and osteoprotegerin (OPG). This system regulates osteoclast formation, differentiation and activity; and these proteins were found to be similarly expressed in the mesenchymal as well as in the epithelial cells of AF.17 This finding is consistent with the potential of bone resorption of the tumour.17

If the AF aggressiveness is influenced by cell proliferation, apoptotic activity is also a key event in the regulation of tumour growth. Calretinin, a calcium-binding protein, plays a possible role as a calcium sensor and inhibitor of apoptosis. This protein was not immuno-detected in AFs, which was not surprising, as the mesenchymal portion of tooth germs was also negative for calretinin immunoreactivity.18 Bcl-2 is an anti-apoptotic protein and its immunoreexpression was investigated in AF and AFS.11 The mesenchymal component of AF remained negative for Bcl-2, with positivity restricted to the epithelial portion.11 On the other hand, the protein showed positivity in the sarcomatous portion of the malignant tumour, while the epithelial strands were negative for its expression.11 On the basis of these findings, it seems that Bcl-2 expression is important in AF progression to AFS, and it would be interesting to investigate this gene using other molecular approaches. The investigation of the apoptotic activity of AF as well as AFS is relevant for a better characterisation and understanding of their molecular pathogenesis.

The immunoreexpression of some other molecules in AF has been investigated. It is suggested that AF develops at an early stage of odontogenesis, in view of the vimentin staining in some areas of immature dental papilla-like cells as well as in the odontogenic epithelium of AF.19 Nestin is a protein expressed in ectomesenchymal tissues during tooth development. From the early stages until the tooth is complete and its immunoreactivity was demonstrated in 2/2 cases of AF.20 However, the function of this protein and its role in AF pathogenesis is unclear.

As the odontogenic tumours often show histopathological features similar to structures involved in amelogenesis, a recent study assessed the immunohistochemical expression of odontogenic ameloblast-associated proteins, amelitin, ameloblastin and amelogenin in diverse odontogenic tumours, including AF.21 Among these four proteins, AF was positive only for amelogenin. On the basis of their results, the authors suggested that the tumour cells are as undifferentiated as the dental lamina cells.21 However, such conclusions need to be further confirmed, as this study relied only on one AF sample.

Genetic alterations

A few investigations of the molecular alterations in AF and AFS have been conducted. Aneuploidy results from chromosomal instability, which is an early event in cancer pathogenesis and one of the hallmarks of cancer. DNA ploidy of three AFs and five AFSs was investigated by flow cytometry.22,23 All three cases of AF were diploid and the DNA content of the odontogenic epithelium and the mesenchyme had similar nuclear DNA contents. Four cases of AFS were diploid; however, one case presented a diploid nuclear content in the epithelial component whereas the mesenchymal component demonstrated one diploid and an aneuploid peak.22 These findings reinforce the idea that the mesenchymal component is the one that undergoes malignant transformation.

Our group has recently conducted a study to investigate genetic instability in the odontogenic mixed tumours (AF, AFO and AFS).24 We used a panel of microsatellite markers for the chromosome regions 3p, 9p, 11p, 11q and 17p, which contain important tumour suppressor genes found to be altered in many human neoplasms. The average fraction of allelic loss (FAL) in AF was 13.2%, whereas FAL average was 36.6% in AFO and 74.6% in AFS. The TP53 marker showed the highest FAL (62%) as 5/8 informative lesions showed loss of heterozygosity (LOH) at this marker. AF only showed LOH at the genetic locus 17p13 (markers p53 and AFM238WF2).

In terms of AFS, the cKIT, a proto-oncogene involved in the transcription of growth factors in stem cells, has been investigated but no mutations were identified in exons 9, 11, 13 and 17.16

The PRKAR1A gene encodes the regulatory subunit of protein kinase A (PKA), an important mediator in the eukaryotic cells, which is involved in cell proliferation, differentiation and apoptosis.25,26 Our group has previously demonstrated mutation and decreased expression of PRKAR1A in odontogenic myxomas.27 Now we are

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