Molecular etiopathogenesis of ameloblastoma – current concepts revisited

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Abstract

The ameloblastoma (AB) is a true neoplasm of enamel organ type tissue which does not undergo differentiation to the point of enamel formation. AB is locally invasive and recurs despite adequate surgical removal. It is of varied origin, although the stimulus initiating the process is unknown. The study of molecular and genetic alterations associated with the development and progression of the AB will help to predict the course of the tumor and lead to the development of new therapeutic concepts for their management. An attempt has been made at compiling about molecular pathogenesis of AB.

Keywords

Ameloblastin, ameloblastoma, matrix metalloproteinase, molecular and genetic alterations, signaling molecules

Introduction

Ameloblastoma (AB) is the second most common benign epithelial odontogenic tumor. ABs are clinically classified into solid/multicystic, unicystic, desmoplastic, and peripheral types, and based upon the pattern of arrangement of tumor cells they may be divided into follicular, plexiform, acanthomatous, granular, basal cell types, etc.[1] AB histologically resembles the epithelial odontogenic apparatus, such as enamel organ and dental lamina, in some respects; however, the detailed mechanism of oncogenesis, cytodifferentiation, and tumor progression remains unknown. This review focuses on molecular and genetic alterations that occur in AB which are helpful for better treatment and prognosis.

Clonality

Most odontogenic tumors are monoclonal in nature.[2] An initial mutation/molecular alteration is the first event in the development of the tumor. However, the sequence of events remains unknown.[3]

Stem Cell Related Molecules

Kumamoto et al., (2010) analyzed the expression of stem cell related molecules (Bmi-1, CD133, and ATP-binding cassette subfamily G member 2 [ABCG2]) in ameloblastic tumors and in tooth germs. Positive expression of CD133 and Bmi-1 was observed in odontogenic epithelial cells adjacent to basement membrane in tooth germs, ABs, and metastasizing ABs, and most of the neoplastic cells in clear cell odontogenic carcinomas and ameloblastic carcinomas showed reactivity for CD133 and Bmi-1. When compared to the tooth germs and ABs, malignant ameloblastic tumors showed significantly increased expression of CD133. ABCG2 was expressed in some ameloblastic tumors but not found in tooth germs. Malignant ameloblastic tumors showed significantly stronger expression of ABCG2 than that in tooth germs. They suggested that stem cell-related molecules might have role in cell differentiation, oncogenesis, and malignant potential of odontogenic epithelium.[4]

Cell Adhesion Molecules

Leocata et al., (2007) conducted a study in intraosseous ABs and it was found that syndecan-1 (SDC1) expression by tumor epithelial cells and subsequent shifting to stromal cells and extracellular matrix, which might be the reason for the local invasiveness of some intraosseous AB subtypes.[5] Bologna-Molina et al. (2008) found reduced expression of SDC1 in solid ameloblastoma (SA) and correlated with its more aggressive biological behavior when compared to unicystic...
Ameloblastin and Other Enamel Matrix Proteins

Expression of ameloblastin, sheathlin, and enamelin proteins were not found in AB, suggesting that ameloblasts have not attained functional maturation in tumor cells. However, Snead (1992) found that amelogenin transcribed only by differentiated ameloblasts, was expressed by AB epithelial cells. Toyosawa et al., (2000) reported potential mutations in ameloblast transcript although these mutations may not be primarily related to the development of AB.[5]

Perdigao et al., (2004) also found an association of mutation in ameloblastin gene in epithelial odontogenic tumors. The exact mechanism of tumor formation in mutant mice is unclear but due to deficiency of ameloblastin there may be deregulation of ameloblast differentiation which might be the likely cause of the tumor.[5]

Molecules Involved In Cell Cycle Proliferation

Ki-67

Florescu et al., (2012) in their study observed an increased expression of Ki-67 in peripheral cells of tumor islands when compared to the central cells suggesting that the peripheral cells are more proliferative. Ki-67 indices were similar in both UA and SA, despite some difference in various subtypes: highest values in the luminal UA which could be attributed to the presence of less number of stellate reticulum-like cells when compared to other subtypes, and consequently, most of the cells counted corresponded to basal, and parabasal layers which are more prone to be positive.[13]

Proliferating nuclear cell antigen (PCNA)

PCNA expression is more in UA compared to SA. Funaoka et al., found higher PCNA labeling index for follicular AB than that of plexiform ameloblastoma. Ueno et al., (1989) and Reichart et al., (1995) also stated that the high expression of PCNA in follicular AB can explain its increased potential of recurrence, but Salehinejad et al., (2011) found the highest mean Index of positivity for PCNA in acanthomatous AB.[14] The possibility of malignant transformation in recurrent cases of AB can be determined by PCNA values. There was an increase in the expression from UA to follicular AB to plexiform AB and with maximum positivity seen in ameloblastic carcinoma, which may be correlated with biological behavior of the tumor. Li et al., and Shear et al., stated that Ki-67, PCNA are the markers for recurrence and aggressive behavior of the tumors.[15]

Cyclin D1

Nuclear and cytoplasmic expression of cyclin D1 was predominantly seen in both peripheral cells as well as cells of stellate reticulum-like tissue suggesting their role in proliferation in peripheral cells and differentiation in central cells.[16]

Telomerase

Telomerase activity is associated with the proliferative potential of AB cells. Expression of c-Myc protein similar to telomerase reverse transcriptase (TERT) suggested that c-Myc might induce the telomerase activity in ABs.[17] High expression of human telomerase RNA and hTERT was closely related to the clinical behavior of AB and regulated by p53.[18]

Growth factors

Increased epidermal growth factor receptor levels in AB when compared to radicular cyst suggested their role in tumorigenesis. Hepatocyte growth factor and c-Met show increased expression in ameloblastic carcinoma and clear cell odontogenic carcinoma implying their association with the malignant potential of epithelial odontogenic tumors. Immunohistochemical evaluation of fibroblast growth factor (FGF)-1 and -2, showed similar staining in AB and normal dental follicles, where as intense reactivity seen in the cytoplasm of cultured AB epithelial cells. Ameloblast-like cells and the cells of stellate reticulum-like tissue presented high expression of FGF-1, whereas FGF-2 was seen in basement membrane implying their distinct roles. Further studies are
needed to confirm their role.[19] Platelet-derived growth factor (PDGF) AA isoform and its receptor PDGF-α receptor levels were more in malignant ameloblastic tumors when compared to non-metastasizing AB. PDGF ligand and receptor system might participate in malignant transformation of odontogenic epithelium.[20]

**Apoptotic Markers**

Kumamoto et al., (1999 and 2004) found expression of bcl-2 family proteins, bcl-2 and bcl-x, and inhibitor of apoptosis protein (IAP) family proteins, survivin, and XIAP in neoplastic cells neighboring the basement membrane suggesting their role in suppression of apoptosis in those cells. Later in 2005 it was observed that the expression of apoptotic protease-activating factor-1, cytochrome-c, apoptosis-inducing factor, and caspase-9 in tooth germ and ABs and it was suggested that apoptotic cell death of normal and neoplastic odontogenic epithelium is by mitochondria mediated apoptotic pathway. Increased apoptotic cell death in keratinizing cells in acanthomatous AB, granular cells in granular cell AB variants and neoplastic cells of ameloblastic carcinoma was found suggesting their role in cytodifferentiation and malignant transformation of odontogenic epithelium.[3]

Luo et al., (2006) studied the expression of the Fas/FasL and concluded that it might have a role in the disposal of terminally differentiated or degenerative tumor cells in ABs. Many studies conducted on apoptosis demonstrated that anti-apoptotic proteins (bcl-2) and cell proliferation markers (Ki-67) were expressed in the peripheral basal cell layers of ABs, which might be the reason for their progression.[21]

**Tumor Suppressor Genes**

**p53**

Aberration of p14ARF-MDM2-p53 cascade strongly correlates with neoplastic transformation. Kumamoto et al., (2004) found an elevated expression of p14ARF, MDM2, p53 in benign and malignant ABs and suggested that the alteration of p14ARF-MDM2-p53 cascade leads to oncogenesis or malignant transformation of odontogenic epithelium and that it is also associated with tissue structuring and cytodifferentiation of ABs. However, mutations in the exons 5-8 in 10 samples were not observed and concluded that mutations of p53 might play a minor role in the neoplastic change of odontogenic epithelium.

Kitkumthorn et al., (2010) evaluated the association of p53 codon 72 polymorphism with AB and found that the Arg allele of p53 gene codon 72 might increase susceptibility, and p53 may be important in the pathogenesis of AB.[22]

**Rb**

Kumamoto et al., (2006) demonstrated increased expression of retinoblastoma (Rb) and phosphorylated Rb in AB than in tooth germs which might have a role in cell proliferation and differentiation of odontogenic epithelium. However, another study by Lim and Ahn et al., (2006) using DNA microarray and reverse polymerase chain reaction (more sensitive compared to IHC) revealed downregulation of Rb1 in AB in comparison with dentigerous cyst.[3]

**PTEN**

Scheper et al., (2008) found decreased expression of PTEN in AB compared to dental follicles and elucidated the role of PTEN/AKT/mTOR pathway in pathogenesis and aggressiveness of AB.[3]

**MMPs**

Ribeiro et al., (2009) evaluated the expression of MMP-1, -2, and -9 in AB and AOT and suggested that MMPs are related to growth and progression of tumors and particularly in AB, its aggressive behavior may be due to active participation of the stromal cells and their products (MMPs).[23]

Shen et al., (2010) studied immunohistochemical expression of osteonectin/secreted protein acidic and rich in cysteine (SPARC) and MMP-1, -2, and -9 in 23 cases of AB and found an association between the SPARC and MMP-9 in AB to regulate tumor invasion. MMP-2, -9 degrade type IV collagen component of the basement membrane.[24] Hence, lesions with an intense expression of MMPs show more invasive behavior.

**Osteoclastic Markers**

Abdelsayed et al., (2004) found an increased expression of parathyroid hormone-related protein (PTHrP) in AB and suggested that it has role in local bone resorption and also provided an explanation for infiltrative growth and destructive behavior of AB.[3]

Kumamoto et al., (2004) analyzed the expression of osteoclast differentiation factor/receptor activator of nuclear factor-κB ligand (RANKL) and osteoclastogenesis inhibitory factor/osteoprotegerin, PTHrP in AB and tooth germs and observed that these factors regulate bone metabolism and dynamics in tooth as well as in prognosis of AB and also found their involvement in tumor cell differentiation and tumor structuring in AB. Sandra et al., (2005) found that the RANKL, tumor necrosis factor-α secreted by AB cells could induce the osteoclastogenesis, which in turn provide space for it to expand.[3]

**Other Signaling Molecules**

The PTCH1 gene encodes a receptor for Sonic Hedgehog (SHH). SHH regulates growth and determines the shape of the tooth, but its signaling is not essential for the differentiation of ameloblasts or odontoblasts.[25]

Kumamoto et al., (2004) found lower expression of SHH in stromal cells compared with the mesenchymal cells in tooth germ. Zang et al., (2006) found strong cytoplasmic expression of...
SHH in cellular components of the neoplastic tissues, mainly in the peripheral or cuboidal cells and suggested that they regulate the proliferation of tumor epithelial cells.[25]

Gao et al., (1997) conducted an immunohistochemical study in 20 cases of AB to determine the expression of bone morphogenic protein (BMP). Although positive expression was seen in the ameloblasts of the tooth germs in the controls, none of the tumor samples showed expression for BMP. As no detectable levels of BMP found in immature tumor cells, and the identification of such proteins might be influenced by the degree of differentiation of the odontogenic epithelium, present in ABs (Gao et al., 1997). Conversely, another investigation (Kumamoto and Ooya, 2006) revealed expression of BMP-2, -4 and -7, BMPRI and BMPRII in AB samples. In all types of AB tested, BMPs and their receptors were identified. Immunostaining for these molecules was observed in neoplastic cells neighboring the basement membrane of the tumors, suggesting their role in the cytodifferentiation of neoplastic odontogenic epithelium. In addition, in keratinizing cells of acanthomatous AB there was a strong reactivity for BMP-7 which suggests its association with cell death of neoplastic odontogenic epithelium.[25]

An aberrant Wnt pathway was suggested to occur in oncogenesis. β-catenin molecule was found in the cytoplasm and membrane of most neoplastic cells of ABs, and it was thought to be associated with signal transduction and cell-cell adhesion in the neoplastic odontogenic epithelium. Kumamoto and Ooya, (2005) found nuclear β-catenin expression in some ABs, but it was not identified in tooth germ.[25]

Alaeddini et al., (2008) studied expression of calretinin in 55 odontogenic tumors including AB, AOT, CEOT, ameloblastic fibroma, and odontogenic myxoma; it was expressed only in ABs. Another study by Sireesha et al., (2010) in KCOT, dentigerous cyst and AB, revealed that only cells of stellate reticulum-like tissue of SA and UA expressed calretinin. Thus, calretinin can be considered as the specific IHC marker for neoplastic ameloblastic epithelium which is expressed only in SA and UA and not in any other odontogenic cysts/tumors. Furthermore, it can be used as a diagnostic marker to differentiate UA from other cystic lesions.[26]

### Conclusion

The development and progression of AB are affected by alterations of many kinds of genes and molecules. Further molecular studies, including genomic and proteomic-based profiling, are required to clarify the etiology and pathogenesis of AB. A better understanding of underlying molecular mechanisms will help to predict the course of AB and lead to the development of new therapeutic applications such as molecular-targeted treatment and patient-tailored therapy.

<table>
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<tr>
<th>Marker</th>
<th>Expression in AB</th>
<th>Suggestive of</th>
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<tbody>
<tr>
<td>ABCG2, CD-133, Bmi-1</td>
<td>Increased</td>
<td>Oncogenesis</td>
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<tr>
<td>Syndecan-1</td>
<td>Decreased</td>
<td>Aggressiveness, Invasiveness</td>
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<tr>
<td>α5β1 integrin</td>
<td>Increased</td>
<td>Invasiveness</td>
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<tr>
<td>Podoplanin</td>
<td>Increased</td>
<td>Invasiveness through collective cell migration</td>
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<td>MT</td>
<td>Increased</td>
<td>Invasiveness, High recurrence</td>
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<tr>
<td>Ki-67</td>
<td>Increased in peripheral cells</td>
<td>Aggressiveness</td>
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<td>PCNA</td>
<td>Unicystic ameloblastoma, Follicular ameloblastoma, Plexiform ameloblastoma, Ameloblastic carcinoma</td>
<td>Recurrence, Aggressiveness</td>
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<tr>
<td>Cyclin D1</td>
<td>Increased</td>
<td>Invasiveness</td>
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<td>p14ARF, MDM2, p53</td>
<td>Increased</td>
<td>Neoplastic transformation</td>
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<td>Rb</td>
<td>Increased</td>
<td>Cell proliferation and differentiation</td>
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<td>PTEN</td>
<td>Increased</td>
<td>Aggressiveness</td>
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<td>MMP-1, -2, -9</td>
<td>Increased</td>
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<td>PTHrP</td>
<td>Increased</td>
<td>Infiltrative growth and destructive behavior</td>
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<td>RANKL, TNF-α</td>
<td>Increased</td>
<td>Osteoclastogenesis tumor expansion</td>
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<td>PTCH1</td>
<td>Increased</td>
<td>Proliferation of odontogenic epithelium</td>
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<td>BMP-2, -4, -7</td>
<td>Increased</td>
<td>Cytodifferentiation, apoptotic cell death</td>
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References
