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Review Article

A Review on Molecular Markers in the Pathogenesis of Ameloblastoma

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ABSTRACT:

Ameloblastoma is a benign odontogenic tumor of jaw bone. The tumor arises from the residual epithelium of the tooth germ, epithelium of odontogenic cysts, stratified squamous epithelium and epithelium of the enamel organ. It represents approximately 1% of oral tumors. It is a highly invasive and destructive tumor that shows a high rate of recurrence despite adequate surgical removal. Molecular studies have offered recent updates and very significant findings regarding the pathogenesis of ameloblastoma. This review is to focus on the current updates in the molecular biology of ameloblastoma for it plays an essential role in identifying the prognostic markers of ameloblastoma behaviour and to develop more effective alternative approaches to the treatment of aggressive odontogenic tumor.

Key Words: Ameloblastoma, Molecular pathogenesis, Immunohistochemical markers, odontogenic neoplasm.

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INTRODUCTION

Ameloblastoma is the most common odontogenic neoplasm, it is a slow growing and locally invasive tumor. It occurs exclusively in the jaws with the majority of lesions occurring in the posterior region of mandible. It is a benign tumor, but highly destructive and locally invasive. According to WHO, Ameloblastoma is classified into:

- Solid/multicystic
- Extraosseous/peripheral
- Desmoplastic ameloblastoma
- Unicystic.

In the present review, the most recent updates on the molecular biology of ameloblastoma were addressed to establish new diagnostic tools for the differential diagnosis of the variants of ameloblastoma and with future perspectives of translational studies for few cases of ameloblastic carcinoma has been reported to arise from the malignant transformation of ameloblastoma by spontaneous dedifferentiation or by repeated surgical procedures and therapeutic radiations.(1)

THE MOLECULAR MARKERS INVOLVING IN THE CELL ADHESION AND MIGRATION

Cell adhesion and migration are performed by cell adhesion molecules, CAMs. The CAMs are essential for the maintenance of stratified epithelial structures because they play a vital role in the processes of cell renewal , mobility, ECM interactions. Dysregulation of cell adhesion interaction promotes tumor progression, recurrence, invasion and metastasis. The loss of cell adhesion allows neoplastic cells to migrate, degrade the ECM, invade tissues and possibly cause metastasis.

SYNDECAN-1

It is a transmembrane heparan sulfate proteoglycan. Its altered expression in ameloblastoma suggests that, this molecule could have a prognostic value in assessing the clinical outcome of ameloblastoma. Tumor epithelial cells expresses syndecan 1, which indicates unfavourable prognosis in epithelial tumour. Leocata et al found a significant correlation between the percentage of Intraosseous ameloblastomas-bearing Syndecan 1-positive stromal cells. It can be hypothesized as a critical factor for carcinogenesis and local invasiveness of ameloblastoma.(2)

The reduced expression of syndecan-1 indicates that Solid Ameloblastoma has a more aggressive biological behavior than the Unicystic Ameloblastoma.[3]

CADHERIN

Cadherins are expressed on cell membranes in the adherens junction and have the ability to communicate with different intracellular controls. E-cadherin is an important regulator of cell adhesion; therefore, the loss of E-cadherin could be associated with tumor advancement in AMs. E-cadherin expression was diminished in poorly differentiated tumours, this result indicates that the malignant neoplasms are capable of spreading by invasion and metastasis due to loss of adhesions between the tumor-forming cells.[4]

INTEGRINS

Integrins constitute an important family of transmembrane receptor proteins that bind to cell surfaces and to ECM ligands, where they participate in anchoring to the ECM proteins and in the modulation of multiple molecules that are involved in growth, adhesion, migration, proliferation, apoptosis and cell morphology[5]. The integrins can activate multiple molecules required for cell survival. Therefore, the dysregulation of these molecules might be related to tumor invasion. A strong expression of the α1, α2, α3, α5, αv, β1, β3 and β4 integrins in follicular, acanthomatous and plexiform SMAs, as well as in luminal UAs were observed by modulo et al[6]. It suggest that decreases in integrin expressions are related to tumor growth and the invasion of the neighboring structures.

CLAUDIN

Claudins are the proteins of tight junctions, they are present in epithelial and endothelial cells and are important in functions of barrier, embryogenesis and organogenesis and mainly in the epithelial-mesenchymal transition[7]. An intense immunoreactivity of claudins 1, 4 y 7, principally in stellate reticulum like cells of ameloblastoma is observed by Bello et al. It indicates the effort of these proteins to maintain cell-cell adhesion. the expression of claudins 1, 4 y 7 is generally weak or moderate in ameloblastic carcinoma and it can be correlated to aggressive behavior of these carcinomas. Consequent changes in the expression of claudin maybe responsible for the events related to tumoral development of AM

PODOPLANIN

Podoplanin, a mucin-type transmembrane glycoprotein expressed by lymphatic vascular endothelial cells, mediates a pathway leading to collective cell migration and invasion in vivo and in vitro. Immunohistochemical reactivity for podoplanin was detected in the cell membrane and cytoplasm of most odontogenic tumor epithelial cells in AMs. Expression of Podoplanin was strongly observed in peripheral columnar cells and slightly in central stellate reticulum-like cells. Expression of podoplanin in AMs is considered to be associated with neoplastic odontogenic tissues; this molecule might play a role in the collective cell migration of tumor nests in AMs.[8]

METALLOTHIONINE

Metallothionein is a protein associated with tumorigenesis, serving as a prognostic factor in different neoplasms. Metallothionein was observed in the columnar cells, in the periphery and in the central polyhedral cells of all the samples in a study done by Reibero et al.[9]

MOLECULAR MARKERS INVOLVED IN CELL PROLIFERATION

P16

P16 (cyclin-dependent kinase inhibitor) is a tumor suppressor protein, which acts as a negative regulator of proliferation of normal cells. Methylation of CDKN2A gene functions as a major mechanism of tumorigenesis in ameloblastoma and many other odontogenic tumours. In a study of Suzuki et al, the highest expression of p16 in follicular ameloblastoma followed by acanthomatous and the least in plexiform type is observed. Correlation between p16 expression and rate of recurrence was also established such as, in the plexiform ameloblastoma, the rate of recurrence was lowest [10]

Ki-67

Ki-67 is a nonhistone nuclear protein which is the most reliable marker of cellular proliferation. Ki-67-positive nuclei are located in peripheral ameloblast-like cells in the follicular and the plexiform areas of solid ameloblastoma and in the basal cells of unicystic ameloblastoma.[11]

Cyclin D1

Cyclin D1, a member of G1 cyclins, controls the cell cycle. The dysregulation and overexpression of cyclin D1 has been correlated with rapid growth and proliferative activity, histologic aggressiveness, tumor invasiveness and poor prognosis. Cyclin D1 was expressed by the peripheral columnar and central stellate reticulum-like cells of ameloblastomas[12]

Telomerase

Telomerase is a DNA polymerase that stabilizes the chromosomal structure. Telomerase reverse transcriptase (TERT) is a catalytic subunit of telomerase, whose expression is closely correlated with telomerase activity. Telomerase activity is believed to be crucial for cell immortalization and cancerization. In a study conducted by Kumamoto et al, telomerase activity was positive in all the samples of ameloblastoma, and TERT expression was detected in the nuclei of neoplastic cells but not in those of stromal cells. The cuboidal cells, sporadic central polyhedral cells and some granular cells in ameloblastomas were found reacting with anti-TERT antibody.[13] These results suggest that telomerase activity is associated with the oncogenesis or proliferative potential of odontogenic epithelium. The c-myc protein is suggested to

induce telomerase activity in ameloblastoma for its expression was found in a similar distribution pattern of TERT.[13]

PCNA

PCNA is a 36kD nuclear protein and a polymerase expressed during late G1 and S phases. It is a good marker of aggressiveness, recurrence and malignant potential of ameloblastoma. The proliferative activity was assessed by proliferating nuclear cell antigen (PCNA) labeling in some studies. The study of Maya et al showed that ameloblastic carcinoma had the maximum proliferative capacity. The maximum proliferative capacity was found in plexiform ameloblastoma, followed by the follicular and unicystic types among all the variants of ameloblastoma. Hence proliferating cell nuclear antigen is suggested to be associated with the biological behavior and proliferation of tumor cells in the variants of ameloblastoma and ameloblastic carcinoma.[14]

MOLECULAR MARKERS INVOLVING TUMOR GROWTH AND ANGIOGENESIS

FIBROBLAST GROWTH FACTOR

The **fibroblast growth factors** are cell signalling proteins involved in normal development. Fibroblast growth factor FGF3, FGF7 and FGF10 are expressed by the neural crest-derived ectomesenchymal cells that induce the proliferation of odontogenic epithelial cells during tooth development. The fibroblast growth factors act as extracellular signalling molecules that activate cell surface receptors. In a study of Myoken et al FGF-1 was found in epithelial cells of ameloblastomas, whereas FGF-2 was mainly found in the basement membranes with only moderate staining in epithelium. These data demonstrate the contribution of FGF-1 and FGF-2 in the growth and development of ameloblastomas. It suggests that FGF 1 has autocrine mechanism of tumor growth and FGF 2 is responsible in tumor growth and invasion by inducing the proteases.[15] Nakao et al also found the expression of FGF7 and FGF10 in ameloblastomas thereby suggesting a role in the growth of ameloblastomas through MAPK pathway[16]

EPIDERMAL GROWTH FACTOR RECEPTOR

EGFR when disrupted, produces receptor proteins that results in abnormal cell growth or tumorigenesis. In a study of Vered et al, anti-EGFR agents were found to reduce the size of large tumors and to treat unresectable tumors that are in close proximity to vital structures.[17]

VASCULAR ENDOTHELIAL GROWTH FACTOR

Vascular endothelial growth factor (VEGF) is a key regulators of angiogenesis. VEGF receptor pathway when activated, promote endothelial cell growth, migration and survival from pre-existing vasculature. Hence associated with tumor angiogenesis. In a study of Bhavana Gupta, a strong expression of VEGF in all cases of ameloblastoma by stellate reticulum-like cells were found at the center of the follicles and suprabasal layers of epithelium.[18]

MOLECULAR MARKERS IN APOPTOSIS

Apoptosis-related proteins play critical roles in cell proliferation, differentiation, and death. Their expressions are frequently altered in cancer cells by gene mutation, deletion, rearrangement, inactivation, or overexpression. These apoptotic factors are possibly involved in oncogenesis and cytodifferentiation of odontogenic epithelium in odontogenic tumors.

Fas & Fas-Ligand

In a study of Kumamoto et al 2001, downregulation of Fas expression and upregulation of FasL expression was observed in malignant ameloblastomas, when compared with benign ameloblastomas,which indicates escape from cell death attack by immune cells.

CASPASE

Caspase-3 expression was found in high intense in malignant ameloblastomas than in tooth germs and benign ameloblastomas. Less frequent apoptotic reactions were observed in malignant ameloblastomas compared to benign ameloblastomas, which indicates an abnormal regulation of cell turn over in odontogenic epithelial cells.[19]

BCL2

The bcl-2 proteins prevent apoptosis and its expression is seen in development, in sites of epithelial-mesenchymal interactions. In the developing tooth germ, bcl-2-protein is expressed in the epithelial component. In cases of ameloblastoma, bcl-2 protein was negative in inner cells (stellate reticulum like cells and squamoid cells) and found only in the outer layer of tumor cells, Hence the bcl-2 protein is suggested to maintain the stem-cell population in the peripheral layers of the tumor nests from which proliferating cells can be recruited[20].

SURVIVIN

Survivin, belongs to the family of antiapoptotic proteins, which inhibits apoptosis and promotes cell proliferation in malignant tumors. It can be detected in nuclei of tumor cells, which may indicate a poor prognosis in several malignant tumors. [21]

MOLECULAR MARKERS IN TUMOR SUPPRESSION

P53

Mutations and loss of heterozygosity of p53 gene have been associated with increased cellular proliferation and malignant potential. Elevated expression of p53 is observed in benign and malignant ameloblastomas in a study done by Kumamoto et al, It suggests that alteration of the p53 cascade is involved in oncogenesis and/of malignant transformation of odontogenic epithelium. p53 mutation is suggested to play a minor role in neoplastic changes of odontogenic epithelium. p53 immunoreactivity is suggested

to be associated with tissue structuring and cytodifferentiation of ameloblastomas.[22]

PTEN

An allelic loss of PTEN is found to occur in ameloblastomas in a study by Scheper *et al*. In carcinogenesis, loss of PTEN allows for uncontrolled cell proliferation, apoptosis inhibition and cell cycle deregulation. Aberrant signaling in the PI3K/AKT/mTOR pathway is considered to be the cause of aggressiveness of ameloblastomas.[23]

MOLECULAR MARKERS INVOLVED IN EXTRACELLULAR MATRIX DEGRADATION

MMP

In a study of Pinheiro et al, the result of zymography and western blotting showed expression of MMPs 1, 2 and 9 in ameloblastoma. These may digest bone matrix and release mitogenic factors, which would increase tumour proliferation. Hence MMPs are suggested to play a major role in contributing to the local invasiveness of ameloblastoma by an interdependent mechanism involving MMPs and proliferation of ameloblastoma cells.[24]

HEPARANASE

Heparanase plays an important role in ECM remodeling by cleaving heparin sulphate. Strong expression of heparanase was detected in all ameloblastomas in a study by Nagasutka et al. An intense staining with heparanase antibody is observed in cystic areas and squamous metaplastic areas, proposing the possible contribution of heparanase in the local invasiveness and secondary morphologic changes of ameloblastoma.[25]

MOLECULAR MARKERS INVOLVED IN BONE REMODELLING

CYTOKINES

Interleukin 1α, interleukin 1 β, interleukin 6 and TNF α were the cytokines that possess osteolytic activity, that are suggested to be implicated in the growth and expansion of the ameloblastoma.

PARATHYROID HORMONE RELATED PROTEIN (PTHrP)

Both normal and neoplastic odontogenic epithelial cells show immunohistochemical reactivity of PTHrP in a study of Kumamoto. In ameloblastomas, peripheral columnar or cuboidal cells were recognized with stronger reactivity than in the central polyhedral cells, and keratinizing cells showed increased PTHrP reactivity. Which suggests that PTHrP possibly regulate bone metabolism and dynamics in tooth development as well as in progression of ameloblastomas.[26]

OSTEOPONTIN

In a study of Wang et al to examine the expression of osteopontin and its receptors, integrin alpha and CD44v6 in ameloblastomas, a positive staining rate of 87% for OPN, 45% for integrin alpha(v), and 90% for CD44v6 were found. A high expression of OPN and CD44v6 were observed in both ameloblast-like and stellate reticulum like cells, which implies that binding of OPN to osteoclast cell membrane receptor integrin alpha(v) can activate the osteoclast and increase its osteolytic activity and binding of OPN to ameloblastoma tumor cell membrane receptor CD44v6 can enhance tumor cell migration, invasion and spread. The local aggressiveness and high osteolytic ability of ameloblastomas in the jaw bones is suggested to be associated with high expression of OPN and CD44v6 in ameloblastoma cells and the high expression of integrin alpha(v) in osteoclasts.[27]

MOLECULAR MARKERS INVOLVED IN THE FUNCTIONING OF TUMOR STROMAL CELLS

MYOFIBROBLASTS

Myofibroblasts can influence tumor infiltration and progression by the expression of proteinases. In a study of Fregnani et al, to examine the presence of myofibroblasts and expression of matrix metalloproteinase-2 (MMP-2) in intra-osseous solid multicystic ameloblastomas, myofibroblasts were found in close contact with stromal neoplastic cell islands, which is suggested to have a significant correlation between presence of myofibroblasts and MMP-2 expression. Which in turn significantly correlated with rupture of the osseous cortical, it is considered as an important prognostic marker of aggressiveness of ameloblastoma and suggested to be associated with a more aggressive infiltrative behavior.[28]

CONCLUSION

Ameloblastoma is usually with poor symptoms and low prevalence, which often leads to its late diagnosis. The treatment may include resection and reconstruction. Histological classification of the ameloblastoma is essential for its morphological characterization and a better treatment. A better understanding of the pathogenesis of ameloblastoma aids in constituting proper treatment of choice at an early stage thereby preventing morbidity associated with extensive therapy. The molecules involved in the pathogenesis can serve as markers in later follow-up to check for recurrence. Genetic and molecular research of oral cancers has led to an increasing amount of knowledge and understanding of their physiopathological pathways. Markers known to be induced in response to growth factors, tumour promotors, cytokines, bacterial endotoxins, oncogenes, may indeed shed new light on the biological mechanisms involved in the development of the tumors.[29]

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