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Insight into the molecular pathogenesis of odontogenic tumours

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***Abstract*---**Odontogenic tumors, derived from epithelial, ectomesenchymal, and/or mesenchymal elements of the tooth-forming apparatus, constitute a heterogeneous group of lesions, including hamartomas, benign and malignant neoplasms with metastatic capabilities. This review provides a comprehensive overview of the molecular events in the pathogenesis of odontogenic tumors. The reasonable and better understanding of the molecular components may prompt new ideas for their detection and administrating a better prognosis of odontogenic tumors.

***Keywords*---**ameloblastoma, molecular profiling, odontogenic cysts, odontogenic tumors.

Introduction

Molecular profiling is the classification of tissues or other specimens for diagnostic, prognostic and predictive purposes based on multiple gene expression, it is a technology that holds major promise for optimizing the management of patients. Developmental biology is a field of rapid progress and in recent years advanced gene technology have led to an explosion in the information and understanding of the molecular mechanisms regulating embryonic development. The presence of growth factors and their receptors in embryos and their differential expression at various developmental stages suggest that these growth-promoting polypeptides are involved in the regulation morphogenetic and differentiation events.[1]

There are various molecular markers for studying developmentally related genes like BMP, FGF, WNT, Nestin; for bone destruction are MMP-9, MMP-2, PTHrP, various markers for demonstrating cell adhesion and cell migration are Syndecan, Integrins, markers used involved in bone remodelling are like IL-1, IL-6.[5] Other markers found to be playing an important role in immunoprofiling are -NR0B1, DAX1, DNA- and RNA-binding homeodomain protein, Pitx2, lhx6, Msx2, Ki67, p53, CD34, Bax, bcl-2, Ki-67, Su5402, fibroblast growth factor, Platelet derived growth factors (PDGF), Transforming growth factor, Insulin-like growth factors (IGFs), Bone morphogenic protein, Tenascin, Amelogenin, Ameloblastin, β-catenin. Odontogenesis is a highly co-ordinated and complex process which relies upon cell- cell interactions that results in the initiation and generation of tooth. The gross histological processes are well documented but the mechanisms that are involved at the molecular level are only beginning to be elucidated, this is largely due to the revolution in molecular biological techniques that has occurred over the last decade and their continued application in developmental biology.[2]

Odontogenesis is under the strict genetic control of regulators that determine the positions and shapes of the teeth, such as Msx-1, Msx-2, Dlx-2, Barx-1, and Pax-9, or that are involved in the morphogenesis and cytodifferentiation of the teeth, such as Sonic hedgehog (SHH), bone morphogenetic protein (BMP), Wnt, HGF, and FGF(Fig. 3). Aberrant functions of these specific genes cause various dental anomalies. SHH signals control cell–cell interactions and cell proliferation in tissue patterning of various organs, including the teeth. Patched (PTC), whose product is one of the SHH signal transduction molecules, is responsible for basal cell nevus syndrome (BCNS), characterized by basal cell carcinomas and odontogenic keratocysts and mutations of PTC have been identified in both BCNS-associated and sporadic odontogenic keratocysts. Expression of SHH signaling molecules, SHH, PTC, smoothened (SMO), and GLI1, has been detected in several odontogenic tumors. These findings suggest that SHH signaling pathway plays a role in epithelial–mesenchymal interactions and cell proliferation during the growth of odontogenic tumors as well as during tooth development. Wnt signal transduction controls diverse developmental processes by regulating cell proliferation, morphology, motility, and fate in various organs, including the teeth. Mice targeted for the Wnt signaling molecule LEF-1 show inhibition of tooth morphogenesis. Wnt signaling is regulated by the levels of β-catenin, and activation of this pathway results in cytoplasmic accumulation and nuclear translocation of β-catenin. Aberrations of Wnt signaling pathway are involved in

oncogenesis and cytodifferentiation of odontogenic epithelium via dysregulation of cell proliferation. Other regulators of tooth development, such as HGF and FGF, have been found in odontogenic tumors.[3]

Amelogenins are enamel matrix proteins secreted by the ameloblasts and constitute over 90 % of the developing enamel extracellular matrix during odontogenesis. Amelogenins are mostly 20 to 25 kD proteins, primarily hydrophobic and contain proline, glutamine, leucine and histidine amino acid residues. This protein molecule consists of three distinct regions: a hydrophobic core sequence of 100-130 residues enriched in prolines and glutamines, a N-terminal region (45 residues) and an acidic hydrophilic C-(carboxy-) terminal region (13-15 residues). The N-terminal domain is rich in tyrosine and therefore it is referred to as the TRAP segment (tyrosine-rich amelogenin peptide). The N- and C- terminal regions have been proposed to be highly conserved across mammalian species, as opposed to the variable hydrophobic central region of the molecules.

Interspecies conformity of amelogenin

The amino-acid sequences of amelogenin found in dog tissue have shown 88% homology with the human amelogenin derived from the X chromosome and 85,1% homology to the human Y chromosome derived amelogenin. It has also been suggested that the splicing pattern in the dog is more closely related to that of man or pig than that of mice.[4]

Functions of amelogenin

A variety of functions have been proposed for amelogenin, but its primary role is to provide the necessary milieu for the development of the mineralized enamel. Amelogenin has been proposed to act as a proton buffer that absorbs the large amounts of hydrogen ions generated during hydroxyapatite formation and to inhibit crystal growth, without changing the crystal morphology. As enamel crystals first grow in their length during the secretory phase of amelogenesis and then in thickness during the maturation stage, it was proposed that full length amelogenin molecules bind to developing enamel crystals to prevent premature crystal fusion during early stages of amelogenesis. The stepwise processing of amelogenins was shown to affect its affinity to bind to apatite crystals, but the persistence of large quantities of amelogenin cleavage products as well as the conserved C-terminus have been suggestive of the intact protein as well as cleavage products functioning in enamel formation.[5] However, it was recently suggested that the nanosphere supramolecular structure of amelogenin could promote the heterogeneous nucleation of calcium phosphate by acting as a nucleation template and concentrating charge at the nanosphere surface. With regard to the above mentioned functions, amelogenin has been implicated in the pathogenesis of Amelogenesis imperfecta as well in dental flourosis.[6]

The role of amelogenin in epithelial-mesenchymal signalling during tooth development has been proposed by various authors. Through this signalling interaction amelogenin expression in pre-odontoblasts has been implicated to inhibit the secretion of ameloblast matrix and the maturation thereof until after

the initial layer of dentin has been mineralized. It has also been suggested that vitamin D regulates amelogenin expression in odontoblasts and is therefore part of this interactive process.[7] Amelogenin expression has been observed on the dentin surface at the apical end of developing human tooth roots. When porcine enamel matrix was placed in experimental cavities created in extracted monkey incisors, a tissue identical to acellular extrinsic fibre cementum was formed and therefore it was proposed that amelogenin could have an inductive effect on some cells in the dental follicle. These findings led to the development of a porcine enamel matrix derivative called EMDOGAIN, essentially a mixture of amelogenins.[8] EMDOGAIN was developed as a treatment for periodontal repair and it has shown to result in increased levels of bone formation as well as increased periodontal attachment. In a study on amelogenin-null mice it was also indicated that amelogenins may prevent abnormal resorption of cementum. Low-molecular-weight amelogenins have been suggested to have a positive effect on cell-cycle progression of dental pulp cells. This effect has been proposed to have potential use to promote proliferation of dental pulp tissue in the presence of injury, resulting in reparative dentin formation.[9]

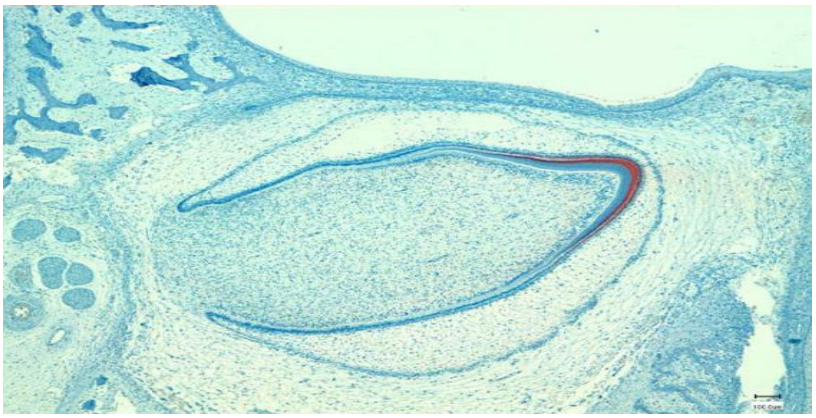

Diagram showing diffuse amelogenin staining observed in the secretory ameloblasts of a bell stage tooth germ (group 2 foetus)

Ameloblastin

Ameloblastin (AMBN) acts as a cell adhesion molecule essential for amelogenesis. This protein plays an important role in maintaining the ameloblasts in secretory stage of differentiation by binding to them and inhibiting their proliferation. Ameloblastin, enamelin and sheathlin proteins were not expressed in ameloblastoma, suggesting that the tumour cells do not attain functional maturation as secretory phase ameloblasts. Perdigao et al (2004) demonstrated that AMBN gene mutations are associated with the development of ameloblastoma, AOT, squamous odontogenic tumor (SOT) and CEOT. Mutations in the AMBN gene are responsible for the tumorigenesis of epithelial odontogenic tumors without odontogenic ectomesenchyme.[10]

Mineralized matrices of dentin, cementum, and bone contain type I collagen and numerous non-collagenous proteins, such as bone sialoprotein (BSP),

osteonectin, osteocalcin, osteopontin, and dentin matrix protein 1. BSP, osteonectin, osteocalcin, and osteopontin are found in many types of odontogenic tumors, suggesting that these proteins play a role in pathologic mineralization and/or tumor formation.

Mammalian Neuronal Differentiation Factors

p75 Neurotrophin Receptor (p75NTR)

A complex interplay between diffusible molecules and their cell surface receptors are responsible for the development and maintenance of the nervous system.[39] The neurotrophins are the best characterized mammalian neuronal differentiation factors [39] and are comprised of four proteins: nerve growth factor (NGF), the prototype of the neurotrophins, brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4 (NT-4).[200] The diverse biological functions of the neurotrophins are mediated by signal transduction systems.

p75NTR expression in odontogenic tissue

In 1990 it was reported that in bud stage rat molars, cells of the dental lamina, epithelial cells at the tip of the growing tooth bud, as well as associated mesenchymal cells stained positive for p75NTR. In contrast, it was later described that p75NTR does not stain the dental epithelium of bud stage rat molars, but faint staining was observed in the condensed mesenchyme.[11] In the cap stage intense staining has been observed in the IEE of rat molars and less intense staining of the OEE and stellate reticulum cells. Later on, cap stage rat molars also revealed p75NTR staining in cells of the IEE with added staining of the stratum intermedium, dental papilla and dental follicle. In the bell stage staining has been observed in proliferating cells of the IEE, some cells of the stratum intermedium and in preodontoblasts/ polarizing odontoblasts. Absent staining has been observed in pre-ameloblasts before terminal differentiation into ameloblasts, outer enamel epithelium, cervical loop cells, Hertwig's epithelial root sheath (HERS) cells, cementoblasts and functional odontoblasts. The expression of p75NTR has been described to be lost progressively as the cells of the IEE became post-mitotic, polarized and differentiated into ameloblasts.

More recent studies revealed immunoreactivity against p75NTR to be restricted to the IEE, the dental papilla and the dental follicle of rat incisors and therefore p75NTR has been used as a marker for IEE cells. In adult rat tissue the ectomesenchymal cells of the pulp revealed strong p75NTR immunoreactivity that became concentrated in the sub-odontoblastic regions of the crown. These findings were supported by others as the cells underlying the functional odontoblasts (sub-odontoblastic layer cells) stained positively for p75NTR and the nerve fibers of the sub-odontic plexus also revealed positive staining for p75NTR. One study has also reported the presence of p75NTR in the basal epithelial cells of the junctional epithelium and in adjacent sulcular epithelium as it has been proposed to be a marker for intraepithelial nerve fibres and their associated epithelial cells in adult rats.

p75NTR in epithelial cells

In bud stage dental organs no staining of the epithelium was observed. Focal positivity for p75NTR was observed in cap stage IEE as well as in some bell stage tooth organs as the IEE in the region of the cervical loop stained positive for p75NTR. No staining was observed in OEE, stellate reticulum, stratum intermedium, differentiated ameloblasts, dental lamina, Serres rests or overlying alveolar epithelium.[12]

Calretinin is a proliferative marker but not a marker for ameloblasts and inner enamel epithelium in teeth, it can be regarded as a marker for odontogenic epithelium as the overlying alveolar epithelium, dental lamina and Serres rests revealed positive staining in the majority of cases. It should however be kept in mind that various factors alter the expression pattern of calretinin and one could therefore question the reliability of results found under various conditions.

Calretinin expression in odontogenic tissue

A study on calretinin expression in the rat dental organ revealed focal expression of calretinin in the dental lamina, outer enamel epithelium, stellate reticulum and stratum intermedium. Diffuse and intense expression was however encountered in the IEE and presecretory ameloblasts with less intense staining observed in the cytoplasm of secretory ameloblasts. The authors even suggested calretinin to play some role in enamel formation as calretinin expression remained negative in rat molar teeth over the cusp tips, where enamel is never formed. At the late cap and bell stages staining in the papilla was interpreted as representing neural elements with no staining of odontoblasts and other ectomesenchymal cells. In accordance with a previous postulation regarding calbindin, the authors postulated that calretinin may act as a “calcium ferry” as its presence was only detected in cells that reside directly in the path of calcium transition towards the enamel matrix, resulting in the dynamic temporal and spatial distribution thereof.[13]

Tachykinins are neurotransmitter and neuromodulatory peptides in the mammalian central and peripheral nervous systems, and consist of five distinct peptides. Four of these-substance p, neurokinin a, and its two extended derivatives neuropeptide y and neuey arop. The ptide y- are proteolytically processed from a single gene product precursor, preprotachykinin or ppt-a. They are thought to be removed from the extracellular space by the action of a cell-surface-bound neutral endopeptidase, termed nep. Both ppt-a and nep are strongly co-expressed condensed dental mesenchyme from the bud through late-cap stages of molar tooth development, with expression diminishing by the bell stage. These expression patterns can be maintained in molar tooth rudiments explanted at e.10 and cultured for nine days, in some cases reaching the late-cap stage.[14]

Expression pattern of various markers observed in developing odontogenic tissue					
	Calretinin	Keratin 14	Keratin 19	Amelogenin	P75NTR
IEE	-	D++	F+	-	F+
Presecretory	-	D++	D++	D++	-

Ameloblasts					
Dental Lamina	D++	D++	D++	-	-
Serres Rests	D++	D++	D++	-	-
Alveolar Epithelium	D++	D++	F+	-	-
Oral Epithelium	-	D++	F+		
Pre-Odontoblasts	-	-	-	-	F+
Odontoblasts	-	-	-	S+*	-

Unicystic ameloblastoma

Vimentin expression was confined to the subepithelial region of the lining odontogenic epithelium. This may be due to the odontogenic inductive effect exerted on the connective tissue by the proliferating odontogenic epithelium.

KCOT- Various CKs expression were seen by various authors (CK10/11, CK13/16, CK 19, CK 5,8) but expression of CK 16 has been found to be intense, a cytokeratin that has been associated with proliferative activity. Expression of CEA & EMA is weak and patchy of surface layer.
Cyclin D1 was detected in the nuclei of basal and parabasal cells in the lining. Epithelium of OKCs suggest that the cyclin D1 expression reflects cellular proliferative potential in lining epithelium of OKCs.[15]
Ki-67 expression was seen in the nuclei of basal and suprabasal cell layers of lining epithelium suggestive of its greater proliferative activity.
Immunohistochemical reactivity for podoplanin was detected in the cell membrane and cytoplasm of most of the basal and suprabasal layer, areas of budding basal cell proliferation, epithelial nests and peripheral cells of daughter cysts in the stromal connective tissue in KCOTs, it suggests the possible contribution of podoplanin in the local invasiveness and the neoplastic nature of the KCOT.

IL-1α has been strongly expressed in the lining epithelial cells of KCOT and the intracystic fluid levels of IL-1α has been found to be significantly higher than the levels of other inflammatory cytokines IL-6 and TNF-α. A strong relationship has been present between the intracystic fluid pressure and IL-1α expression in epithelial cells and the released IL-1α play a crucial role in the growth of KCOT by stimulating proteolytic enzyme production and osteoclastogenesis.

Adenomatoid odontogenic tumour- They have unique keratin expressions, immunohistochemical detection of CKs presented intense positivity for CK14 in all epithelial elements, they had an interesting CK14 immunohistochemical profile. Variation in the expression of CK14 indicated variable differentiations of the tumoral cells, it was suggested that this particular thin cystic epithelium probably originates from undifferentiated odontogenic epithelium or stratum intermedium cells.[16]

Calcifying epithelial odontogenic tumour- tumours cells had shown expression of wide spectrum of CKs like CK8, 13 and 19 indicating odontogenic epithelial origin of CEOT.
Odontogenic fibroma- positive immunohistochemical expression of CK 19, CK14 and CK5 are present indicating its origin from odontogenic epithelium.
Odontogenic myxoma- Immunohistochemical (IHC) studies proved that most of tumors of mesenchymal origin like odontogenic myxoma (OM) do not express CK 14 and 19. Thus, CK 14 and 19 can be used as markers for tumors of odontogenic epithelial origin.[17]
Ameloblastic fibrodontoma- high number of LOH seen in comparison to AF showing differences in tumorogenesis and may present a distinct genetic profile despite showing histological similarities.
PCNA expression was higher in the mesenchymal component of ameloblastic fibrodontoms than in epithelial component suggesting mesenchymal component has more proliferative potential.[18]
Ameloblastic fibroma- Notch expression was found in both epithelial and mesenchymal components, staining pattern in epithelial tumour nests is similar to that of enamel organ.
Odontogenic myxoma- Notch expression was not present in the tumour tissue, this finding suggests that the development stage of odontogenic myxoma is less advanced than ameloblastic fibroma.
The cells of the odontogenic epithelium in show positivity for p53.

Lastly, an additional gene implicated in human tooth development is that encoded by the genetic locus responsible for x-linked anhidrotic (hypohidrotic) ectodermal dysplasia (eda) Patients with mutations in the eda gene, which encodes a novel transmembrane protein, express hypo- and anodontia along with sparse hair and lack of sweat glands, suggesting a general role for the eda gene product during development in epithelial-mesenchymal interactions.[19] Given the large number of signaling molecules which appear to have important morphologic roles in tooth morphogenesis, one obvious conclusion is that the developmental program is highly complex.

The current research on dental stem cells in odontogenesis is expanding at an unprecedented rate. Tissue engineering is an emerging field of science aimed at developing techniques for the fabrication of new tissues to replace damaged or lost tissues / organs of living organisms; and is based on the principles of cell biology and biomaterial sciences. The three main components believed to be essential for tissue regeneration are growth factors, scaffold and stem cells.[20] Henceforth, the emerging field of 'personalized medicine' has become quite popular, which refers to new medical technologies derived from a patient's own stem cells and the use of genomic diagnostics.

Conclusion

The development and progression of odontogenic tumours depend upon multiple factors. A better understanding of the pathogenesis of the odontogenic tumours may help in predicting the prognosis of patients.

Future scope

Future studies in this regard may devise better targeted therapeutic treatments, thus, reduce the morbidity and mortality rates of patients.

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