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Review Article

Recent Concepts in Odontogenesis

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Abstract

Stem cell therapy is a potentially revolutionary new way to repair damaged and diseased tissues with healthy new cells provided by stem cell transplant. It offers an opportunity to treat many degenerative diseases arising as a consequence of death, destruction or malfunction of specific cells, in the context ofOdontogenesisthe is body'sa highly co-ordinated & complex process which relies upon cell-to-cell interactions that result in the initiation & generation of the tooth. Stem-cell-based tissue engineering has been performed in the animal model for many types of tissue regeneration, such as articular cartilage, bone, tendon, muscle & adipose tissues. The application of stem-cell-based tissue-engineering approaches to create organs and tissues for transplantation.

Keywords: Stem cells, Genetics, Tissue engineering, Odontogenesis, Apoptosis, Growth factors, Signalling centres

Introduction

In 1999, Beardsley wrote "A flurry of startling discoveries in stem cell biology in past months has shattered preconceptions about how cell specialization is controlled in the body and has boosted the field to the top of scientific, political and commercial agendas. The excitement has raised hopes that the long sought goal of being able to regenerate human tissues may be closer than had been thought"[(1)]. Five years later, stem cell therapy research is still in its early stages, however, promising results with experimental transplants have been achieved, and first clinical applications are expected in 5-10years. What is stem cell therapy? It is a potentially revolutionary new way to repair damaged and diseased tissues with healthy new cells provided by stem cell transplant. It offers an opportunity to treat many degenerative diseases arising as a consequence of death, destruction or malfunction of specific cells, in the contex inability to repair or restore them. Currently, the only interventions available to treat wasting disorders include transplant surgery, or therapeutics to delay the onset and relieve the symptoms of the underlying malady[(2)].

Genetics of Tooth Development

Early events in tooth development depend upon epithelial–mesenchymal interactions that involve the secretion of diffusible signaling molecules that induce the expression of transcription factors in the responding tissue. Epithelial–mesenchymal interactions are a common means of organ differentiation and similar gene networks regulate the development of teeth and other organs. Functional failures involving key participants in these signaling systems arrest tooth development, and hypodontia and familial tooth agenesis are a feature of many syndromes. Because of their expression during the development of other tissues, it is perhaps surprising that the loss of potent transcription factors such as Pax9 and Msx1 would be manifested as non-syndromic familial tooth agenesis, with only some teeth being affected and the pattern of tooth agenesis varying among affected individuals in the kindred. Regardless of the broadness of the clinical manifestations, the key concept is that early tooth formation depends upon a network of signaling molecules and transcription factors that drive cell proliferation and differentiation. A series of epithelial–mesenchymal interactions ultimately leads to the terminal differentiation of odontoblasts and ameloblasts and mineralization of the tooth.

After the basic shape of the tooth crown is established, odontoblasts initiate their secretion of a collagen-based matrix beneath the basal lamina of pre-ameloblasts, which start, slowly at first, to secrete enamel matrix proteins. Much has been published about the roles of these extracellular matrix molecules, particularly amelogenin, in the final differentiation of odontoblasts and ameloblasts, but the genetic studies prove they serve no critical role in cell differentiation. Odontoblasts terminally differentiate normally and produce normal dentin in patients with AI caused by defined mutations in the amelogenin(AMELX), enamelin(ENAM), enamelysin(MMP20) and kallikrein 4 (KLK4) genes. Similarly, ameloblasts terminally differentiate and produce normal enamel in patients with osteogenesis imperfecta, dentinogenesis imperfecta, and dentin dysplasia caused by defined mutations in COL1A1 (17q21.31–q22), COL1A2 (7q22.1), and DSPP. The genetic evidence suggests that changes in odontoblast and ameloblast activities secondary to their exposure to enamel or dentin extracellular matrix molecules must be involved in late events, such as in synchronizing and fine-tuning secretions to meet the requirements of biomineralization.

Root formation starts after the crown has been defined. The inner and outer dental epithelium fuse to form Hertwig's epithelialgrowsdownrootto form the sheath root, presumably by inducing the differentiation of odontoblasts. The role of the Dlx3 transcription factor in these processes is unknown, but the taurodontism observed in its absence suggests there is one. Again, the consistent lack of clinical and radiographic evidence of root defects in AI kindreds with AMELX, ENAM, MMP20, and KLK4 mutations argues strongly against enamel matrix proteins playing a vital role in the differentiation of cementoblasts or root odontoblasts. Cementum forms after the root sheath disintegrates and its mineralization is necessary for attachment of the periodontal ligament. Alkaline phosphatase is an enzyme that is covalently bound to the outer membrane of cells expressing it. Cementum deposition in the primary teeth is sensitive to inhibition by pyrophosphate, possibly due to higher concentrations of enzymes that produce it[3,4].

The Genetic Control Of Early Odontogenesis

Odontogenesis is a highly co-ordinated & complex process which relies upon cell-to-cell interactions that result in the initiation & generation of the tooth. Whilst the gross histological processes are well documented[5], the mechanisms that are involved at a molecular level are only now beginning to be elucidated[6].

I t is the mouse that has become the principle organism used to study mammalian development because of its suitability for both genetic & embryo logical manipulation[7]. Engineered genes can be permanently inserted into the germline to produce

transgenic mice which allows a direct method of studying the function of a gene during development.

Fundamental to the study of tooth development is the manipulation of tooth germ explants from wild type & mutant mice. These explants can be cultured in vitro as far as odontoblast & ameloblast differentiation with the early stages of both dentin & enamel secretion beginning to occur. The technique of in situ hybridization, using labelled mRNA probes allows the domains of expression of specific genes to be visualized in these developing tooth germs. Cultured tooth germs can now be transferred into the kidney capsules of adult male mice, allowing tooth development to proceed to full crown formation & localized alveolar bone differentiation. Dental epithelium & mesenchyme can be separated, & recombined with tissues of different origins, developmental stages, & altered genetic constitutions. Agarose or heparin acrylic beads expressing protein signalling molecules, & growth factors can be implanted into cultured epithelium & mesenchyme. The resulting effects of these techniques on downstream gene expression & odontogenic phenotype can then be evaluated[8].

Stem Cell Biology

Stem cells are specialized cells with the unique potential of self-renewal & cell specification following stimulation with appropriate biochemical/biophysical cues. Initially uncommitted, following specific signals, these cells have the capacity to differentiate into lineage-committed cells. Three broad categories exist to classify these cells:

1. Following fertilization of an egg, the zygote replicates numerous times, ultimately giving rise to the 216 different cell types, which comprise the human body. The first three divisions of the zygote, however, yield 8 cells, each of which is capable of developing into a human being. These cells are referred to as totipotent stem cells.

As the cells continue to divide from the 8 cell stage, the number of stem cells yielded increases, however, their capacity to transdifferentiate into different cell types becomes more limited. Five days post-fertilization, the blastocyst forms. The outer cell layer generates the placenta & the inner cell mass of approximately 50 cells creates the embryo. These latter cells are designated pleuripotent embryonic stem, or ES cells. Although each is capable of generating most embryonic cell types, they are not capable, individually, of creating a human being.

3. As the embryo continues to develop, the cells become more & more specialized & commit to specific cell types. In order for this differentiation to occur, as the embryo develops, genes necessary for earlier stages of development "switch off", until only those required for a specific tissue function/phenotype remain active. However, a small number of only partially differentiated stem cells persist in some adult tissues, & are referred to as multipotent stem cells. These are capable of forming a limited number of specialized cell types, & generally function locally to replace fully differentiated cells lost through depletion or damage.

Sequencing of the human genome has contributed to rapid advances in cell biology, improving our understanding of the physical cues & biological signals that control cell phenotype, & also enable us to manipulate stem cells in culture & utilize the healing process in patient tissues. Indeed, a key goal of stem cell research, whether in adult or embryonic stem cells, is to understand how differentiation is controlled & to learn how to direct its progression both in & ex vivo. ES cells are capable of generating more cell types than the adult variants, they grow more quickly & are easier to differentiate, they are more abundant & easier to isolate & ultimately ES research may speed the development of adult stem cell therapies. Despite current hurdles, potential clinical applications for stem cell technologies include: the production of cardiomyocytes to replace damaged heart tissue, the manufacture of insulin-producing pancreatic cells for patients with diabetes & the generation of neurones for the treatment of patients with Parkinson’s disease. Indeed, the use of bone an

example of stem cell therapy already in practice.

Furthermore, recent studies have highlighted the potential of ES cells to become cancerous with age, adding additional complexity to these cells. Post-natal stem cells may not develop this problem, & can be used for autologous transplants. Progress is being made in the potential use of these adult cells, particularly for the treatment of muscle & connective tissue diseases & damage[2].

Tissue Engineering

The embryonic oral epithelium is a simple, two-cell-thick ectoderm, & it is conceivable that this could be replaced with epithelial cells from another source. If this epithelium can be engineered to express the appropriate signals to initiate odontogenesis, a complete tooth primordium could be produced entirely from cultured cells. The identification of stem cells in dental pulp & from exfoliated deciduous teeth also raises the possibility that a patient's own tooth cells could be used to generate new tooth primordia[9,10]. The ability to tissue-engineer an organ rudiment such as a tooth primordium constitutes a major component of a regenerative medicine procedure[11]. However, such organ primordia must be capable of developing into the complete organ in situ, in the appropriate site in the adult body. The renal capsule & anterior chamber of the eye are two adult sites that have been routinely used to support ectopic organ & tissue development, because they are immunocompromised & can provide an adequate blood supply to the transplanted tissue. Transfer of embryonic tooth primordia into the adult jaw resulted in complete tooth development. The adult mouth is thus an appropriate environment to support development of an embryonic tooth primordium [12].

Tissue engineering of teeth & associated tissues requires that the scaffolding of the extracellular matrix be faithfully duplicated. Both developing & adult teeth are surrounded by the PDL & anchored to alveolar bone by cementum. These connective tissues have in common an extracellular matrix that includes collagen, proteoglycans & an assortment of noncollagenous glycoproteins. Studies in one of their laboratories, have shown the critical role of the extracellular matrix substrata in bone induction by BMP's, particularly in craniofacial morphogenesis & regeneration, which is contact-mediated & occurs over a short range[16,17]. The rate of release of growth factor from the scaffold can profoundly affect the results of tissue engineering strategies[18]. Polylactic-glycolic acid & polyethylene glycol copolymers, which are commonly used in implantable devices, are effective in releasing growth factors & thus are useful in BMP delivery[19]. The mineral phase in alveolar bone, cementum & teeth is carbonate-rich hydroxyapatite. The affinity of proteins for hydroxyapatite makes it a natural protein delivery system[20].

Stem-cell-based tissue engineering has been performed in the animal model for many types of tissue regeneration, such as articular cartilage, bone, tendon, muscle & adipose tissues. Studies, including direct cell-pellet implantation[12,21], & tissue engineering in combination with biocompatible scaffolds[22,23], have enabled them to contemplate new & promising strategy for hardtissue repair, particularly for tooth reconstruction. DPSCs (dental pulp stem cells) & BMSSCs(bone marrow stromal stem cells), which can differentiate into multiple mesenchymal cell lineages, are putative candidate cells for tooth & bone-tissue engineering respectively (9,24,25,26,27,28,29).

The application of stem-cell-based tissue-engineering approaches to create organs and tissues for transplantation requires an understanding and manipulation of the developmental processes that direct organ/tissue formation in the embryo, a source of cells with multipotential that can be easily cultured, and an ability of an organ rudiment to form the complete organ in the adult environment[30]. In common with most organs, teeth develop from interactions between epithelial cells(oral epithelium) and mesenchyme cells (cranial neural-crest-derived ectomesenchyme)[31].

Bone Morphogenetic Proteins (BMP)

The addition of BMP4 to intact second-arch explants resulted in the development of organized structures containing layers of cells that express marker genes of tooth-specific cells, odontoblasts & ameloblasts. Thus, although overt tooth development did not occur, BMP4 has the ability to stimulate organized differentiation of epithelial & mesenchymal-derived dental-specific cells from non-dental primordium[32].

The Fibroblast Growth Factors

The fibroblast growth factors(FGF's) are a large family of heparin binding proteins that are known to mediate the growth and differentiation of cells from a wide variety of developmental origins[33]. Comprehensive in situ hybridization studies have shown that Fgf-4, Fgf-8, and Fgf-9 are all expressed in epithelial cells of the developing tooth germ at times when epithelial-mesenchymal interactions are known to be regulating odontogenic morphogenesis[34]. Expression of Fgf-8 and Fgf-9 is seen initially in the primitive oral epithelium, at the time of odontogenic initiation this expression becomes restricted to the area of the presumptive dental epithelium and persists until the beginning of the bud stage. Both Fgf-4 and Fgf-8 expression then becomes up-regulated later on at the cap stage of development in the enamel knot, and later again in the secondary enamel knots that form at the sites of future cuspal morphogenesis. These findings suggest roles for Fgf-8 and Fgf-9 in mediating the initiation of tooth development, and for Fgf-4 and Fgf-9 in determining coronal morphology.

Sonic Hedgehog

Sonic hedgehog(Shh) is the vertebrate homologue of the Drosophila hedgehog(hh) segment polarity gene which is involved in defining the identity of parasegment borders in the developing fly embryo. In the vertebrate, Shh encodes a signal peptide that mediates both long and short range patterning in a number of well known developmental signalling centres. During odontogenesis, Shh is expressed strongly in the epithelial thickenings of the future tooth forming regions[35], and also at a later stage of development in the enamel knot[36]. This expression pattern has led to speculation that Shh is involved in epithelial signalling both during the initiation of tooth development and at a later stage during cuspal morphogenesis. Targeted disruption of Shh in knockout mice results in severe defects of the central nervous system, the axial skeleton, and the limbs. These mice are cyclopic and have holoprosencephaly, a failure of midline cleavage of the developing forebrain and they die before birth. As Sonic hedgehog is required for embryonic viability prior to the onset of odontogenesis in these mutant mice they reveal little regarding its role in tooth development. However, the role of some downstream target genes in the Shh signalling pathway has recently been investigated in relation to odontogenesis. The Gli zinc finger transcription factors(Gli-1, - 2, - 3) are known to act downstream of Shh. Analysis of mice with the expression of Gli-2 knocked out revealed abnormal development of the maxillary incisors, possibly due to a mild form of holoprosencephaly. However, Gli-3 mutants showed normal development of their dentitions. Double homozygous knockout mice for Gli-2/Gli-3 had no teeth that developed normally, whereas in double homozygous/heterozygous mutants(Gli-2 −/,Gli−-3 +/)maxillary− incisor development arrested, and all molars and the mandibular incisors were microdont. These results have confirmed an essential role for Shh signalling in odontogenesis and suggest a degree of functional redundancy between some members of the downstream target genes[37].

Apoptosis In Odontogenesis

Apoptosis is an active process of cellular self-destruction and the importance of this process is increasingly recognized both in physiologic regulation and in pathologic conditions. Apoptosis is usually manifested as the death of an individual cell or cells in a given population. The process of tooth development is an example of epithelial mesenchymal interactions and this results in the various stages which exhibits different morphologic patterns. This process exhibits certain distinct morphologic and molecular features such as cell shrinkage, chromatin condensation, internucleosomal DNA fragmentation and activation or inactivation of specific gene functions[38].

Apoptosis is essential to balance mitosis[39]. In other words, it is a physiologic mechanism of cellularity control regulating the size of tissues in an inverse situation of mitosis[40]. In early bud stage apoptotic cells are found in the budding epithelium in particular in the cells facing the oral cavity in rodent studies[41]. When the tooth germ prolongs its central axis at the late bud stage apoptotic cells become concentrated at the tip of the tooth bud. At the bud stage, no apoptotic cells are seen in the mesenchyme .In the cap stage, this cluster of apoptotic cells are localized within the enamel knot. As development continues the enamel knot does not show any evidence of loss of cell mass suggesting a rapid replacement by proliferating cells surrounding the enamel knot. With disappearance of the primary knot, apoptosis is no longer observed in this area but is detected in the gubernaculum(epithelium joining the enamel organ to the buccal epithelium). At the cap stage, a few apoptotic cells are detectable in the condensed mesenchyme but these show no restricted pattern. During bell stage, apoptosis is evident in secondary enamel knots, stratum intermedium cells adjacent to the enamel knots and adjacent mesenchyme. All teeth pass through the same developmental stages and consist of the same tissues(41,42,43).

Enamel knot is considered to act as the signaling centre for tooth morphogenesis[44]. The signals of the primary enamel knot is believed to instruct the formation of the secondary enamel knots on the future tips. It is also suggested that there may even be a cellular continuity between the primary and secondary enamel knot due to the migration and division of surviving cells from the former to the latter. The epithelial mesenchymal interactions are largely mediated by exchange of cell signaling proteins and downstream activation of gene transcription. The instructive signals for tooth formation comes from the oral ectoderm and include members of the FGF, BMP, Wnt and Shh families[31]. These proteins bind to receptors on mesenchymal cells which respond by sending signals back to the dental ectoderm. Within the oral ectoderm and mesenchyme planar signaling is also evident. A passive functional role of apoptosis in enamel knots is believed to be the mechanism whereby the function of enamel knots is terminated.

Multiple roles for apoptosis in odontogenesis have been suggested. Apoptosis may

a) play a role in the disruption of dental lamina
b) occur in the central cells of the invaginating epithelium during the early and middle bud stage, which may support proliferation of underlying basal, mucosal cells.
c) play a role in deciding the final position and size of the tooth in the jaws
prevent tooth appositions in edentulous areas by preventing epithelial overgrowth between the teeth.
e) play a role in deciding the final number of teeth .
f) be an important morphogenic mechanism in shaping the final crown tooth morphogenesis[42].

The formation of tooth buds of appropriate size and shape at the correct position(odontogenesis) involves regulated cell division and cell death. Apoptosis plays both passive and active roles in bud formation, morphogenesis, in reduction of the dental lamina and silencing of the enamel knot signaling centres. Apoptosis also has roles in dental diseases and dismorphology but whether these are from primary defects in apoptotic pathways or due to secondary consequence is not yet known. An understanding of the same could provide new modalities of treatment for genetic diseases like hypodontia and agenesis[45].

Steps in Regenerating a Tooth via a Cap-Stage Implant

Snead et al (2008), proposed a number of progressive steps:

1) harvest adult stem cells (bone marrow stromal stem cells or tooth-derived postnatal stem cells)
2) expand the cells in culture, with cell banking for future organ regeneration needs;
3) seed the cells into an intelligent peptide amphiphile-based scaffold that provides an optimized biochemical & biomechanical environment;
4) instruct the cells with spatially targeted, soluble molecular signals &/or induce with

porcine sources of odontogenic tissue;
5) confirm that the gene expression profile of the cells demonstrates readiness for the next stage in the odontogenesis pathway; &
6) repeat these steps until the cells have expressed genes associated with the cap stage of odontogenesis[46].

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