Study on Wnt signalling pathway in tooth formation from mesenchymal stem cells.

M.D. Kaamini
Department of Biotechnology, Amity University, Haryana, India
drkaaminidhanabalan@gmail.com

Abstract
Tooth is produced by the differentiation of pluripotent cell, which in turn gives rise to the formation of the entire tooth in the jaws. The stem cells are appropriately induced using specific proteins, growth factors etc., and grown in the lab to form a bud of cells. This procedure works unique by attracting stem cells already in the person's body rather than requiring an injection of stem cells from donor. The quick recovery time and natural use of the body's own resources would make this an attractive dental treatment in one day. Amongst the various growth and transcription factors required for differentiation of these stem cells, Wnt gene also plays a major role. A number of Wnt genes are expressed in the developing teeth, with most of them restricted solely to the dental epithelium. When tooth forming sites and tooth patterning are determined, Wnt genes are expressed in the oral epithelium except the presumptive dental epithelium. Wnt genes interact with Shh signalling to set up the ectodermal boundaries between oral and dental ectoderm, positioning the sites of tooth formation. Other Wnt genes are expressed in molar and incisor epithelium, in the enamel knot, a putative signalling center for tooth patterning. Thus understanding the expression and functioning of the Wnt gene in tooth formation can pave better ways for successful tooth regeneration from stem cells in lab as this gene plays a vital signalling role during early stages of tooth development. One can expect in the near future that once a tooth is extracted, it will be possible to have it regenerated. This will be major game changer in dentalcare. It will be like not having lost the tooth at all. This project aims to study the detailed function by expression analysis for each Wnt member in tooth development from mesenchymal stem cells.

Keywords: Tooth regeneration, Transcriptional factors, Wnt gene, Growth factors

Introduction
Tooth loss is a major problem for many from younger to older age people. People who have lost some or all of their adult teeth typically look to dentures, or more recently, dental implants to bridge the gap between a toothless appearance due to unaesthetic appearance, disturbance in phonetics, other functional interference like mastication, food impaction etc. However, this appearance can have a host of unsettling psychosocial ramifications and a tooth-filled grin that is not without pain and discomfort. And also lots of tedious work is involved in fabrication as well as when placed in the missing tooth area can cause further deterioration of the remaining teeth and surrounding periodontium. As the saying goes, “nothing can replace the natural teeth”, having a natural teeth in place would enhance aesthetics, better proprioception and a good healthy periodontium as a vital tooth is in place.

Numerous research works are in progress in developing the natural teeth at lab, which can be replaced in the missing tooth area or a surgically opened site. In the process of tooth regeneration from stem cells, lots of transcription factors as well as growth factors acts on numerous genes to modulate and nudge the stem cells in the path of producing tooth-forming cells. Amongst them Wnt gene is found to play a major role in the signalling processes in stem cells and is found to play a major role in the differentiation of stem cells into different organs like teeth. The major role played by this Wnt gene through the signalling pathway in regulation of stem cell differentiation into tooth will be discuss in this review.

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Discussion

Mammalian tooth development is largely dependent on sequential and reciprocal epithelial-mesenchymal interactions. These processes involve a series of inductive and permissive interactions that result in the determination, differentiation, and organization of odontogenic tissues. Multiple signalling molecules, including BMPs, FGFs, Shh, and Wnt proteins, have been implicated in mediating these tissue interactions (Yan Ding ZHAN et al., 2005). Transcription factors participate in epithelial-mesenchymal interactions via linking the signalling loops between tissue layers by responding to inductive signals and regulating the expression of other signalling molecules. Adult stem cells are highly plastic and multipotent. These cells including dental pulp stem cells and bone marrow stromal cells could be reprogrammed into odontogenic fate and participated in tooth formation. Recent progress in the studies of molecular basis of tooth development, adult stem cell biology, and regeneration will provide fundamental knowledge for the realization of human tooth regeneration in the near future.

In this review as we are going to deal with the Wnt gene, the further sections will entail broader details into the gene and about the various researches undergoing with respect to this role of Wnt gene relevant to tooth differentiation.

Role of Wnt gene in stem cells differentiation

Recent research has supported this hypothesis. There are data to suggest that Wnt signalling induces differentiation of pluripotent stem cells into mesoderm and endoderm progenitor cells (Willert et al., 2003). Wnt signalling was first identified as a potential component to differentiation, because of its established role in development.

There are several pieces of evidence to suggest that Wnt signalling is important in stem cell differentiation. TCF3, a transcription factor regulated by Wnt signalling, has been shown to repress Nanog, a gene required for stem cell pluripotency and self-renewal (Pereira et al., 2006). Over expression of another gene associated with pluripotency, OCT4 leads to increased $\beta$-catenin activity, suggesting Wnt involvement (Nusse, 2008).

Research published in the Journal of Biological Chemistry has suggested that activation of the Wnt pathway in mouse embryonic stem cells induces differentiation into multipotent mesoderm and endoderm cells. This study showed that upon inducing Wnt signalling in monolayer embryonic stem cell cultures, the cells express high levels of markers associated with mesoderm development, particularly T-brachyury and Flk-1 (Bakre et al., 2007).

A publication from the American Society of Haematology extended the previous study to human embryonic stem cells (hESCs) by demonstrating that Wnt signalling can induce hematopoietic cell development from hESCs. Wnt stimulation is also associated with regeneration of nervous system cells, which is further evidence of a role in promoting neural stem cell proliferation (Woll et al., 2008).

Role played by Wnt gene in physiological tooth formation

The tooth is one of the vertebrate organs in which development at the molecular level is beginning to be understood. Secreted signalling molecules have been identified that mediate sequential and reciprocal inductive interactions between the dental epithelium and mesenchyme. Transcription factors have been found that participate in these signalling cascades. A signalling or organizing centre was also recently evolved in the dental enamel knot that expresses the same signals as other organizing centres in the embryo, and which presumably regulates tooth shape. It has recently become evident that the signalling networks that operate in the development of mammalian teeth are similar to those that are involved in the development of other vertebrate organs. The major signalling pathway involved is found to be the Wnt gene mediated pathway (Thesleff & Nieminen, 1996).
Development of tooth was initiated by thickening of the oral ectoderm and subsequent condensation of neural-crest-derived mesenchyme around the invaginating epithelium to form tooth buds (Tucker & Sharpe, 2004). Signalling between the dental epithelium and mesenchyme modulates the survival and growth of tooth buds. This signalling is crucial for determining tooth number, as rudimentary or vestigial buds initially form in the toothless diastema region between the incisors and molars but degenerate without reaching the cap stage (Peterkova et al., 2006).

At the beginning of the cap stage, a transient epithelial signalling centre, called the enamel knot, was induced at the tip of the tooth bud and it regulates tooth growth and morphogenesis (Tucker & Sharpe, 2004).

Mutations in a number of genes encoding components of major signalling pathways have been shown to influence tooth number. This is consistent with the idea that crosstalk between several major signaling pathways, such as Fgf, Shh, Wnt and Bmp, regulates tissue interactions and modulates tooth formation (Tummers & Thesleff, 2009).

The role of growth factors in tooth development

Growth factors and other paracrine signal molecules regulate communication between cells in all developing organs. During tooth morphogenesis, molecules in several conserved signal families mediate interactions both between and within the epithelial and mesenchymal tissue layers. Different signalling pathways form networks and are integrated at many levels. Many targets of the growth factors have been identified, and mutations in several genes within the signalling networks cause defective tooth formation in both humans and mice (Thesleff & Nieminen, 1996)

Wnt extracellular signalling molecules have essential roles as regulators of cell proliferation, migration, differentiation, and in epithelial-mesenchymal interactions involved in tissue morphogenesis. Frizzled integral membrane proteins have been shown to function as receptors for Wnt signalling molecules. Vertebrates also produce secreted proteins related to frizzled receptors, Frizzled-related proteins (FRPs), which contain the cysteine-rich domain of frizzleds and appear to function as Wnt antagonists (Sarkar et al., 2002).

Tooth development was regulated by a reciprocal series of epithelial-mesenchymal interactions, and many Wnt signalling pathway genes were expressed in the developing tooth at these sites suggesting that Wnt signalling is required early in tooth germ formation and that interference with signalling via addition of an antagonist results in retarded development and formation of smaller teeth (Sarkar et al., 2000).

Wnt canonical signalling through the stabilization of β-catenin and activation of Lef1 are important determinants of normal tooth development. Inhibition of Wnt signalling in the early oral epithelium arrests odontogenesis prior to the bud stage of development and Lef1 is also essential for tooth bud progression beyond the bud stage, being required for WNT-induced expression of Fgf4 in the enamel knot (Kratochwil et al., 2002).

When an analysis of epithelial–mesenchymal interactions in the initial morphogenesis of the mammalian tooth was done it has shown that both bmp and Wnt signalling activate Lef1, making it a candidate for integrating the two distinct signalling pathways. Epithelial–mesenchymal interactions govern the development of epidermal organs such as teeth. During the early stages of tooth development, a local ectodermal thickening, which expresses several signaling molecules, appears. It is believed that this in turn signal the underlying mesenchyme triggering mesenchymal condensation and tooth development.

The role of four epithelial signalling molecules-BMP2, SHH, Wnt10a, and Wnt10b, in the early inductive cascades that govern tooth development. Thus, Wnt gene was found to be

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Review article "Wnt signalling pathway in tooth formation" (Indian J. Med. Healthcare)
one of the major pathways for inducing tooth organogenesis (Helene Dassule et al., 1998).  

*Interactions between Wnt/Shh regulate ectodermal boundary formation during mammalian tooth development*

During the initiation of mammalian tooth development, boundaries that distinguish oral from dental ectoderm must be formed to correctly position the sites of tooth formation.

A study that describes a reciprocal relationship between the expression of Wnt-7b in presumptive oral ectoderm and Shh in presumptive dental ectoderm in mouse embryos that mark boundaries between these cells with different developmental fates was done suggesting that Wnt-7b acts to repress Shh expression in oral ectoderm, thus maintaining the boundaries between oral and dental ectodermal cells (Sarkar et al., 2000).

*Role of Wnt gene in rescuing tooth organogenesis*

In particular, research it has been shown that FGF4, a direct target of LEF1 and Wnt signalling, can rescue the arrest of tooth organogenesis in Lef1 (-/-) mice.

Taken together, these data indicate that a single target of LEF1 can account for the function of LEF1 in tooth development and for a relay of a Wnt signal reception to a cascade of FGF signalling activities, allowing for a sequential and reciprocal communication between epithelium and mesenchyme.

*Increasing the expression of the Wnt gene by using other genes, which can synergise and can increase the expression of this gene can result in more successful tooth regeneration*

In one particular research it has been shown that when Rfz-1 and Xwnt-8 are expressed together in a particular assay, it was observed that a greater induction of these genes occurs, indicating that Rfz-1 can synergize with a Wnt.

Previous studies have shown the relevance and the importance played of Wnt gene in the process of differentiation of stem cells into tooth. This gene can be further analysed, studied and if capable of further modification of the signalling pathway by addition of other genes like Rfz-1, which can be expressed together in this gene, we can possibly produce greater induction of these genes and by further modification of expression of this gene by using Wnt agonist, in tooth buds formed from mesenchymal stem cells which can result in efficient and more successful tooth regeneration in lab.

*Materials and methods*

To understand the role of Wnt signalling in tooth development we can first perform a detailed comparative analysis of the expression profiles of Wnt genes, one receptor and two agonist/antagonists during molar tooth development.

For performing the expression profile analysis of the Wnt gene in the developing tooth buds, In situ Hybridisation can be performed using 35S radiolabelled probes in the mouse Embryo heads which are fixed in buffered paraformaldehyde, embedded in paraffin wax and sectioned in a frontal plane.

As an alternative instead of using Radiolabelled probes, non-radioactive probes or fluorescent in situ hybridisation technique can be followed. Mouse models are preferred as they are considered to be close homologs to humans.

*Possible outcome*

The developing tooth is an excellent model to study the molecular mechanisms involved in epithelial-mesenchymal interactions, and the early arrest of tooth development at the bud stage in Lef-1 mutant mice suggests that Wnt signalling may have an essential role in early tooth development.

From the wide amount of literature of review and research methodology available, Wnt gene has been shown to play a major role in the differentiation of stem cells into tooth forming cells by beta catenin action. Many other signalling molecules and pathways are also seen to be involved together with a Wnt signalling pathway and their means of interactions are still under research.

Studying the expression analysis of the various components of this signalling pathway can also provide insights into the role of other
signalling molecules involved in successful tooth regeneration in lab, which is still a dream under research

Conclusion

In conclusion, of this review, our findings will highlight how the processes of tooth development are highly sensitive to spatiotemporal changes in Wnt signalling activity. Even a small disruption in the signalling network, can be sufficient to change the fate of tooth buds, leading to abnormal tooth number and size. Changes in expression of Wnt signalling pathway might represent an important mechanism that underlies how the spatially restricted and balanced effects of specific components of a signalling network can regulate stem cell proliferation and differentiation into tooth.

Hence, it is possible that variation in the growth capacity of teeth and the extent of enamel deposition in tooth formation can be resulted from fine-tuning of the complex signal network comprising of Wnt genethat regulates the maintenance, proliferation, and differentiation of epithelial stem cells.

Understanding the expression and functioning of the Wnt gene in tooth formation can pave better ways for successful tooth regeneration from stem cells in lab as this gene plays a vital signalling pathway during early stages of tooth development.

In addition, one can expect in the near future, once a tooth is extracted it will be possible to have it regenerated. This will be major game changer in dentalcare. It will be like not having lost the tooth at all.

References