Significance of TGF-β Signal Transduction Pathway in Epithelial Formation: An Area in Tissue Regeneration

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Abstract
Transforming growth factor beta-1 (TGFβ-1) plays a major role in cell proliferation, lineage determination, differentiation, motility, adhesion and cell death. TGFβ-1 has been related with epithelial cells involved in wound healing process. Mesenchymal stem cells (MSCs) have been found to accelerate wound healing process and repair epithelium in vitro through differentiation. Epithelial-to-mesenchymal transition (EMT) and mesenchymal-to-epithelial transition (MET) are thought to be involved in the process of wound healing as TGFβ is known as a chief inducer during EMT. During inflammation, the EMT programme is altered where the MSCs are mobilized to the injury site. Recently, dental pulp stem cells and stem cells from human exfoliated deciduous teeth have gained popularity in tissue regeneration research. This review discusses the involvement of TGFβ-1 signalling pathway during epithelial formation, wound healing and differentiation of MSCs to epithelial-like cells and how dental pulp stem cells could be related and applied in tissue regeneration.

Keywords: Transforming growth factor beta-1, Signalling pathway, Stem cells from human exfoliated deciduous teeth, Dental pulp stem cells, Epithelial-to-mesenchymal transition, Mesenchymal-to-epithelial transition, Tissue regeneration.

Introduction

For many years, researchers have been working to determine the stem cells possibilities to regenerate damaged human cells due to illness, developmental defects and accidents. According to Smith (2006), stem

cells are cells that are able to continuously self-replicate, generate daughter cells with different and more restricted properties and re-populate a host *in vivo*. However, these cells have a limited replicating capacity. Based on their origin and differentiation capabilities, stem cells can be classified into two broad categories; embryonic stem cells which are isolated from inner cell mass of blastocysts and adult stem cells which are from various tissues. Among various adult stem cells, mesenchymal stem cells (MSCs) have been found to be the widest distribution in the human body and have been isolated from diverse tissues and organs (Bobis *et al.,* 2006). MSCs are defined as multipotent cells that can be derived from other non-marrow tissues such as umbilical cord blood, adipose tissue, adult muscle, corneal stroma or dental pulp of deciduous baby teeth. Dotto (1999) and Freedberg *et al.,* (2001) have discovered that epithelial proliferation and differentiation are regulated by an intricate signalling pathway of different peptides (cytokines/growth factors) produced by keratinocytes, stromal cells and infiltrating inflammatory cells and their respective receptors. One of the crucial peptides is transforming growth factor beta (TGF-β) which has been found to play an important regulatory functions in epithelial proliferation and differentiation (Roberts, 1998; Ten Dijke *et al.,* 2002). TGF-β1 has been known as the most prominent among the three TGF-β isoforms (Karatsaidis *et al.,* 2003). This has been supported by Kasai *et al.,* (2005) where he and his co-workers have reported that TGF-β1 is able to induce characterization of motility, proliferation and extracellular matrix (ECM) synthesis observed in mesenchymal cells with a myofibroblast-like phenotype from fibroblast foci. Hence, TGF-β1 has been identified to play a role in regulating cell functionality for survival (Fleisch *et al.,* 2006; Taylor *et al.,* 2009) and in normal wound healing (Desmoulière, 1995). The purview of this review is to discuss the involvement of TGF-β1 signalling pathway during epithelial formation, wound healing process and differentiation of MSCs to epithelial-like cells and to relate the application of dental pulp stem cells in tissue regeneration.

Transforming growth factor beta

TGF-β family consists of a huge number of structurally related polypeptide growth factors, each of which are capable of regulating an array of cellular processes including cell proliferation, lineage determination, differentiation, motility, adhesion, and cell death (Massagué, 1998; Xie *et al.,* 2003). The TGF-β family is highly conserved in mammals and plays a central role in regulating cell functionality for survival (Fleisch *et al.,* 2006; Taylor *et al.,* 2009). Bhowmick *et al.,* (2001) reported that TGF-β plays a role as an autocrine transforming and morphogenic factor in epithelial cells. In mammals, three isoforms of TGF-β, namely, TGF-β1, TGF-β2, and TGF-β3 have been discovered (Saharinen and Keski-Oja, 2000). These TGF-βs are multifunctional cytokines which can regulate cell proliferation and differentiation positively or negatively depending on the cell type (Hauri-Hohl *et al.,* 2008) and have been implicated in such diverse physiological events such as angiogenesis, steroidogenesis, immune function and tissue remodelling and repair (Godkin and Doré, 1998; Leask and Abraham, 2004). The TGF-βs have shown to be involved in a wide variety of cellular processes in cells and tissues such as fibroblast, epithelium, bone and ECM (Govinden and Bhoola, 2003). The TGF-β receptors are TGF-βRI, TGF-βRII, and TGF-βRIII and the biological processes occur when the TGF-β molecule interact with the cell surface receptors (Weis-Garcia and Massagué, 1996). The TGFβ molecule binds to the cell surface receptor TGF-βRII but not TGF-βRI even though both of them are cytoplasmic serine/threonine kinase domains. The TGF-βRI recognizes the heteromeric complex of TGF-β molecule-TGF-βRII and undergoes phosphorylation. Then, the TGF-βRI is phosphorylated and Smad2 proteins are activated. Then, Smad4 proteins attach to Smad2 proteins, undergo phosphorylation and become activated. This Smad2-Smad4 protein complex is translocated to the nucleus and transcription of target genes are activated to mediate the biological process of TGF-β molecule (Godkin and Doré, 1998). The actual function and capability of TGF-βRIII signalling is still under explored. In contrast to TGFB2 and TGFB3, TGFB1 is being intensely studied in the TGF-β signalling pathway and this isoform has been correlated with epithelial formation in many cellular biological processes. Nonetheless, there are many more

signalling pathways that can be involved in cell growth and proliferation such as mitogen-activated protein kinase (MAPK), Smad, Wnt, Hedgehog and Notch signalling pathways.

Epithelial formation and transforming growth factor beta-1

Human body surfaces and cavities are lined with epithelial tissues such as skin, gastrointestinal tract, urogenital system and breast ducts, where these tissues act as a protective barrier. The source of cells for epithelial formation involved in tissue repair after an injury remains controversial and poorly defined (Păunescu*et al.,* 2007). Bhowmick *et al.,* (2001) described the TGF-β1 as a growth factor that modulates growth, differentiation and epithelial transformation in the process of tumorigenesis, wound healing and embryogenesis. It is called a multifunctional peptide, initially characterized as a growth stimulating peptide capable of inducing anchorage independent growth in normal fibroblast cell lines (de Larco and Todaro, 1978). Several researchers have also reported that fibroblast and mesenchymal stromal cells have been applied to accelerate wound healing, have differentiation and paracrine effects (Le Pillouer-Prost, 2003; Satoh*et al.,* 2004; Li *et al.,* 2006). MSCs can be obtained in huge number from variety of tissue sources such as adipose (Nakagami *et al.,* 2006), dermal tissue (Chunmeng and Tianmin, 2004), synovial fluid (De Bari *et al.,* 2001; De Bari *et al.,* 2003), deciduous teeth (Shi and Gronthos, 2003; Pierdomenico *et al.,* 2005; Yamada *et al.,* 2006), cord blood (Bieback *et al.,* 2004; Lee *et al.,* 2004), amniotic fluid (In't Anker*et al.,* 2003; Tsai *et al.,* 2004; De Coppi *et al.,* 2007), and placenta (Igura *et al.,* 2004; Miao *et al.,* 2006). MSCs have been discovered to repair epithelium *in vitro* through differentiation (Spees *et al.,* 2003) which has been supported by Phinney and Prockop (2007) reporting that MSCs promote tissue repair by secreting soluble molecules that modulate inflammation and angiogenesis. MSCs have been reported to spontaneously secrete TGF-β1, vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and IL6 but not interferon gamma (IFNG), IL4 and IL5/IL10 (Wang *et al.,* 2006; Aksu *et al.,* 2008).Shi and Gronthos (2003) managed to isolate the human dental pulp stem cells (hDPSCs) which shows properties that resembles MSCs. Hence, dental pulp stem cells (DPSCs) have been widely used as a new source of stem cells and has been applied to treat a lot of diseases which had been treated using MSCs such as cardiac infarction, Alzheimer and Parkinson's disease, and muscular dystrophy (Gandia *et al.,* 2008; Apel *et al.,* 2009). Since DPSCs and MSCs have similarity in their properties, stem cells from human exfoliated deciduous teeth (SHED) might be one of the stem cell sources since it shares the same location with DPSC which is from the dental pulp tissue. Furthermore, in the study by Karaöz *et al.,* (2011), it was found that human DPSCs (hDPSCs) secreted a higher level of cytokines consisting of TGFβ1 and VEGF compared to human bone marrow stem cells (hBMMSCs).This finding was significant because VEGF may be a critical growth factor in human progenitor cells for the ischemic tissue protection (Wang *et al.,* 2006) in order to prevent the tissue from loss of blood and glucose supply.Hence, hDPSCs hold promises for promoting better wound healing characteristics since VEGF acts as a protective factor and TGFβ1 provides the wound healing characteristics. Based on a mouse model described by Galiano *et al.,* (2004), Nishino *et al.,* (2011) proposed that SHED may offer unique stem cell resources with the potential for novel cell therapies for wound healing due to the formation of epithelium during healing process. In cases of severe mucosal damage, induction of mesenchymal-to-epithelial (MET) transition may give a greater benefit in epithelial healing (Sipos and Galamb, 2012).

Epithelial-to-mesenchymal and mesenchymal-to-epithelial transitions

Epithelial-to-mesenchymal **(**EMT) transition is a biological phenomenon occurring during embryonic development, tumorigenesis, metastasis and wound healing. Epithelial to mesenchymal and mesenchymal to epithelial transitions play a crucial role in tissue organ development and in the pathogenesis of diseases (Sipos and Galamb, 2012). According to Avizienyte *et al.,* (2005) and Hay (2005), the cells exhibit dramatic

shape changes where they may lose many of their epithelial characteristic during EMT. This biological process requires alterations in morphology, cytoskeleton, adhesion and migration capacity (Lee *et al.,* 2006).EMT transition in mostly adult epithelial cells is recognized by the acquisition of spindle morphology and increased motility with changes occurring in cadherin expression and localization (Bhowmick *et al.,* 2001). The process occur when the cells lose the apico-basal polarity and cell adhesion, the repression of E-cadherin, occluding, tight junction protein 1, or cytokeratin expression, and also increasing in their cell mobility. At the same time, the cells obtain the mesenchymal phenotype when the expression of tyrosine kinases is elevated after which the expression of N-cadherin, vimentin, fibronectin, zinc-finger domain proteins (SNA1/SAIL, SNAI2/SLUG, ZEB2/SIP1), matric metalloproteinases, as well as the basic helix-loop-helix domain protein Twist1 expression is upregulated (Thiery 2002; Lee *et al.,* 2006). In the case of palatal fusion, the medial edge epithelial (MEE) cells at the tip of degrading epithelial seam display these characteristics when observed under transmission electron micrographs (Mato *et al.,* 1975; Griffith and Hay, 1992). According to Korinek*et al.,* (1998) and DasGupta and Fuchs (1999), during tooth formation, EMT plays an important role where MSCs seem to rely upon epithelial cues for their survival and differentiation. Furthermore, in EMT, TGF-β is known as a potent inducer when TGF-β directly activates the expression of SNA1/2, Twist and ZEB 1/2 transcription factors which are the key regulators of EMT program (Moustakas and Heldin, 2007) and Snail, Twist, Goosecoid and TGF-β1 induced FOX2 expression when epithelial cells undergo EMT program (Hader *et al.,* 2010; Sano *et al.,* 2010; Hugo *et al.,* 2011). Sipos and Galamb (2012) reported that TGF-β1 has been known as a chief inducer not only in EMT, but also in fibrosis and myofibroblast generation. Meanwhile, MET is known as a reversible biological process which involves the transition from motile, spindle-shaped or multipolar mesenchymal cells to polarized epithelial cells. Spees *et al.,* (2003) reported that during occurrence of inflammation, MET is altered because MSCs are mobilized to the injury site and consequently subjected to the inflammatory responses and that both EMT and MET play a crucial role especially during wound healing process.

Dental pulp stem cells and stem cells from human exfoliated deciduous teeth

Abundant data are available suggesting that the dental tissue-derived MSCs can be an alternative cell source suitable for whole tooth regeneration. Stem cells from teeth can be derived from an individual's primary or permanent teeth. Historically, the first isolated dental stem cells were by Gronthos and his associates which was from DPSCs (Gronthos *et al.,* 2000), and SHED (Shi and Gronthos, 2003). Interestingly, Bluteau *et al.,* (2008) and Sonoyama *et al.,* (2008) have reported that these dental stem cells can also be extracted from apical papilla of shed primary teeth (SCAP). Additionally, periodontal ligament stem cells (PDLSCs) were reported by Seo *et al.,* (2004) and dental follicle stem cells (DFSCs) by Morsczeck *et al.,* (2005). According to Bluteau *et al.,* (2008) and Mitsiadis and Graf (2009), dental follicle, dental pulp and odontoblasts that are responsible for dentin matrix synthesis are derived from cranial neural-crest-derived mesencyhmal cells (CNCCs). CNCCs has been identified as a clonogenic cells because they are capable to differentiate into various types of cells such as odontoblasts, cementoblasts/cementocytes, osteoblasts, chondroblast/chondrocytes, neurons, melanocytes and muscles (Mitsiadis *et al.,* 2011). Yu *et al.,* (2007), Ohta *et al.,* (2008) and Huang *et al.,* (2009) reported that dental tissue-derived MSCs are more committed to be odontogenic cells. Odontoblasts have been recognized morphologically as columnar polarized cells with eccentric nuclei and long cellular processes aligned at the outer edges of dentin (Smith *et al.,* 1995) which will later form a primary dentin after undergoing mesenchymal differentiation. Epithelial cells of the inner enamel organ interacts with mesenchymal cells of the dental papilla during development, which lead to the differentiation of ameloblasts and odontoblasts and later enable them to deposit specialized mineralized matrices, enamel and dentin respectively (Gronthos *et al.,* 2000). When there is an occurrence of injury, they become active, undergo proliferation and finally differentiate into odontoblasts-like cells (Mitsiadis *et al.,*

2011). Mechanisms of apoptosis induction, immune response activation, and alterations in dental tissue physiology will take place when there is occurrence of dental injury (Tziafas *et al.,* 2000; Mitsiadis and Rahiotis, 2004; Mitsiadis *et al.,* 2008). Up-regulation expression of TGFβ has been shown to be accompanied by apoptosis (Kobayashi *et al.,* 2000; Pollack and Leeuwenburgh, 2001) which is important in the formation of reparative dentin (Tziafas *et al.,* 2000; Mitsiadis and Rahiotis, 2004). Gronthos *et al.,* (2000), Shi and Gronthos (2003) and Dominici *et al.,* (2006) reported that cells located in putative dental pulp perivascular niches have the properties of MSC. Generally, MSCs are non-haematopoietic stromal cells that are able to differentiate into wide range of cells, have the ability to double into many populations without loss of function and systematically migrate to the site of injury (Chamberlain *et al.,* 2007). The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy has proposed three minimal criteria to define human MSCs. First, the MSCs must be plastic-adherent when maintained in standard culture conditions when cultured using tissue culture flasks. Second, equal or more than 95% of the MSCs population must express CD105, CD73 and CD90 and lack expression of CD45, CD34, CD14 or CD11b, CD79α or CD19, and HLA class II when measured with flow cytometry. Third, the MSCs should be able to differentiate to osteoblasts, adipocytes and chondroblasts under standard *in vitro* differentiating conditions (Dominici *et al.,* 2006). Gronthos *et al.,* (2000) reported that postnatal dental pulp consists of stem cells that are clonogenic, highly proliferative, and capable of regenerating a tissue both *in vivo* and describes as DPSCs. DPSCs have been indicated to exhibit a higher proliferation rate and growth potential than Bone Marrow stromal cells (BMSCs) *in vitro* and has related to the development stages according to their respective tissues(Gronthos *et al.,* 2000; Shi *et al.,* 2005).This is because the third molars are the last permanent teeth to fully develop and erupt and also an earlier stage of development compared to adult bone marrow (Gronthos *et al.,* 2000). In 2003, Miura and his co-workers found a population with higher proliferation, clonogenic cells that were capable of differentiating into variety of cells such as neural cells, adipocytes and odontoblasts and these stem cells have been termed as SHED. They also found that SHED was able to induce bone formation, generate dentin and survive in mouse brain along with expression of neural markers. In comparison between DPSCs, SHED, PDLSCs, and BMSCs, DPSCs, SHED, and PDLSCs have been found to maintain a higher growth potential (Sloan and Waddington, 2008) and more population doublings (Shi *et al.,* 2005; Huang *et al.,* 2009) than BMSCs except for DFSCs (Morsczeck *et al.,* 2005). Surprisingly, SHED are distinct from DPSCs with respect to their higher proliferation rate, increased cell population doublings and osteoinductive capacity *in vivo* (Miura *et al.,* 2003; Shi *et al.,* 2005). This can be attributed to their developmental process, tissue structure and function (Miura *et al.,* 2003). In contrast, although SHED failed to form the dentin-pulp-like complex, it needs to be considered important since the primary teeth will be removed and become a waste when the permanent teeth erupt.

Tissue regeneration and engineering

Nowadays, modern dental tissues' regeneration approaches offer attractive alternative in comparison with the traditional restorative approaches since the infected and damaged tissues are replaced with the natural tissue obtained from the integral part of the tooth. Generally, the principles of tissue engineering involve combining living cells with a natural or synthetic support that is also biodegradable to build a three dimensional living construct that is functionally, structurally and mechanically equal to or better than the tissue that is to be replaced (Stock and Vacanti, 2001). Recently, there have been establishment of stem cell banks not only with cord stem cell but also dental pulp stem cells of postnatal teeth (Karien, 2009). These stem cell banks have provided lot of benefits as the patients are now able to use their own cells for medical purposes. In future, the medical researchers will be able to use technologies derived from stem cell research to treat various diseases such as cancer, Parkinson's, Alzheimer, spinal cord injuries, diabetes, heart disease, liver disease, blindness, multiple sclerosis, muscle damage and many others (Lindvall, 2003; Fiegel *et al.,*

2006; Timper *et al.,* 2006). Karien (2009) has mentioned that right now the dental research's revolution was really happening when the scientist discovered a population of multipotent mesenchymal dental pulp stem cells which were able to proliferate with a high rate for self-renewal and capable to differentiate into functional odontoblasts and this revolution has opened new avenues in particular generally for reparative and reconstructive dentistry and tissue engineering. It has also been reported that several scientists have recombined the dissociated dental epithelial and mesenchymal tissues that leads to tooth formation both *in vitro* and *in vivo* (Amar *et al.,* 1989; Yoshiba *et al.,* 1998).Recent study by Tran *et al.,* (2003) suggested that BMMSCs can be recruited into the cheek and differentiated into buccal epithelial cells without the host-recipient cell fusion. Marynka-Kalmani *et al.,* (2010) who carried out a research where they transplanted oral mucosa stem cells into nude mice with dexamethasone treatment found that suprisingly, the cells form tumors containing mixed types of tissue. This result shows that caution must be taken when applying stem cells for tissue engineering and stem cell therapy. However, identification of particular cell differentiation and their signal transduction are still unclear and needs to be further investigated.

Conclusions

Recently, research on dental epithelial cells has been widely explored since ameoblasts and ameoblast precursors have been lost after tooth eruption has taken place. MSCs have been discovered in dental pulp, but still there is death of knowledge is known on the molecular regulation of this niche and a lot of research needs to be done for better understanding of their progression. Growth factor, TGF-β1 might play a crucial role in inducing mesencyhmal dental pulp stem cells into epithelial-like cells since the wound healing program is a demanding aspect in life. Moreover, researchers have reported that the TGF-β signalling pathway becomes active subsequent to the treatment of cells with TGF-β1.Also, it has been mentioned in the literature thatTGF-β1 either directly or indirectly modulates the transcription of the mediator on TGF-β pathway, receptor tyrosine kinase pathway, and wnt-pathway and also that the epithelial cells might be more potent in comparison to the mesenchymal cells induced by TGF-β1. These findings demonstrated that TGF-β1 is important in the cellular mechanism of the cells and wound healing properties. Theoretically and practically, stem cells have been shown to be an important source for dental stem cell-based replacement therapy that promises a better option for leading a better life. Although the research progress is promising for regenerative medicine, it is still not ready to be applied for the clinical approaches. This is because there are still a few major problems that need to be addressed like the immune rejection risks from donor to recipient and the need to isolate autologous stem cells with the accessible cell sources without undergoing the surgery. However, knowledge in technology of stem cell is quickly expanding in all medical disciplines with the need for new approaches in all fields, including reparative dentistry. As such, stem cell therapy is still ongoing and constitutes a challenge for both dentists and biologists.

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