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Dental Mesenchymal Stem Cells

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Cover illustration: Lineage traced PLP-CreERT2/R26YFP dental mesenchymal stem cells and odontoblasts (orange) in the adult mouse incisor tooth. Immunohistochemistry staining of HMGA2 (purple), background (cyan).

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Dental Mesenchymal Stem Cells

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To my beloved family Susila, Oiva, Prasad and Diana Kaukua

ABSTRACT

Mesenchymal stem cells have been found in various tissues and act as source for renewal and repair. The mouse incisor tooth continuously grows throughout life, implicating that there are stem cell niches constantly contributing with cells. The composition of these stem cell niches is not fully understood. Here, we show that Schwann cells on the peripheral nerves in the close proximity to the incisor tooth constitute a stem cell niche. Transgenic mouse models were used to label Schwann cells and their progeny *in vivo*. It was also possible to establish that Schwann cell precursors contributed in tooth development during embryogenesis. In the adult incisor tooth, it was demonstrated that there were a continuous replenishment from Schwann cells with dental mesenchymal stem cells and odontoblasts. Moreover, through a multi-color reporter line mouse model it was possible to label individual Schwann cells and show their specific contribution and dynamics to tooth organogenesis in adulthood. The dental mesenchymal stem cells were arranged in highly spatialized domain patterns and competed for the opportunity to form odontoblasts. Furthermore, after tooth injury these Schwann cell-derived dental mesenchymal stem cells could be recruited for repair. Thus, these results advocate a novel source of dental mesenchymal stem cells, the peripheral Schwann cells, that throughout life contribute to tooth growth and become involved in regeneration after tooth damage. This might have important implications for the further understanding of adult stem cell populations and their potential use in tissue engineering.

Dental pulps in deciduous and adult human teeth harbor cells with stem/progenitor cell properties and represent an excellent model system to study aging of stromal populations. Aging is tightly connected to self-renewal and proliferation and thus, mapping potential molecular differences in these characteristics between populations constitute an important task. It was hypothesized that genetic profiles of deciduous pulp cells differ from adult pulp cells, due to ontogeny. Deciduous and permanent teeth were collected for tissue sampling, cell culture and isolation. RNA and proteins were extracted with subsequent microarray, quantitative real-time RT-PCR and Western blot analysis while pulp tissue was sectioned for immunohistochemistry stainings. Results show that there are differentially expressed genes in the deciduous and permanent teeth. Especially genes involved in cell division, mitosis, stemness and ageing are differently expressed in favor of pulp cells from deciduous teeth. Here it is shown for the first time that HMGA2, a neural stem cell marker during embryogenesis, is robustly expressed in deciduous pulp cells. Taken together, the results suggest that cells from deciduous teeth may be more suitable than cells from permanent teeth from a tissue engineering perspective.

LIST OF PUBLICATIONS

I. **Nina Kaukua**[*], Maryam K Shahidi[*], Chrysoula Konstantinidou, Vyacheslav Dyachuk, Marketa Kaucká, Alessandro Furlan, Zhengwen An, Longlong Wang, Isabell Hultman, Lars Ährlund-Richter, Hans Blom, Hjalmar Brismar, Natalia Assaife Lopes, Vassilis Pachnis, Ueli Suter, Hans Clevers, Irma Thesleff, Paul Sharpe, Patrik Ernfors, Kaj Fried, Igor Adameyko.
Glial origin of mesenchymal stem cells in a tooth model system
Nature (2014) 513:551-554

II. **Nina Kaukua,** Mo Chen, Paolo Guarnieri, Markus Dahl, Mei Ling Lim, Tülay Y. Lindberg, Erik Sundström, Igor Adameyko, Jeremy J. Mao, Kaj Fried.
Molecular differences between mesenchymal populations from deciduous and permanent human teeth.
In Revision, Stem Cell Research and Therapy

PUBLICATIONS NOT INCLUDED IN THE THESIS

I. Anders P. Rehn, Radim Cerny, Rachael V. Sugars, **Nina Kaukua**, Mikael Wendel
Osteoadherin is upregulated by mature osteoblasts and enhances their in vitro *differentiation and mineralization.*
Calcified Tissue International (2008), 6:454-464

II. **Nina Kaukua,** Kaj Fried and Jeremy J Mao
Tissue Engineering in Orthodontics Therapy. In: Integrated Clinical Orthodontics. (Ed. V. Krishnan and Z. Davidovitch. Oxford: Wiley-Blackwell, 2012, pp 380-391

III. Vyacheslav Dyachuk*, Alessandro Furlan*, Maryam K Shahidi, Marcela Giovenco, **Nina Kaukua,** Chrysoula Konstantinidou, Vassilis Pachnis, Fatima Memic, Ulrika Marklund, Thomas Müller, Carmen Birchmeier, Kaj Fried, Patrik Ernfors, Igor Adameyko
Neurodevelopment. Parasympathetic neurons originate from nerve-associated peripheral glial progenitors
Science (2014) 345:82-87

*these authors contributed equally to the work

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

BMMSCs	Bone marrow mesenchymal stem cells
BrdU	5-Bromo-2-deoxyuridine
DPSCs	Dental Pulp Stem Cells
E	Embryonic stage
FACS	Fluorescence-Activated Cell Sorting
HES	Human embryonic stem cells
IEE	Inner Enamel Epithelium
IHC	immunohistochemistry
MSCs	Mesenchymal Stem Cells
OEE	Outer Enamel Epithelium
RT q-PCR	Real-Time quantitative Polymerase Chain Reaction
SCP	Schwann Cell Precursor
Shh	Sonic hedgehog

1 INTRODUCTION

Stem cells are at the focus of interest in studies of biological development and regeneration. Stem cells reside in any tissue or organ, and serve to repair/aid the tissue by dividing and differentiating into specific cell types. To investigate somatic stem cells, one needs to examine the environment that they reside in, the stem cell niche.

Unfortunately, there are no definite markers for mesenchymal stem cells (MSCs). The panel of markers to identify them has varied over the years and as the research has progressed, more and more scientists have realized that the somatic MSCs are different in character depending on tissue site and that it might be hard to categorize them into one homogenous group. To date, there is not a single unifying marker for somatic MSCs, especially *in vivo*, although panels of several markers that are commonly used exist. . The mouse incisor is an excellent model system for studies of stem cell niches, since the tooth renews itself throughout life. In particular, the development and generation of odontoblasts from MSCs can be studied in detail.

1.1 NEURAL CREST

Neural crest cells exist in vertebrates. After migration, they generate sensory neurons and glial cells in the peripheral nervous system and cranial ganglia, melanocytes, symphato-adrenal cells, enteric ganglia, stromal tissue and craniofacial cartilage and bone (Bulter and Bronner, 2014). The neural crest arises from the neuroepithelium of the neural plate border and surface ectoderm (Selleck and Bronner-Fraser, 1995). BMP, FGF, Wnt and PAX signaling have been shown to be important for the initiation of neural crest formation in species such as amphibians, avians and fish (Monsoro-Burq et al., 2003; Lewis et al., 2004; Crane and Trainor, 2006). However, with regard to mammalian neural crest formation, the roles of these pathways are more unclear. They do not seem to be indispensable for induction, but important for neural crest survival (Crane and Trainor, 2006). Thus, the signals that orchestrate mammalian neural crest induction still need to be defined.

Depending on the site of birth of the neural crest along the rostro-caudal axis of the neural tube, they can be categorized into four groups: cranial, cardiac, vagal and trunk neural crest cells. The focus in this chapter will be on the cranial neural crest.

1.1.1 Cranial neural crest

Cranial neural crest , unlike trunk neural crest, forms before the closure of the neural folds (Figure 1). Early markers of cranial neural crest cells are the zinc-finger transcription factors Snail1 and Snail2 (Milet and Monsoro-Burq, 2012). They give rise to the widest variety among the four categories of neural crest cells, namely cranial nerve ganglia, the majority of the craniofacial cartilages and bones, smooth muscle, stromal tissue and melanocytes (Bulter

and Bronner, 2014). Also parts of teeth, namely the pulp stromal tissues and dentin-producing odontoblasts originate from cranial neural crest-derived mesenchyme. In addition, other dental tissues such as the tooth periodontium and alveolar bone share this origin (Miletich and Sharpe, 2004; Tucker and Sharpe, 2004). Thus, the most distinguished feature of the cranial neural crest cells in comparison to other types of neural crest cells is their capacity to form unique mesenchymal derivatives (Hall, 1999).

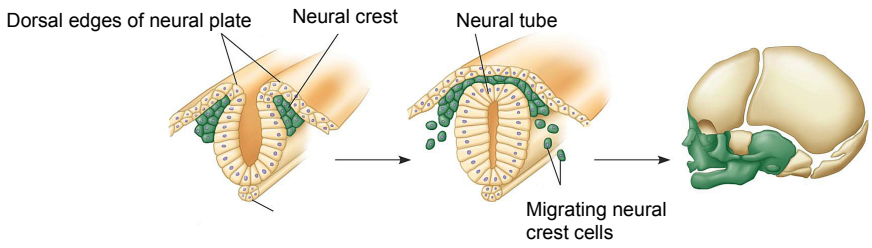

Figure 1. Induction of cranial neural crest formation. Schematic illustration of neurulation and formation of cranial neural crest cells (green). The neural plate bends to form the neural tube and cranial neural crest cells are formed on the edges of it and also from the ectoderm and neuroepithelium. The cranial neural crest cells start migrating extensively to form different craniofacial structures such as cartilage and bone. Modified from http://bio1151.nicerweb.com/doc/class/bio1151/Locked/media/ch34/34_07NeuralCrest_L.jpg

1.2 TOOTH MORPHOGENESIS

Teeth from different species appear in various shapes and numbers. Mammalian teeth are heterodont, which means that they have different sizes and shapes depending on their functional role: incisors, canines, premolars and molars. Both humans and rodents evolved from a common ancestor that have been thought to possess three incisors, one canine, four premolars and three molars per quadrant, teeth that were replaced once (Line, 2003). The mouse eventually lost the canine and premolars completely and has retained one incisor and three molars per jaw quadrant (Tucker and Sharpe, 2004). Additionally, through natural adaptation, the mouse incisor continues to grow throughout life, but the molars are never replaced (Evans et al., 2007). Humans possess each category of tooth type but fewer than earlier in evolution; two incisors, one canine, two premolars and three molars in each quadrant. Also, humans have two sets of teeth, first the deciduous dentition, which from the age of about six is replaced with the permanent dentition.

1.2.1 Building blocks of the mature mouse incisor

Mice are commonly used to study the mammalian dentition. The incisor in each jaw quadrant is separated from three molars by a large diastema (Tucker and Sharpe, 2004) (Figure 2). The mouse incisor is built to cut hard tissue. Enamel, the hardest tissue of the body, is present on the labial part of the tooth only (Figure 2). This uneven distribution of hard tissue makes the lingual part of the tooth, covered by dentin only, to wear off more quickly than the labial part. Accordingly, a sharp pointed tip is formed that enables the mouse to cut hard food. Because the enamel and dentin is arranged in an asymmetrical way there is a constant wear and loss of tissue from the tooth tip, i.e. the incisor becomes shorter. This shortening of the tooth is compensated by a continuous growth from the apical part where epithelial and MSC niches are located.

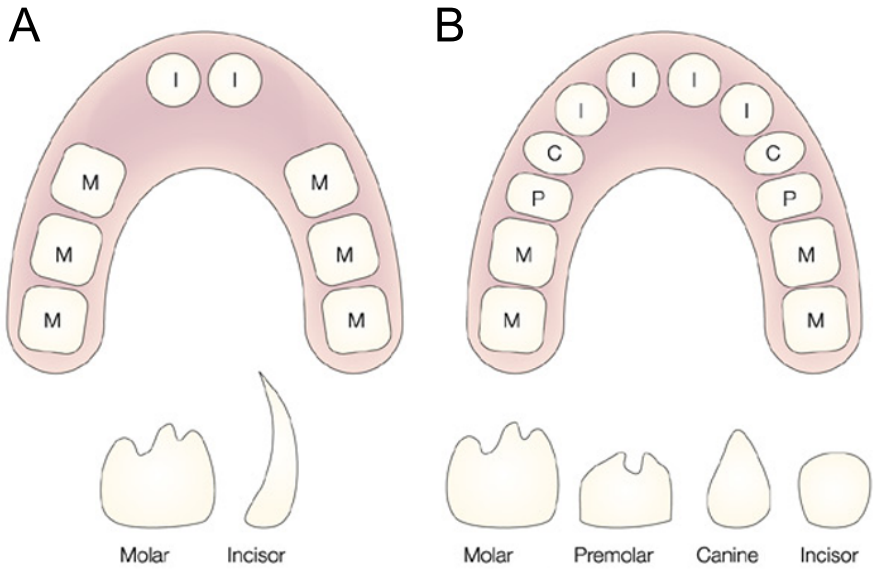

Figure 2. Illustration of the mouse and human dentition. A) The mouse dentition B) The human dentition. M: Molar, I: Incisor, P: Premolar, C: Canine Modified from Tucker and Sharpe, 2004.

1.2.2 Building blocks of the mature human tooth

A human tooth consists of a crown, root(s) and pulp. Both crown and root have a main body of dentin, but the crown dentin is covered by enamel and the root dentin by cementum. At the core of the tooth is the stromal root and crown pulp (Figure 3). The pulp mainly consists of blood vessels, nerves and connective tissue. The walls of the pulp cavity are covered by the dentin-producing odontoblasts.

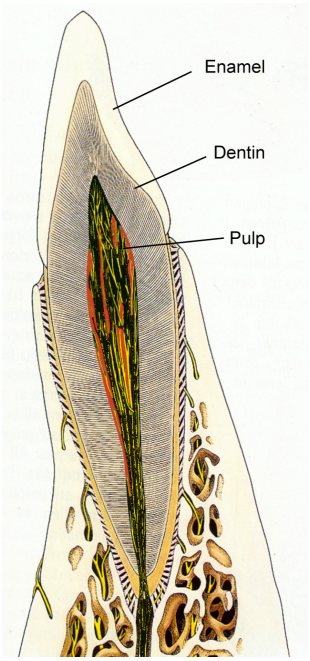

Figure 3. Schematic illustration of a human incisor tooth. Modified from Eriksson, 1980.

1.2.3 Tooth developmental stages

The general gene network that governs tooth development is preserved from early stages of the evolution, already from the time when pharyngeal teeth were developed in jawless fish (Fraser et al., 2009). Thus, the gene regulatory network is similar between mouse and other species (Fraser et al., 2009; Tummers and Thesleff, 2009; Richman and Handrigan, 2011). Not only has the gene regulatory network been conserved between different species, but it is also similar to other ectodermally derived developing appendages such as hair, nails and exocrine glands (Mikkola et al., 2009).

Tooth development passes through several morphological stages where the ectodermally derived dental epithelium interacts with the underlying cranial neural crest-derived mesenchyme (Chai et al., 2000) (Figure 4 and figure 5). It begins with a thickening of the ectoderm, the dental lamina or dental epithelium, which will form a dental placode. The cells in the placode proliferate and form a bud, and consequently this developmental stage is denoted the bud stage. The cells in the bud continue proliferating and the developing tooth transforms into the cap stage. During the transition from bud to cap stage the enamel knot is formed on the border between the epithelium and mesenchyme. The enamel knot consists of a group of cells that do not proliferate, and coordinate the epithelial growth and tooth shape. During the cap stage, the dental mesenchyme becomes partly encapsulated by the epithelium. At its apical part the epithelium has formed cervical loops, and a dental papilla is formed inside. The condensed mesenchyme between the outer parts of the cervical loops will develop into the dental follicle, that eventually will generate cementoblasts, osteoblasts and

periodontal ligament. Cementoblasts will cover the root surfaces with cement, osteoblast will deposit alveolar bone matrix and periodontal ligaments will attach the root of the tooth to the alveolar bone. The epithelial part of the developing tooth during cap stage is called the enamel organ. The enamel organ consists of three different layers of cells. The core is the stellate reticulum, with star-shaped cells, surrounded by the inner enamel epithelium and the outer enamel epithelium (IEE and OEE). Further on, the tooth organ acquires the form of a bell and consequently, this stage is called the bell stage. New cell types appear and hard matrix starts to form. The cells in the dental papilla that appose the IEE differentiate into odontoblasts, which eventually deposit dentin matrix. The epithelial cells adjacent to the odontoblasts differentiate into ameloblasts and produce enamel matrix. Mineralization of the enamel and dentin matrix starts at the cusp tips and moves towards the base. Nerve fibers, although surrounding the developing tooth anlage in basket like-formations at much earlier stages, begin to enter the dental papilla at the late bell/early mineralizing phase (Fried et al., 2007).

At the base of the bell-shaped developing tooth, the IEE and OEE form a bilayer called the Hertwig's epithelial rooth sheet (HERS). This bilayer grows apically, and directs the growth of the root. HERS induce adjacent dental mesenchyme to differentiate into odontoblasts, to form dentin for the root. However, the HERS does not promote ameloblast differentiation, and thus no enamel is formed at the root. (Jernvall and Thesleff, 2000).

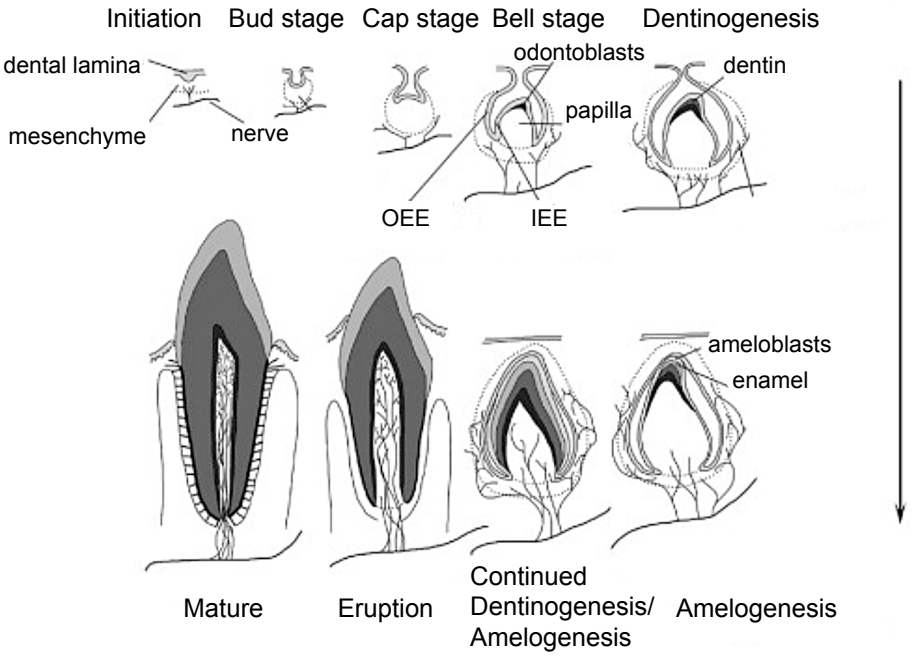

Figure 4. Schematic illustration of human tooth development.
Modified from Avery, 1992.

The entire process of tooth formation takes a long time in the human dentition. The initiation of tooth morphogenesis starts on the fifth week of gestation, and the first deciduous tooth

starts erupting at around age of 6 months. In the mouse dentition, teeth are initiated at embryonic day (E)12 and the incisor erupts at postnatal day 10-12, i.e. a rather fast process (Zegarelli, 1944).

As mentioned above, tooth development goes through the different stages by means of a reciprocal signaling interaction between the epithelium and mesenchyme. This interaction is governed by several signaling pathways: Bone morphogenetic protein (Bmp), Ectodysplasin (Eda), Fibroblast growth factor (Fgf), Hedgehog (Hh) and Wingless (Wnt) (Nieminen et al.,1998; Tummers and Thesleff, 2009; O'Connell et al., 2012).

Shirley Glasstone was one of the pioneers in tooth development research, showing that a tooth bud could be removed and grown *in vitro* to pass through the early developmental stages (Glasstone 1936; Glasstone 1967). These and other similar studies that followed showed that the tissue is predetermined or specified early to have the capacity to become a tooth (Lumsden, 1979).

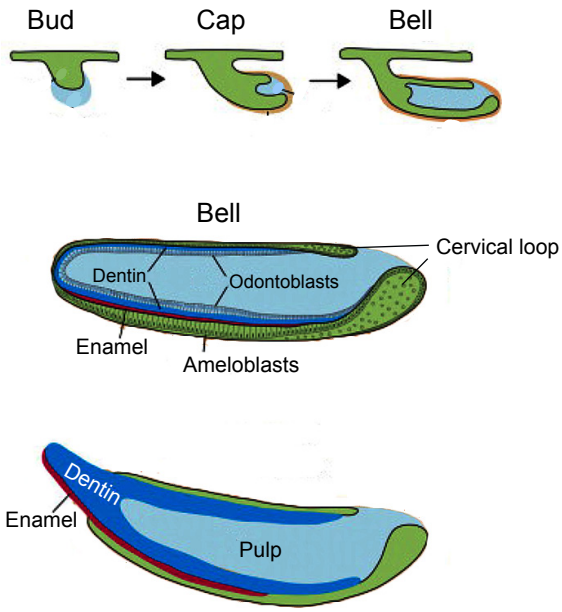

Figure 5. Schematic illustration of mouse incisor tooth development. Modified from Wang et al, 2004.

1.2.4 Tooth initiation and competence

So where lies the competence of the tissue to form a tooth? Pioneering studies showed that the first initial and inducing competence resides in the epithelium. In mouse, this is before E12, the stage when a placode is visible. After E12, the competence has switched to the

underlying mesenchyme. Thus, at this stage this mesenchyme can be combined with epithelium from other sites, e.g. the second branchial arch, and teeth would still be formed (Kollar and Baird, 1970a; Mina and Kollar, 1987). Hence, there are two pre-requisites, an epithelium and a neural crest-derived mesenchyme (Kollar and Baird, 1079a; Lumsden, 1988). A major signaling pathway for tooth initiation is the Wnt/beta catenin system. When this pathway is suppressed by Dkk1, no teeth are formed (Wang et al., 2009). Induction of the Wnt pathway in mutant embryos gives rise to supernumerary teeth, but only in the oral region because the competence to form teeth resides only there (Järvinen et al., 2006; Liu et al., 2008; Wang et al., 2009).

Surprisingly, it is not known how the dental lamina is established, and even more striking is the fact that there are no mutant embryos that are unable to form one. Thus, the initiating factor(s) remain to be identified. The Wnt/beta catenin pathway signaling induces Bmp4 expression in the mesenchyme, which in turn induces Sonic hedgehog (Shh) expression in the epithelium. The condensation of the underlying mesenchyme during the placode stage, is controlled by signaling with Fgf8 and Semaphorin 3 from the dental lamina.

When important mesenchymal transcription factors are depleted, tooth development is arrested at placode or bud stage (Bei, 2009b). Also, when mutant mice are stimulated with different signaling pathways, supernumerary teeth are formed in the diastema region. Such manipulations of signaling pathways and their downstream genes involve overexpression of the gene Ectodysplasin in K14-Eda mouse line, enhanced Fgf signaling in Sprouty gene mutants, enhanced Shh signaling in Polaris mutants and mutation of Sostdc1, which is a gene that modulates both Wnt and Bmp signaling pathways (Mustonen et al., 2003; Kassai et al., 2005; Klein et al., 2006; Ohazama et al., 2009; Ahn et al., 2010).

The gene p63 has been shown to be important for the initiation of ectodermal placode formation. When p63 is deleted, the mutant mice do not develop any type of ectodermal placodes. Nevertheless, there is a dental lamina formed, but the tooth development stops at this stage. The signaling pathways that are impaired are Bmp, Eda, Fgf and Notch (Laurikkala et al., 2006).

Another important signaling pathway in tooth formation is Eda (ectodysplasin). Impairment of genes in Eda pathways leads to defects in ectodermal appendages. It manifests itself as missing or imperfect teeth (humans and mice), or defects in hair and sweat glands (humans) (Pispa et al., 1999; Mikkola et al., 2009).

1.2.5 Odontoblast differentiation

The mesenchymal cells of the dental papilla can differentiate into odontoblasts. Cells from the mesenchyme adjacent to the IEE become aligned to the basement membrane of the IEE and differentiate into preodontoblasts. Preodontoblasts are cuboidal in shape and express Bmp4. When these cells are fully differentiated into odontoblasts, they are elongated and

rectangular, have a polarized nucleus and express Bmp2 (Figure 4 and figure 5) (Nakashima, 1994; Nakashima and Reddi, 2003; Yamashiro et al., 2003). If the Tgf-beta/Bmp signaling pathway is conditionally knocked-down (gene *Smad4*), the odontoblasts are prevented from terminal differentiation and are unable to deposit dentin (Li et al., 2011).

The secretory odontoblasts secrete proteins to form predentin, and during this process, they move towards the central/coronal part of the dental papilla. The odontoblast has a single process extending into the dentin, which is enclosed in a dentinal tubule. When tooth development is over, the odontoblasts remain as a pseudo-epithelium on the dentinal wall, and will continue to secrete secondary dentin, but at a much slower pace. This will cause the pulp chamber to become reduced in size with age. In addition, tertiary dentin can be formed by odontoblasts and odontoblast progenitor cells in the pulp that respond to tooth injury or other insults. This mechanism allows the pulp to protect itself from damage or bacterial metabolites. The dentin consists mainly of dentin sialophosphoprotein (dspp), dentin phosphoprotein (dpp) and collagen type I. Developmental defects in these genes may cause dentinogenesis imperfecta in humans (Shields et al., 1973).

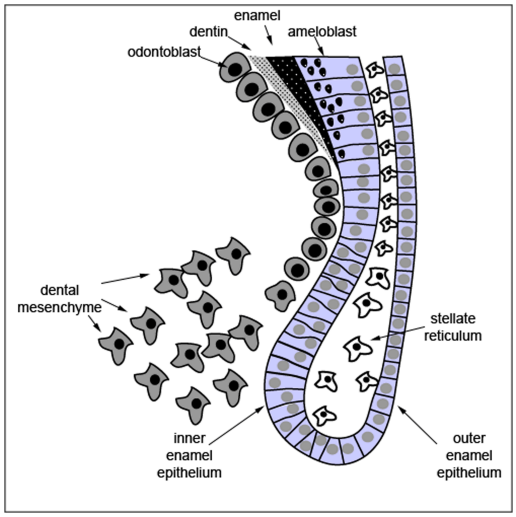

Figure 6. Illustration of mouse incisor labial cervical loop region. Stem cells for ameloblasts reside in the labial cervical loop. Modified from Thesleff, 2009.

1.2.6 Ameloblasts

Ameloblasts originate from the IEE. They begin to differentiate in response to signals from preodontoblasts , which are formed earlier. Ameloblasts, like, odontoblasts, are columnar cells with a polarized nucleus. They disappear after eruption of the human tooth, hence there is no possibility to renew enamel (Zeichner-David et al., 1995; Bei, 2009a). The Bmp signaling pathway is important for ameloblast differentiation. When the Bmp pathway

inhibitor Follistatin is introduced to the dental epithelium, no ameloblasts or enamel is formed (Wang et al., 2004). The canonical Wnt pathway influences the differentiation of ameloblasts. This is evident since when Wnt3a is overexpressed, there are no ameloblasts in the mouse incisor (Millar et al., 2003). Shh is present in the stellate reticulum cells and preameloblasts and is important for the differentiation and maturation of ameloblasts (Dassule et al., 2000; Gritli-Linde et al., 2002). SiRNAs also play a role in ameloblast development. When *Dicer-1* is conditionally knockdown, there are defects in ameloblast and consequently also defects in enamel formation (Cao et al., 2010; Michon et al., 2010).

1.3 MESENCHYMAL STEM CELLS

Initially, mesenchymal stem cells (MSCs) were identified as cells in the bone marrow that could differentiate down the osteogenic pathway when transplanted. In vitro culture of them showed that they could adhere to plastic and were clonal (Friedenstein et al., 1968, Friedenstein et al., 1970, Friedenstein et al., 1976). The phrase mesenchymal stem cells were proposed by Caplan, and he also proposed that these cells could differentiate down the mesodermal lineage (Caplan, 1991). Since then, extensive research around these cells in various tissues have been performed, and with different results and rather showing heterogeneity than homogeneity among MSCs. Meaning that MSCs seems to be tissue specific, and thereby confusing what the criteria for what a mesenchymal stem cell is. Therefore, the Mesenchymal and Tissue Stem Cell Committee of the ISCT (the International Society for Cellular Therapy) proposed a minimal of terminology and criteria for multipotent MSCs (Horwitz et al., 2005; Dominici et al., 2006). Adherence to plastic culture during *in vitro* culture, a panel of specific cell surface markers and differentiation potential down the adipogenic, chondrogenic and osteogenic pathways. The markers included CD105, CD73, CD90 (Thy1) and that the cells were devoid of hematopoietic cell markers (Dominici et al., 2006). However, these criteria are based on in vitro culture and not the actual situation in vivo. More studies are needed to define MSCs in the in vivo situation.

1.4 RODENT DENTAL STEM CELLS

The first adult stem cells associated with teeth were found in the dental epithelium (Harada et al., 1999). There are both epithelial and mesenchymal stem cells that contribute to the perpetual self-renewal of the mouse incisor.

1.4.1 Epithelial stem cells of the mouse incisor

Early label-retaining studies on mouse and rat incisors demonstrated that there was a rapid turnover of differentiated cells, indicating that there had to be a stem cell reservoir that was feeding the system with new cells. The mouse incisor tooth renews very fast, between 2-2.8 micrometers per day (Zegarelli, 1944). In 1980, it was shown with label-retaining techniques that stem cells were residing in the cervical loop of the rat incisor (Hwang and Tonna, 1965; Smith and Warshawsky, 1975; Smith, 1980). The stem cells for ameloblasts in the labial cervical loop (Figure 6), are transformed to transit amplifying cells, (TA cells), which then differentiate into enamel-producing ameloblasts (Thesleff and Tummers, 2009).

Label retaining methods have been developed and refined throughout the years. Most commonly, BrdU, 5-Bromo-2-deoxyuridine is used. BrdU can be injected into mice and incorporate itself into the DNA of the animal, and then the cells can be visualized with antibodies against BrdU. The cells that divide rarely, i.e. quiescent stem cells, will retain BrdU in a higher degree than cells that are dividing rapidly and the label is diluted. There is a consensus that stem cells can be quiescent for long time periods and that a cue can activate them. If cells have incorporated BrdU after a long chasing period, they are slowly dividing cells, and probably represent a stem cell niche. In the tooth system, BrdU slow cycling cells have been found in the labial cervical loop (Harada et al., 1999).

An alternative as a labeling retaining system is to use transgenic mice that have a tetracycline-sensitive histone H2B-GFP cassette under the control of an activator (Tumbar et al., 2004). In these animals, all cells in a tissue of interest have H2B-GFP activated and then the transgene is suppressed by exposure to doxycycline, leaving the label to be diluted over time if the cells are dividing. Label-retaining cells have been detected in the mouse incisor; OEE and stellate reticulum of the labial cervical loop with this technique (Seidel et al., 2010). Lineage tracing with transgenic mouse models has demonstrated that epithelial stem cells located to the cervical loop express the stem cell marker Sox2. Such Sox2-expressing cells give rise to all epithelial-derived lineages of the tooth (Juuri et al., 2012; Juuri et al, 2013).

As mentioned earlier, the signaling pathways that are involved in incisor development and homeostasis are Bmp, Fgf, Notch, Shh and Wnt. The Fgf signaling pathway has been especially interesting when it comes to epithelial stem cell maintenance and differentiation. Fgf10 has been shown to be important for stem cell maintenance during incisor development, while Fgf2b controls the regenerative capacity of the adult mouse incisor (Harada et al., 1999; Harada et al., 2002; Parsa et al., 2010). Also, when Sprouty genes, which are downstream targets of Fgf signaling, are lost, lingual cervical loop and side begin to produce ameloblasts and enamel while the labial side of the tooth system loose this ability (Klein et al., 2008). Down-regulation and loss of Fgf3 leads to hypoplastic labial cervical loops, while loss of epithelial Follistatin leads to up-regulation of Fgf3. However surprisingly enough, on the lingual side the cervical loop increases in proliferation and expansion (Wang et al., 2007). The loss of Alk5 receptor leads to the down-regulation of Fgf3, Fgf9 and Fgf10 and reduces

the size of the labial cervical loop (Zhao et al., 2011). These findings indicate that Fgf signaling is important in maintaining the quantity of epithelial stem cells.

Another very important signaling pathway for the development and survival of epithelial stem cells is Notch. Notch1 and Notch 2 are expressed in the epithelium and mesenchyme while Notch 3 is expressed in the mesenchyme only (Harada et al., 1999). *In vitro* inhibition of Notch signaling leads to reduction of the labial cervical loop (Felszeghy et al., 2010), while a deletion of *Jag*2, which is upstream from notch ligands, causes defects in the cellular morphology of the incisors (Mitsiadis et al., 2010).

Concerning the Wnt signaling pathway, this canonical pathway is not activated during renewal of the incisor tooth, unlike in organs such as skin, intestine and cornea (Huelsken et al., 2001; He et al., 2004; McGowan et al., 2007; Suomalainen and Thesleff, 2010).

Yet another key protein is encoded by Shh, which is expressed by differentiating progeny and signals back to the epithelial stem cells to generate more progeny. When this hedgehog pathway is inhibited, stem cells cease to produce more progeny and there is an arrest in ameloblast production (Seidel et al., 2010). This is not limited to only the epithelial stem cells and ameloblast production, but also holds true for dental MSCs, the compartment that odontoblasts are produced from.

MicroRNAs (miRNAs) are also involved in tooth development and renewal. When DICER1, an enzyme that process miRNAs, is deleted in the epithelial compartment, no enamel is formed and there is a change in incisor form and patterning (Cao et al., 2010).

1.4.2 Mesenchymal stem cells of the mouse incisor

Mesenchymal stem cell populations that reside within the tooth are an easily accessible cell source (in comparison to bone marrow) for potential therapeutic purposes. The mouse incisor provides an excellent model to study MSCs which are enganged in both renewal and repair of the tooth. Genetic lineage tracing has enabled us to examine MSCs in ways that are not possible in humans. In this way we have retrieved information not only on how a tooth grows and repairs itself, but also in general how MSCs behave.

It is problematic to identify MSCs *in vivo* since there are no defined markers. Many of the markers used for MSCs are also present in perivascular cells. One method that has helped greatly is genetic tracing with transgenic mouse lines. Here the target cell and its progeny are permanently labeled.

It is known that stem cells often but not always are in a quiescent state until mobilized. Their immediate progeny, the transit amplifying cells are on the contrary dividing and proliferating. In the mouse incisor, the slowly cycling cells have been found at the apical end of the cervical loop, and the rapidly cycling cells adjacent to the cervical loop (Seidel et al., 2010;

Lapthanasupkul et al., 2012). Thus, this method has helped to pinpoint the location of the dental mesenchymal stem cell niche.

The transit amplifying cells express the polycomb repressor complex (PRC1) group of proteins, amongst which Ring1a and Ring1b are included. Previous studies have shown that these proteins are important for maintaining embryonic stem cells in an undifferentiated state (Endoh et al., 2008; van der Stoop et al., 2008). When these proteins are conditionally knocked-down during adulthood,, the incisor stops growing and the ameloblasts and odontoblasts are compromised (Lapthanasupkul et al., 2012). The can be explained by the fact that there is a loss of Fgf3 and Fgf10 in the pulp mesenchyme, which leads to loss of transit amplifying cells for odontoblasts and reduced Fgf signaling to the epithelial stem cell niche in the cervical loop. This will then lead to failed ameloblast development (Harada et al., 2002; Lapthanasupkul et al., 2012).

Numerous studies show that MSCs are activated to migrate to the site of tissue damage (Caplan and Correa, 2011). In injured teeth, pulpal cells in the transit amplifying cell zone migrate towards the damage, but the stem cells in the slow cycling area do not (Feng et al., 2011).

Pericytes surround capillaries and function as support cells. The tooth pulp is highly vascularized and thus harbors many pericytes. Pericytes express several markers in common with MSCs, making it difficult to distinguish them from each other. This hurdle can be removed with genetic lineage tracing, using the pericyte marker NG2. This has been done, and the results showed that a only small amount of traced cells contributed to the formation of odontoblasts in the adult incisor. However, in the case of a tooth injury, more pericytes (up to 15%) were involved, proliferated and differentiated into odontoblasts. Still, this shows that there has to be another dental mesenchymal stem cell source in addition to pericytes (Feng et al., 2011).

A quite recent study, showed with Gli^{+} lineage tracing that cells surrounding the arterioles (neurovascular bundle) in the apical region of the mouse incisor are a stem cell niche that give rise to pulp mesenchyme (Zhao et al., 2014).

Thus, it seems as the homeostasis of the ever-growing tooth is controlled by several stem cell niches.

1.5 HUMAN DENTAL MESENCHYMAL STEM CELLS

Stem/progenitor cells are present in both deciduous and permanent tooth pulps. Gronthos and collaborators were first to isolate stem cells in the pulp of permanent third molars. These cells were transplanted into immune-compromised teeth, where they differentiated and produced dentin (Gronthos et al., 2000). They termed these cells (that came from permanent teeth)

Dental Pulp Stem Cells (DPSCs). Subsequently, pulpal stem cells from human exfoliated deciduous teeth (SHED), were isolated (Miura et al, 2003).

1.5.1 Dental Pulp Stem Cells

Previous studies have shown that DPSCs have characteristics similar to bone marrow mesenchymal stem cells (BMMSCs). They share the ability to adhere to plastic culture surfaces and to form clonogenic cultures (which indicates a potential to self renew). When using the clonogenic assay on plastic culture dishes, only twenty percent of the seeded cells are clonogenic. Flow cytometry sorting for STRO-1, 3G5 and CD146 in dissociated pulp cells and culture of these cells, has resulted in clonogenic colonies (Gronthos et al., 2002; Shi and Gronthos, 2003). These markers are typical for smooth muscle cells and pericytes, and these DPSCs have been found to be associated with blood vessels. They also share some bone marrow stem cell-associated markers such as CD44, CD105, CD146 and STRO-1 (Huang et al., 2009).

The difference between BMMSCs and DPSCs is that DSPCs can differentiate into odontoblast-like cells and can help form pulp-dentin complex when transplanted *in vivo* (Gronthos et al. 2000; Huang et al., 2006). One study on dogs has shown that DPSCs can be used to regenerate the pulp after a pulpectomy (Iohara et al., 2011).

DPSCs are multipotent and have the capacity to differentiate and become myocytes, chondroblasts/chondrocytes and osteoblasts/osteocytes. Another potential of DPSCs, although debated, is their ability differentiate into neuronal-like cells, and be useful for axonal guidance (Arthur et al., 2008; Arthur et al., 2009).

If cells are isolated from inflamed pulp, they are termed DPSCs-IP. These cells share many characteristics with cells from normal pulps and express markers such as CD73 and CD146. They can differentiate and deposite dentin, but their potential for this use is reduced, probably due to the inflammation (Alongi et al., 2010).

1.5.2 Stem Cells from Human Exfoliated Deciduous Teeth

SHED is known to be different from DPSCs. First and foremost, they are unable to form a complete pulp-dentin complex when transplanted *in vivo* and instead recruit osteoblasts into the site with new bone formation as a result. Another feature which differs, is that they can be cultivated not only on plastic with adherence, but also as neurospheres, much like neural stem cells (Miura et al., 2003).

SHED have multipotent capacity to differentiate down adipogenic, odontogenic and neurogenic pathways, can also repair critical sized calvarial defects and help recover locomotor function after spinal cord injury (Seo et al., 2004; Sakai et al., 2012).

Actually, several types of stem cells have been isolated from deciduous teeth. Among these, one type is the immature dental pulp stem cells (IDPSCs), that are CD34 negative and express typical embryonic stem cell markers such as OCT4, NANOG, SSEA3, SSEA4, TRA-1-60 and TRA-1-81. These cells have been able to follow the differentiation paths of smooth muscle, neurons, cartilage and bone (Kerkis et al., 2006).

1.5.3 Stem cells and aging of the pulp

Senescence and aging are associated with the loss of self-renewing capacity of stem cells. This principle is valid for multiple locations in the body including the nervous system, connective tissue and bone marrow, and plays a significant role in the regenerative potential of stem cells (Enwere et al., 2004; Sharpless and DePinho, 2007; Choumerianou et al., 2010). There are several elements that contribute to the aging of stem cells. These are changes in the systemic environment of factors from the blood or the niche being altered, or/and intrinsic factors within the stem cell such as protein accumulation, damage to mitochondrial as well as nuclear DNA, telomere attritition and cell cycle inhibition that eventually leads to failure to function and aging (Jung and Brack, 2014). Identification of potent tissue-specific stem cells and their banking is, for obvious reasons, crucial for regenerative medicine and teeth host pulpal mesenchymal stem/progenitor cells (Gronthos et al., 2002; Yang et al., 2010; Ma et al., 2012; Smith et al., 2012). These cells have been proven in animal models to be useful in spinal cord injury regeneration and other medically relevant procedures (Sakai et al., 2012; Yang et al., 2010; Ma et al., 2012). Previous work on genetic profiles of dental pulp cells has yielded several important clues. Comparisons of gene expression between fast growing and slow growing cell populations showed robust expression of transcription factors with critical roles in cell growth and survival, in dental pulp cells, just as they are in the periodontal ligament and bone marrow (Tete et al., 2008). Deciduous and permanent teeth represent an excellent model for studies of molecular differences between mesenchymal cell populations, especially in relation to their age.

1.6 SCHWANN CELL PRECURSORS AND SCHWANN CELLS

Schwann cell precursors (SCPs) are derived from the neural crest. During development/embryogenesis, the migrating neural crest cells come from the dorsal part of the folding neural plate. These neural crest cells give rise to an array of different type of cells (Le Douarin et al., 2004) and among them are the SCPs cells that eventually mature into Schwann cells (Figure 7).

Schwann cells protect and support the neurons and build myelin sheaths to enable fast propagation of action potentials in the peripheral nervous system. During adulthood, several

types of Schwann cells exist, such as myelinating and non-myelinating Schwann cells, terminal Schwann cells and satellite glial cells (Kaucká and Adameyko, 2014).

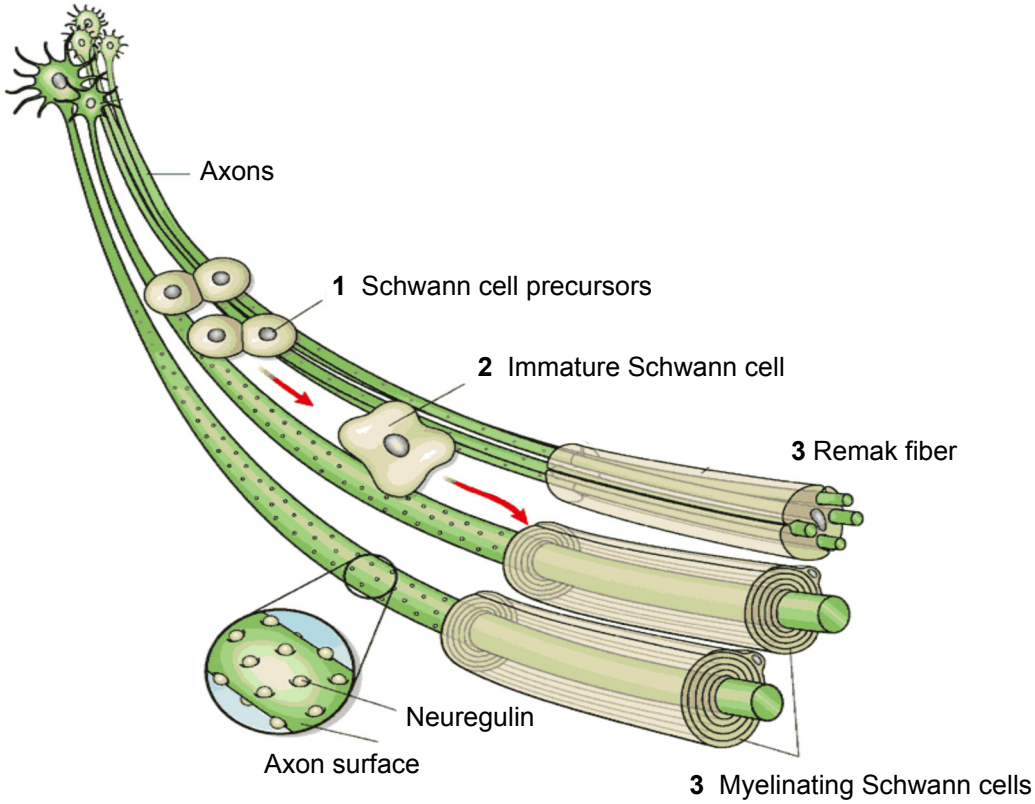

Figure 7. Stages of Schwann cell differentiation.

Schematic illustration on different stages of Schwann cell differentiation. Stage 1) Schwann cell precursors 2) Immature Schwann cells 3) Myelinating Schwann cells or Remak fiber (unmyelinating Schwann cell). Modified from Nave and Schwab, 2005.

During embryogenesis the early glia of the peripheral nerves are called SCPs. These cells eventually differentiate into the different types of Schwann cell mentioned above. However, it has been revealed that SCPs also give rise to other types of cells. Genetic lineage tracing has demonstrated that SCPs are the origin of melanocytes, endoneural fibroblasts and parasympathetic neurons during embryogenesis (Joseph et al., 2004; Adameyko and Lallemend et al., 2009; Adameyko et al., 2012; Dyachuk and Furlan et al., 2014).

The SCPs survive and maintain their glial phenotype through binding of neuregulin 1, which is secreted from the axon (Figure 7). The differentiation of SCPs into immature Schwann cells is controlled by Fgf2 and Notch signaling. Nevertheless, mature non- and myelinating Schwann cells are still considered as reversible states.

The differentiation of neural crest cells into SCPs is not fully understood but there are some characteristics. First, SCPs are dependent on the axon to remain in their state. Secondly, neural crest cells can migrate for long distances, while the SCPs seem to be locally positioned in nerves. The common feature, though, is that SCPs also express the transcription factor Sox10 (Kubu et al., 2002; Riethmacher et al., 1997). The $Sox10^{+}$ cranial neural crest cells cease to exist around E10 in the mouse. Therafter, at E11.5, the only neural crest-derived cells that are $Sox10^{+}$ are SCPs, Schwann cells along the peripheral nerves and melanoblasts (Dyachuk and Furlan et al., 2014). The SCPs mature into Schwann cells through endothelin and Notch signaling (Brennan et al., 2000; Wakamatsu et a., 2000). During development, Schwann cells support axonal growth by secreting nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor and neurotrophin-3 (NT3). The role of Schwann cells is multifaceted. Some, non-myelinating Schwann cells, segregate the axons into bundles, and form Remak fibers (Figure 7), with retained proliferation capability (Kidd et al, 2013). In prospective myelinating axons, transcriptional factors such as Krox20, Sox10 and Oct6 promote myelination and a subsequent mature differentiated state. The importance of Krox-20 has been shown in knockout mice where the Schwann cells fail to myelinate (Topilko et al, 1994). Upon nerve injury, factors like c-Jun, Notch, Sox2, Krox24 and Pax3 cause Schwann cells to de-differentiate into an immature state in order to assist in nerve regeneration (Jessen and Mirsky, 2005, Jessen and Mirsky 2008). In addition, Schwann cells of non-myelinating peripheral nerves of the bone marrow support and maintain the hibernating hematopoeitic stem cell niche (Yamazaki et. al., 2011). Throughout life, both myelinating and non-myelinating Schwann cells can reverse and dedifferentiate into a state where they proliferate, secrete growth factors and help regenerate axons upon nerve injury.

2 AIMS OF THE THESIS

General aim

The overall aim of the work in this thesis was to study progenitor/mesenchymal stem cells in the tooth pulp.

Specific aims in each paper

Study I

To investigate the role of Schwann cell precursors and Schwann cells as putative dental mesenchymal stem cells.

Study II

To compare stromal cell populations of human deciduous and permanent teeth, and map potential molecular differences of importance for stemness, proliferation, differentiation and signaling.

3 RESULTS AND DISCUSSION

3.1 PAPER I: GLIAL CONTRIBUTION TO DENTAL MESENCHYMAL STEM CELLS AND ODONTOBLASTS

Proteolipid protein 1 (PLP1) and sex-determining region Y-box 10 (Sox10) are expressed in cranial neural crest during embryogenesis. However, after migration around embryonic days (E)9–10, they are retained only in neural crest-derived SCPs (which eventually will mature into Schwann cells), but not in cranial mesenchyme (Adameyko et al., 2012).

Lineage tracing experiments with PLP-CreERT2 and Sox10-CreERT2 mice where recombination was induced at E12.5 and/or later showed that SCPs were present in peripheral nerves in close proximity to the tooth placode during development. At later stages of tooth development, around early and late bell stage, we found that SCP-derived YFP^{+} progeny formed streams of cells in the apical region of the developing tooth, consisting of dental mesenchymal stem cells, pulp cells and odontoblasts.

The fact that SCPs contribute to the pulpal cell populations represents an entirely novel concept; classical oral developmental biology has taught for decades that dental mesenchyme and odontoblasts are derived directly from migrating cranial neural crest cells.

We aimed to examine in detail the mechanism behind this process, and therefore used confetti strains crossed to PLP-CreERT2 mice. This enabled us to monitor individual recombined SCPs displaying different colors, and specifically to study the fate of the progeny from each individual cell. It was found that the structure of the streams of cells from each SCP was highly clonal.

Having established that SCPs contribute to pulpal cells, we wanted to examine the ectomesenchymal contribution. If expression is induced in PLP-driven and Sox10-driven CreERT2 at E8.5, all neural crest cells and their progeny are traced. We performed such experiments, using the PLP-CreERT2/ R26RConfetti strain, before segregation of CreERT2 expression into glial lineage. to examine the general contribution of neural crest cells to tooth formation. We found that migrating neural crest cells formed both dental MSCs and odontoblasts in the same patterns as SCPs.

To ensure the Schwann cell identity of the PLP- or Sox10-traced nerve-associated cells and exclude the possibility that they were a pool of cells unrelated to nerves several analyses were performed in the embryo.

First it was confirmed that Schwann cell precursors were present even before tooth placode formation through immunostaining with a BFABP marker at E11.5. Furthermore, all Sox10-positive cells in close vicinity to the cervical loop of the developing tooth were associated to peripheral nerves, and PLP-driven CreERT2 was expressed in a subpopulation of nerve-associated Sox10 positive cells.

In the adult, as expected, all traced nerve-adjacent Sox10+ cells expressed Schwann cell-specific markers such as GFAP, p-c-Jun and S100. In addition, P0, MBP and Krox20, markers that are characteristics of myelinating Schwann cells, were expressed in several, but not all traced cells. We wanted to address whether these Sox10- positive Schwann cells could be a potential stem cell source for the continuously growing mouse incisor. Indeed, with PLP-

CreERT2 and Sox10-CreERT2 *in vivo* genetic tracing it was discovered that the $Sox10^+$ Schwann cells in the peripheral nerves, in close vicinity of the apical part of the incisor, were the origin of dental MSCs, pulp cells and odontoblasts. Individual Schwann cell progenies formed streams and occupied highly organized areas in the developing pulp. Again using the PLP-CreERT2/ R26RConfetti strain, it was found that these streams were connected to the odontoblast layer. The progeny of different Schwann cells which displayed different fluorescent labels formed cohorts of odontoblasts in the odontoblast layer which intermingled with each other at borders. The results indicate that different clones compete for the opportunity to generate odontoblasts. The contribution to the odontoblast layer from individual clones appeared to vary, ranging from none to about 57%. Thus, the vast majority of the progenies became pulp cells. The probability for a clone to breed odontoblasts seemed to depend on the proximity of its cell stream to the cervical loop. The closer to the cervical loop the clone was positioned , the larger its contribution was to the odontoblast layer.

It was further asked how adult dental MSCs generation would be affected if the dental nerve with its resident Schwann cells was interrupted. We thus sectioned the inferior alveolar nerve, which supplies the mandibular incisor, in genetically labeled mice. The results showed that there were almost no Schwann cell-derived dental MSCs in the denervated teeth, while the contralateral side control teeth contained abundant YFP^+ odontoblasts and cells in the pulps. Thus, generation of SCP-derived progeny of dental MSCs and odontoblasts was impaired after denervation.

This led us to the question of the size of the Schwann cell contribution to the pulp cells and odontoblasts . Therefore, counting of the number of PLP^+ Schwann cell-derived YFP positive odontoblasts in tooth sections was performed. The results showed that the amount varied between 8.23 - 47.28%, depending on the number of tamoxifen injections. When measured with Flow-Activated Cell Sorting (FACS), the amount of traced YFP positive cells in the mesenchymal compartment of the tooth was 10.3%.

Thus, since less than half of the pulpal cells have a Schwann cell origin, other sources of dental MSCs that supply the pulp with stromal cells and odontoblasts must exist. Other studies have shown that pericytes is a dental MSC source, from which cells can be recruited after injuries. Hypothetically the pericyte could be an intermediate form that is derived from SCP-derived dental MSCs . However, our immunohistochemistry (IHC) stainings demonstrated that YFP^+ Schwann cells and their progeny were never positive for $NG2^+$, an established pericyte marker . This rules out the possibility that pulpal pericytes have differentiated from Schwann cells.

To further understand the characteristics of the pulpal SCP-derived traced mesenchymal cells, we used FACS to analyze the expression of established MSC markers such as CD44, CD45, CD146, and CD90 (Thy1). These markers were also checked with antibodies or in situ hybridization in the adult incisor and we discovered with both FACS and IHC that only Thy1 (CD90) was expressed in the mesenchymal cells in the apical incisor region. This was not wholly surprising, since the panel of antibodies used for identifying MSCs is based on in

vitro studies. with only few in vivo observations available where these markers are expressed. This calls for caution when attempts are made to translate in vitro findings to in vivo situations in studies of MSCs, and merits further studies in order to find reliable markers. However, one of the commonly used MSC indicators, Thy1 was expressed in the dental stem cell niche as shown by both FACS, in situ hybridization and immunohistochemistry. Flow cytometry analysis sorted out a population of 1.5% Thy1 positive cells in the tooth. Of these, 0.4% were proliferative, as determined by their $Ki67^+$ labeling, and YFP^+. Consequently, to demonstrate that the $Thy1^+/YFP^+/Ki67^+$ positive cells might be dental MSCs we did genetic tracing in Thy1-Cre/R26YFP animals. A robust amount of traced Thy1 traced cells both in the odontoblast layer and in the pulp was found. Additionally, we analyzed Thy1-Cre/R26Confetti animals and found that the Thy1 progeny was clonal (confetti initially labels individual cells with different colors, so the progeny from each cell could be easily tracked). The existence of Thy1 expression in slowly cycling cells (EdU retaining) in the apical tooth mesenchyme was confirmed with FACS and IHC.

MSCs are known to respond and migrate upon injury. When we inflicted damage to the incisor tooth, we found that many YFP^+ traced dental MSCs were recruited to the injury site. These cells differentiated into odontoblasts and produced mineralized matrix. To ensure that the traced dental MSCs had the ability to differentiate into odontoblasts and secrete hard-forming matrix, we performed *in vitro* culture. Thus, we expanded the cells, FACS-sorted the YFP^+ cells and cultured them under osteogenic conditions. Alizarin R staining confirmed that these cells could deposite mineralized matrix, similar to differentiated odontoblasts.

Our observations demonstrate that Schwann cells constitute a continuous source of dental mesenchymal stem cells. Is this unique for the tooth, or might Schwann cells from any PNS nerve contribute to MSCs in a range of different tissues? This is in fact indicated by our recent findings that during embryogenesis, a proportion of parasympathetic neurons originate from SCPs (Dyachuk, Furlan et al., 2014). Further studies in embryonic, adult as well as injured tissue are obviously needed in this field.

3.2 PAPER II: EXPRESSION PATTERNS OF HUMAN DENTAL PULP STROMAL CELLS WITH FOCUS ON STEM CELL DYNAMICS

In this paper, using microarray technology, we found that gene expression profiles were different between deciduous and permanent pulp cells. In total there were 70 genes that were differently expressed, notably genes associated with proliferation, extracellular matrix, differentiation and ageing. The expression of the gene HMGA2 (HMGIC) was especially strong in deciduous pulp cells. This is a gene that previously was known to be expressed in neural stem cells during embryogenesis. Furthermore, to confirm the microarray analysis, RT q-PCR and Western blot experiments were performed on a subset of genes, which corroborated and substantiated prior results. The ingenuity pathway analysis revealed that there were several stemness-, tumorogenic- and cell regulatory genes associated with HMGA2. To further investigate the role of HMGA2 in the tooth pulp we conducted siRNA–mediated loss-of-function experiments that revealed a down-regulation of the stem cell marker NANOG, indicating that HMGA2 is involved in stem/progenitor maintenance.

Additionally, we wanted to analyze the HMGA2 expression in deciduous pulp cells in comparison to BMMSCs and human embryonic stem cells (HES). Deciduous pulp cells had significantly higher HMGA2 expression than BMMSCs, although expression in HES, as expected, was much stronger. Stem cell markers such as NANOG and OCT4 were similarly expressed by deciduous pulp cells and BMMSCs, but again, at much lower levels than in HES.

Thus, deciduous pulp cells harbor a stromal cell population that expresses the stem/progenitor cell marker HMGA2. According to immunohistochemistry stainings it seems as though this is a population scattered throughout the connective tissue.

Since not all cells in the deciduous pulp express HMGA2, but only a subpopulation of 16.28%, expression of HMGA2 cannot be a regarded as a general feature of cells in immature or young tissues. However, it could be a feature of immature rather than mature cells. This issue was addressed by collecting sections of embryonic and adult gingiva and perform immunohistochemistry. HMGA2 was abundantly expressed with 24.76±9.57% both in epithelial gingiva and underlying mesenchyme of the embryo, while the adult gingiva and stromal tissue was devoid of HMGA2 expression. Thus, this indicates that young somatic tissues, both epithelial and mesenchymal, express HMGA2 while expression is lacking in adult tissues. Previous studies have shown that there is a stem/progenitor population in deciduous teeth that is associated with blood vessels. However, our data clearly demonstrated that HMGA2-positive cells were not perivascular cells. These results indicate that the tooth pulp harbors another proliferative non-vascular cell population that expresses HMGA2 and displays at least some stem/progenitor characteristics.

4 CONCLUSIONS AND FUTURE PERSPECTIVES

- Schwann cell precursors residing in sensory nerves are the origin of dental mesenchymal stem cells, dental papilla cells and hard-matrix producing odontoblasts of the developing tooth during embryogenesis.

- Spatial domains occupied by dental papilla cells and odontoblasts in the developing tooth are populated by single clones originating from individual SCPs.

- Ectomesenchymal neural crest-derived dental MSCs give rise to the same fates, and form similar spatial patterns of progeny in the pulp and corresponding domains of odontoblasts as SCP-derived MSCs do during tooth development.

- In adulthood, Schwann cells contribute to the self-renewal process of the continuously growing mouse incisor by providing dental MSCs, pulp cells and odontoblasts. Thus, Schwann cells are multi-potent stem cells with a niche in the dental peripheral nerves.

- The progeny of individual Schwann cells form separate streams and occupy different domains in the odontoblast layer of the adult tooth with some overlapping border regions.

- Schwann cell-derived dental MSCs are recruited after *in vivo* tooth injury to form odontoblasts that produce mineralized matrix for tissue repair. They also have the capacity to differentiate into odontoblasts and deposite hard dentin/bone-like matrix *in vitro*.

- Damage to the inferior alveolar nerve impedes the generation of Schwann cells-derived dental MSCs and odontoblasts in the adult mandibular incisor.

- A proportion of Schwann cell-derived dental MSCs expresses the classical mesenchymal stem cell marker Thy1. When traced, this population of cells contribute to dental MSCs and odontoblasts according to the same same patterns as observed after PLP and Sox10-driven CreERT2 lineage tracing.

- The amount of Schwann cell-derived progeny constituted up to less than half of the total number of cells in the pulp and odontoblast layer. This demonstrates that additional sources of dental MSCs to these cell populations must exist..

- In general, human deciduous pulp cells express more proliferative, progenitor- and cell cycle-related genes than permanent teeth.

- Deciduous teeth harbor a stromal cell population that expresses the neural stem cell marker HMGA2.

- HMGA2 siRNA knock-down in cultured pulp cells leads to a down-regulation of the stem cell marker NANOG, which suggests that HMGA2 is involved in stem cell maintenance.

The discovery of a glial origin of odontoblasts in the tooth raises new questions about how this process is controlled *in vivo*. Further work will aim to resolve intrinsic mechanisms (transcriptional factors network) together with extrinsic signaling pathways (e.g. soluble ligands and their receptors) underlying the transition from peripheral glial cells to odontoblasts. The discovery of the stem cell marker HMGA2 in primary but not permanent human tooth pulps indicates that this gene could constitute a vital part of an important signaling system in pulpal cell differentiation, an issue which clearly needs further attention. The medical implication of the present studies includes a better understanding of tooth regeneration, and provide new approaches to dentin recovery and tooth restoration following trauma. Technical advancements and novel data gained through these studies might be helpful for attempts to artificially grow teeth for natural implants in human jaws. Further advances in the knowledge on the connection between peripheral glial cells and odontoblasts as well as the understanding of the signaling pathways involved in their cell fate transitions will also yield better knowledge of the origin and development of odontoma tumors. Moreover, novel information about genes selectively expressed in odontoblasts and their precursors will provide benefits to the odontology research community in general.

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