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Potential Stem Cell Therapy of Radiation Induced Salivary Gland Dysfunction (A Review)

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ABSTRACT

Salivary glands play an important role in the maintenance of healthy oral cavity through the production of saliva. Hyposalivation, frequently experienced in certain patients specially those receiving radiation therapy for head and neck cancers. Radiotherapy treatment often results in unavoidable co-irradiation of normal tissues surrounding the tumor, such as the salivary glands . The ability to speak, swallow, masticate, taste food, and maintain a healthy oral cavity is heavily reliant on the presence of saliva. Reduction in saliva results in many symptoms whose combined effect can drastically reduce quality of life. Although artificial lubricants and drugs are available to ameliorate the consequences of hyposalivation, their effects are at best transient. Such management techniques do not address the source of the problem resulting from radiation-induced stem cell sterilization. Therefore, recent reseach work paid attention to stem cell replacement therapy to treat radiation-induced hyposalivation. Substantial progress has been made lately in the understanding of cell turnover in the salivary glands and the recent identification of stem and progenitor cell populations provides a basis for studies toward development of a stem cell-based therapy for xerostomia. Knowledge on the embryonic development, homeostasis and regeneration after atrophy of the salivary glands has provided important knowledge on the location of the salivary gland as well as on the factors that influence proliferation and differentiation. This knowledge has helped to locate, isolate and characterize the salivary gland stem cell in an attempt to be mobilized and transplanted in salivary glands. Hence, this review aims to survey the current state of knowledge of salivary gland stem cells and their potential in prevention or treatment of radiation induced salivary gland damage and hyposalivation in patients .

Key words: Hyposalivation, Radiotherapy, Regeneration,Stem/progenitor cells, Stem cell therapy.

Introduction

Xerostomia, the subjective feeling of dry mouth, contributes for a more than 50% reduction in salivary flow. In old age people and especially in patients using drugs, suffering from Sjogren's syndrome and those being treated for head and neck cancer with radiotherapy, prevalence of hyposalivation is particularly high. In addition,a number of other systemic conditions including uncontrolled diabetes mellitus, sarcoidosis and renal diseases can cause hyposalivation related xerostomia (Napenas *et al*., 2009). Induced by radiation, salivary glands dysfunction and the resultant hyposalivation causes many post-treatment complications, including hampered speech, dental problems, difficulties with swallowing and food mastication, impaired taste, and nocturnal oral discomfort. Although protocols have been developed to minimize early and late loss of gland function following radiotherapy, 40% of head and neck cancer patients will still experience moderate or severe xerostomia (Burlage *et al*.,2001;Vergeer *et al.,*2009). Considering the severe impact xerostomia may have on patients quality of life, there is an unmet clinical need for an efficient treatment (Vissink *et al.,* 2003a,b). Stem cell therapy could provide an option to prevent and repair damage of tissues induced by degenerative processes due to auto-immune responses, radiation-side effects or other cytotoxic events. Basically, three stem cell types are currently being investigated for their potential use in stem cell therapy: embryonic stem cells, induced pluripotent stem cells and adult stem cells. Induced pluripotent stem cells which closely resemble embryonic stem cells may evolve to be useful in the near future but first the control of cell differentiation and development into salivary glands specific lineages needs to be assessed to prevent the formation of teratomas. Thus, this review will cast light on the recent progress of research work for the characterization of rodent and human adult salivary gland stem cells, and advances in design of an adult stem cell-based therapy for long-term treatment of salivary gland dysfunction and hyposalivation induced by radiotherapy in patients.

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Development and embryogenesis of the salivary glands, a key for regenerative therapies.

The parotid gland and the submandibular / sublingual gland develop from different embryonic origins. The parotid gland is derived from the ectoderm, whereas the submandibular rand and sublingual glands are derived from endoderm. From the stem cell point of view this could make them unique and potentially not interchangeable. However, all types of salivary gland develop in a similar pattern of morphogenesis driven by cytokines, growth factors and extra cellular matrix components. Transplantation of submandibular gland stem cells in parotid glands and vise versa could shed light on the interchange ability of the stem cells. For the clinical application of stem cells this may be very important, since then after the dissection of the submandibular gland during a neck dissection procedure, its stem cells could be used for therapy of the parotid gland. What is clear, however, is that both the parotid and submandibular / sublingual glands are enclosed within a well defined capsule of neural crest derived mesenchyme and that interaction with the surrounding mesenchyme is essential for the initial budding of the salivary gland (Jaskoll *et al.,* 2002).

The role of growth factors in the development of salivary glands

Fibroblast growth factor (FGF) signalling is of major importance during development of the salivary gland (Patel *et al.,* 2006). Secretion of members of the FGF family by the mesenchyme, for example FGF8 and FGF10, appears to control the branching of the developing salivary gland but not the initiation process. In parallel the epithelial cells of the salivary gland express the FGF2b receptor at this stage (Jaskoll *et al.,* 2005).A whole range of growth factors, cytokines, extracellular matrix proteins and anti-apoptotic proteins are involved in the development of the salivary glands. When provided at the appropriate time, FGF7, FGF8 and tumour necrosis factor-alpha (TNFa) increase budding while FGF10, epidermal growth factor (EGF) transforming growth factor-b (TGF-b), TNFa, bone morphogenetic protein 7, ectodysplasin and sonic hedgehog homolog expression induced branching (Tucker, 2007). Many of these factors have been shown to play a role in regeneration as well. The specificity of salivary gland mesenchyme is of eminent importance (Kratochwil, 1969; Kusakabe *et al.,* 1985). However, mesenchyme of submandibular and parotid gland seems to be interchangeable (Ball, 1974) even between species (Nogawa and Mizuno, 1981), meanwhile maintaining the characteristics of the epithelial cells of origin (Tyler and Koch, 1977). Epithelium and mesenchyme are separated by the basement membrane of which components such as glycosaminoglycans, collagens, fibronectins, integrins, laminin-c1 and nidogen-1 play a crucial role in branching morphogenesis (Ekblom *et al.,* 1994; Kadoya *et al.,* 1997; Kadoya and Yamashina, 2005; Patel *et al.,* 2006, 2007; Ho *et al.,* 2008). It appears that once cytodifferentiation has started, the presence of mesenchyme is no longer required (Cutler, 1980). As a result it has been shown in adult mice that specificity of mesenchymal cells is less important since even bone marrow derived mesenchymal cells were able to stimulate regeneration (Lombaert *et al.,* 2006). For future development of regenerative therapies, knowledge of the development of the salivary glands, their stem / progenitor cells and the interactions with the environment in cell fate decision is of eminent importance (Lombaert and Hoffman, 2010).

What are the adult stem cells of salivary glands ?

Adult stem cells are generally organ restricted and only form cell lineages of the organ from which they originate (unipotent) and therefore do not form teratomas. Like any other adult stem cell population, salivary gland adult stem cells are undifferentiated but reside between differentiated cells (stem cells niche). Adult stem cells are able to self-renew and can differentiate to yield all specialized cell types. Formation, maintenance and repair of the tissue in which they reside are the primary roles of the adult stem cell. Regretfully, adult stem cells are not easy to find, but by using well-established specific stem cell characteristics it is possible to distinguish them from the other cells in the tissue. Adult stem cells are closely related to or remnants from the embryonic development. Interestingly, during adult recovery from injury cells with an embryonic like phenotype have been associated with the regeneration process (Cotroneo *et al.,* 2008, 2010; Carpenter and Cotroneo, 2010). Additionally, like during embryonic development (Cutler and Chaudhry,1973) the interaction between mesenchymal and epithelial cells also seems important for the regeneration of the radiation injured salivary gland tissue (Lombaert *et al.,* 2006).

The role of stem/progenitor cells in response of salivary glands to radiation

The salivary glands of rodents and humans are composed of saliva producing mucous and serous acinar cells, myoepithelial cells and a ductal cell system which modifies saliva composition (Fig.1) (Pringle *et al.,* 2013). Also, cholinergic and adrenergic nerve fibers stimulate saliva production and indirectly affect salivary secretion through innervation of their blood vessels. The whole cells are invested in supporting stromal tissue

[(Pavolv, 1906; Proctor and Carpenter, 2007); Fig.1]. The impact of radiotherapy on function of salivary glands is bifaceted. Saliva-producing acinar cells are largely postmitotic in nature, and according classical radiobiology theory not predicted to be radiation-sensitive (Sreebny, 2010). However, radiotherapy of the salivary glands induces severe early (phases 1 and 2, 0–10 days and 10–60 days, respectively) loss in saliva production, suggesting that the salivary gland is more radiosensitive than anticipated (Coppes *et al*., 2001). It is not sure whether this observed early radiotherapy induced hyposalivation is attributable to apoptosis or to membrane damage-induced dysfunction of the acinar cells (Stephens,1989; Burlage *et al*.,2001; Coppes *et al*., 2001). The later phases of radiotherapy-induced hyposalivation (phases 3 and 4, from 60 to 120 and 120 to 240 days, respectively), wherein functionally mature acinar cells senesce and are not replenished with new ones, are now suggested to be due to radiotherapy induced sterilization of a salivary gland stem/progenitor cell population (SSPCs) [(Liu *et al* 1993; Denny and Denny,1999; Coppes *et al*., 2001; Konings *et al*.,2005); Fig.1]. Stem or progenitor cells are characterized by their self-renewal and differentiation capabilities, can replenish damaged cells, and have been identified in many tissues within the mouse and human (Shackleton *et al*.,2006 ; Barker *et al*., 2007). In this hypothesis therefore, the number of remaining undamaged SSPCs will determine the regenerative capacity of the gland after irradiation. Recovery and compensatory responses in nonirradiated regions (presumably containing SSPCs) have been observed after radiation, indicating the potential of surviving SSPCs to regenerate the tissue (Braam *et al*.,2005; Konings *et al*.,2006). The evidence for the existence of such a SSPC population that is both responsible for salivary gland homeostasis and regeneration, as well as for long-term hyposalivation when sterilized is reviewed.

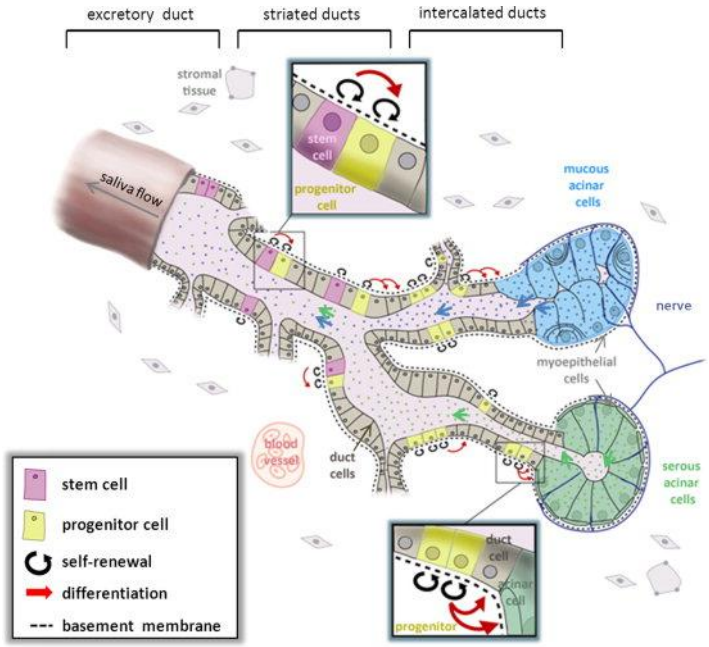

Fig. 1: Schematic representation of a generic salivary gland showing component cell types and theorized stem and progenitor cell locations.Visualization of proposed location of primitive stem cells, within larger excretory and striated ducts, and progenitor cells, within the striated and intercalated ducts. The stem cell pool supplies in the progenitor cell pool, which in turn replenishes the population of functionally mature duct and acinar cell types. Both stem and progenitor cells have the capacity to self-renew and differentiate. Schematic diagram generated based on available duct ligation, label-retaining cell, and stem/progenitor cell marker expression data.

The role of adult salivary gland stem/progenitor cells in gland homeostasis and regeneration

Cell loss due to aging or cytotoxic insults needs to be compensated by proliferation and differentiation of the tissues progenitor and ⁄ or stem cells. In most tissue the normal homeostasis involves proliferation of progenitor cells, whereas the stem cell is relatively quiescence and becomes only active after substantial depletion of differentiated cells. Many studies have been performed to investigate the cells that are involved in the homeostasis of salivary glands. It has been shown that nearly all of the differentiated cell types of the adult salivary gland appear to retain the ability to replicate (Denny *et al*., 1993), but which cells have the potential to produce other types or all types of cells?. Additionally, salivary glands have an excellent capacity to regenerate after partial extirpation (Hanks and Chaudhry,1971) or duct obstruction (Burford Mason *et al*., 1993), it seems

likely that they in fact do contain stem/progenitor cells. Both extirpation and duct obstruction have been used to characterize and isolate stem/progenitor cells (Scott *et al*., 1999; Takahashi *et al*., 2004, 2005).

Through label-retaining cell (lRC) studies using nucleotide analogs such as bromodeoxyuridine and ^{3}H-thymidine, proliferating cells have been localized mainly to the excretory and intercalated ducts in the salivary glands[(Denny and Denny, 1999; Man *et al*., 2001; Takahashi *et al*.,2001; Ihrler *et al*., 2002); Fig1.]. Nucleotide analogues are incorporated in the DNA after a period of continuous administration, and label all dividing cells. During a subsequent chase period in which no nucleotide analogue is administered, the label will be diluted with every cell division. The less frequently dividing cells will therefore retain the label;the LRCs are considered to contain the stem cells (Potten *et al*., 2002).

Ligating the major excretory duct of the salivary gland, creating a dysfunctional/apoptotic acinar cell environment, results in the proliferation of intercalated and excretory duct cells (Osailan *et al*., 2006; Katsumata *et al*.,2009).The initial functional ablation in ligated glands can be rescued after deligation through proliferation and suggested differentiation of these ductal cells, and saliva flow will rather rapidly return to pre-ligation levels. Label-retaining cell studies have also demonstrated that acinar cells themselves display a limited degree of proliferative ability, but the total ablation of acinar cell function in ligation experiments suggests that acinar cell proliferation is unlikely to account for the rescue of function.The above studies imply that cells capable of proliferation and differentiation reside within the ducts of salivary glands and may represent a potent SSPC population. Further studies have also suggested that these putative SSPCs are responsive to growth factor-mediated stimulation,wherby radiotherapy-induced hyposalivation was rescued through administration of keratinocyte growth factor (KGF), or GFs secreted by bone marrow cells (BMCs) mobilized to the salivary gland via granulocyte-colony-stimulating factor (G-CSF) (Lombaert *et al.,* 2006; Lombaert *et al*., 2008c). Tissue recovery in G-CSF- or KGF-treated animals was markedly higher than that in control animals, as assessed by a significantly higher acinar cell content in and saliva production from treated glands (Lombaert *et al*.,2006;Lombaert *et al*., 2008c). However, reducing the number of surviving putative SSPCs by increasing the radiation dose prevented these growth factors from rescuing salivary glands function (Lombaert *et al*., 2006; Lombaert *et al*., 2008c). These data serve to emphasize further the importance of a functional residual SSPC population in the salivary glands for hyposalivation recovery.

Pre-radiotherapy isolation of SSPCs followed by post-radiotherapy replacement into the patient could therefore increase the regenerative potential of the salivary gland and potentially completely restore tissue homeostasis following radiotherapy. In order to develop such a cell-based therapy for hyposalivation, determine the most potent SSPC population, and further characterize these cells, the ability to manipulate putative SSPCs *in vitro* is paramount (Pringle *et al*.,2013).

Selection and characterization of salivary glands stem cells

Label retaining studies have provided important knowledge of the cells involved in maintenance and regeneration of a tissue. However, it is very difficult to pin point the exact location of the stem cell. Another approach to detect the exact stem cell is genetical labelling of cells for example, using two knock-in alleles, Lgr5 or Lgr6 were exclusively expressed in cycling cells of the intestine (Barker *et al*., 2007), stomach (Barker *et al*., 2010) or in the hair follicle (Snippert *et al.,* 2010). With these powerful tools stem cells could be traced after division and differentiation, demonstrated to form all tissue lineages and shown to take part in the regeneration after wound infliction (Snippert *et al.,* 2010). It is anticipated that when creating a mouse with a genetic label of a gene involved in the biology of salivary glands, it will possible to locate their real stem cell. However, even with these techniques, additional novel cell types have been identified that are able to self renew and differentiate in all lineages of the tissues. These data query the existence of one stem cell population in the whole tissue. Further evidence for the existence of several potent stem cell populations within a tissue niche is provided by the field of hematopoietic stem cells (Kent *et al.,* 2009). An alternative strategy for the isolation of tissue stem is to detect cells that express certain stem cell markers. A wide variety of such well-established markers have been characterized. In salivary glands attempts at cell culturing after dispersion of the tissue have yielded several different cell types, of different origin, that may have stem cell properties. Therefore, primary cultures of dispersed cells will always contain all these cell types making it difficult to pick out the specific salivary gland stem cell.The exact stem cell of a tissue would be the selection of cells carrying a specific marker or selecting the cells labelled with induced reporter proteins, as mentioned above. Although the genetic expression of reporter proteins in salivary glands has not been established yet, several attempts have been undertaken to select for specific stem cells of the salivary gland using stem cell markers.

Rodent SSPCS

Recent studies have demonstrated that *in vitro* culture of processed salivary gland tissue is possible, a summary of which can be found in Table 1 (Pringle *et al*.,2013). Some of these studies have used a monolayer

culture technique, where adherent, proliferative colonies of presumed SSPCs were cultured from rat salivary glands, and after7days of culture with added epidermal growth factor and hepatocyte growth factor demonstrated expression of ductal (cytokeratins 18 and 19 and c-Met), acinar (amylase and aquaporin-5), and myoepithelial (vimentin and α-smooth muscle actin) differentiation marker proteins (Table 1). Also, CD24/CD49f (α 6 β1 integrin) and CD117(c-Kit) stem-cell-associated proteins were found at frequencies of 90% and 6%, respectively, in these cultures(Kishi *et al*., 2006; David *et al*.,2008) . Recent researches developed a nonadherent method for culturing potential murine SSPCs [(Lombaert *et al*., 2008a; Nanduri *et al*., 2011; Pringle *et al*., 2011);Table1].After mechanical and enzymatic digestion, aggregates of cells cultured in suspension, which were named salispheres, increased in size over time and contained proliferating cells (Lombaert *et al*., 2008a; Pringle *et al*.,2011). Murine salispheres were found to express the adult stem cell marker proteins CD117, CD24, CD29, CD49f, Sca-1, Musashi-1, CD44, CD90, and CD34, expression of most of which has been localized to ducts in naïve salivary glands [(Lombaert *et al*., 2008a; Banh *et al*., 2011); Fig.1], with the exception of CD44, whose expression was also suggested to be associated with differentiated serous acinar cells (Maria *et al*.,2012). Interestingly, CD117 expression in 3-day cultured salispheres (>0.6%) was markedly higher than that immediately following salisphere isolation (< 0.01%), suggesting that salisphere culture represents a form of lineage selection and could be used as a tool to enrich for stem cells prior to therapeutic use (Maria *et al*., 2012). Spontaneous differentiation into cells expressing acinar (α-amylase) and ductal cell (cytokeratins 7 and 14) marker proteins during culture was also reported in salisphere cultures (Lombaert *et al*., 2008a; Banh *et al*., 2011).Thus,through ligation, label retaining cell, growth factor, and culture-based studies,It has been confirmed that a stem cell-like population is likely to be contained within salivary gland duct cells. For the development of a (stem) cell therapy for hyposalivation, ductal-like cells from salisphere or monolayer cultures may be promising candidates.

Table 1:Summary of current salivary gland stem cell phenotypic studies presented in chronological order within species

			Tested for			
Species	Marker/of interest	Culture method	*In vitro* differentiation?	*In vitro* function?	First author	Ref.
Mouse	CD117	Salispheres	Yes	Yes	Lombaret	44
	CD49f, CD29, CD24,CD117	Salispheres	No	Yes	Nanduri	45
	CD117 and ALDH	Salispheres	Yes	No	Banh	46
	SP cells,Sea-1, clusterin	No culture	No	Yes	Mishima	47
	Ascl-3	Salispheres	Yes[a]	No	Rugel-Stahl	48
Rat	No marker	Monolayer	Yes	No	Kishi	49
	CD49f, CD29,	Monolayer	No	No	David	50
	CD49f, CD29, ,CD117	Monolayer	Yes	No	Neuman	51
Human	CD49f, CD90	Monolayer	Yes[b]	No	Sato	52
	CD117	Salispheres	Yes	No	Feng	53
	CD34, CD117, ALDH,CD90, CD44	Salispheres	Yes	No	Banh	46
	CD44, CD166	No culture	-	-	Maria	54
	CD49f, CD29,	Salispheres	No	No	Palmon	55

-, Indicates not applicable in study.[a] Lineage tracing data. [b]Pancreatic like differentiation shown. CD nomenclature are given where possible. Pseudonyms are :CD49f =integrin α6 =CD117=c-Kit CD=Thy-1=CD166=activated leukocyte cell adhesion molecule (ALCAM);CD24=heat stable antigen (HAS);cd29=integrin β1. Abbreviation: ALDH, aldehyde dehydrogenase ; CD, cluster of differentiation; SP, Side population celles.

Human SSPCS

Preliminary data suggests that salisphere-based culture principles and the employment of protein markers can be utilized in the study of human SSPCs (hSSPCs) [(Lombaert *et al*., 2008a; Feng *et al*.,2009; Banh *et al*., 2011); Table 1]. It has been demonstrated that CD117, CD24, CD29 and CD49f are expressed by a proportion of cells in 3-5 days old human salisphere cultures (Fig. 2) (Pringle *et al*., 2013). Preliminary data showing some human salisphere differentiation into three-dimensional organoid structures containing acinar and ductal-like regions is also encouraging in terms of the potential differentiation capabilities of these cells . Alternatively, cells grown in monolayers have also been shown to express a panel of stem-cell-associated marker proteins (CD44, CD49f, CD24/CD49f, CD90, CD104,and p75NGER). The colocalization of two such markers, CD49f and CD90, in the periductal region of a native gland was further suggested to be evidence for the ductal location of hSSPCs (Sato *et al*., 2007; Palmon *et al.,* 2012).

Potential stem cell therapies

Application of agents that stimulate the division of progenitor and stem cells

Stimulation of proliferation and differentiation of radiation surviving stem cells using growth factors, cytokines and other drugs proved to be beneficial in many organs. In the salivary gland cytokines like EGF

(Ohlsson *et al*., 1997; Limesand *et al*., 2009), insulin growth factor (Thula *et al*., 200; Limesand *et al*., 2009) and bFGF (Thula *et al*., 2005) have been suggested to inhibit apoptosis and/or enhance proliferation. Recently, the effects of Keratinocyte growth factor of KGF or FGF7 on radiation-induced salivary gland damage in the mouse submandibular gland were studied (Lombaert *et al*., 2008c). DN23-KGF treatment for 4 days prior to irradiation induced salivary gland proliferation of all cell types but especially stem/progenitor cells, increasing the stem- and progenitor cell pool. Postirradiation treatment with DN23-KGF further improved gland function, seemingly through accelerated expansion of the pool of progenitor/stem cells that survived the irradiation treatment. These results are very promising, also because, DN23- KGF has been shown to ameliorate radiation-induced damage in oral mucosa (Dorr *et al*., 2002), potentially attenuating radiation-induced xerostomia by reducing both hyposalivaton and mucositis. However, there is a possibility that KGF, may stimulate tumour proliferation and may potentially interfere with anti-cancer therapies. Interestingly, postradiation KGF treatment also showed a small but significant increase stem/progenitor proliferation and salivary flow. From these studies it can be concluded that salivary gland that do survive the radiation-insult are able to proliferate and regenerate the tissue when stimulated properly. Appreciation of these facts suggest new strategies for the enhancement and regeneration of radiation damaged tissue.

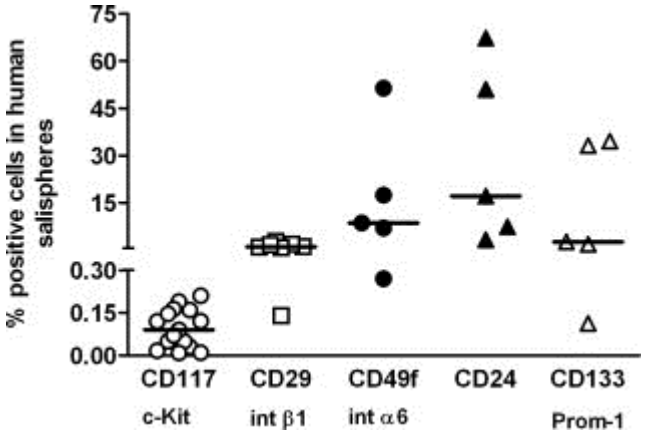

Fig. 2: Human salispheres express adult stem cell marker proteins. Adult human salivary gland biopsies were subject to mechanical and enzymatic digestion and cultured as previously reported (Pringle *et al*., 2011; Maria *et al*.,2012). Flow cytometry was performed between 3 and 5 days post-isolation, to detect expression of the CD117, CD29, CD49f, CD24, and CD133 stem cell-associated proteins. Data points within each marker protein are from separate patient isolations. Bars represent mean percentage expression. Alternative nomenclatures if applicable are also stated.Abbreviation: int, integrin prom-1, prominin-1.

Stimulation of vascular regeneration

Blood vessels are of eminent importance for the function of the salivary glands. It has been shown that by only 4 h after irradiation the microvessel density in salivary glands decreases by 45% (Cotrim *et al*., 2007). Whereas 90 days after irradiation blood vessel dilation contributes to decreased blood perfusion of the salivary gland (Ahlner and Lind, 1994). This impaired regeneration capacity results from reduced endoglin expression (Lombaert *et al*., 2008b) a factor important for neoangiogenesis (Arthur *et al*., 2000) and vascular repair (van Laake *et al*., 2006).The early loss of microvessel density and function could be reduced by pretreatment with serotype 5 adenoviral vector-mediated transfer of basic fibroblast growth factor or vascular endothelial growth factor complementary DNAs (Cotrim *et al*., 2007), stimulating blood vessel growth. The late vascular damage could be ameliorated by postirradiation mobilization of bone marrow derived cells (Lombaert *et al*., 2008b). The subsequent homing of mesenchymal cells (MSCs) and endothelial progenitor cells (EPCs) not only resulted in an improvement of the parenchyma but also in a reduction of vascular damage. The latter is due to the role of bone marrow derived cells(BMCs) in differentiating into vascular cells and also in secreting factors that directly stimulate the existing blood vessel cells (Lombaert *et al*., 2006, 2008b). Regretfully, preirradiation treatment with growth factors may not be applicable for clinical use due to their pro-tumorigenic potential (Cotrim *et al*., 2007). However, postirradiation mobilization of MSCs and EPCs from the bone marrow may not be tumorigenic. Similar to what has been suggested for other diseases (Khoo *et al*., 2008) autologous EPCs derived from the bone marrow could serve to prevent radiation induced vascular damage.

Adult tissue stem cell transplantation

One of the first encouraging reports of adult stem cell transplantation raised the issue of stem cell plasticity (Orlic *et al*., 2001).Adult stem cells are capable, under suitable circumstances, of producing a wide variety of cell types regardless of their germ layer. Cell-cell fusion has been proposed as a more appropriate interpretation for this phenomenon. Additionally, transplanted adult stem cells may induce repair by stimulation of proliferation of endogenous tissue stem cells (Duffield *et al*., 2005) through factors secreted by these cells (Krause and Cantley, 2005). Although adult stem cells may not be as powerful or diverse as embryonic stem cells, yet, they offer many advantages for the development of cellular therapeutics including lack of ethical problems, the possibility to using autologous cells, accessibility, stable phenotype and tissue type compatibility. Adult tissue specific stem cells have now been identified in salivary glands (Lombaert *et al*., 2008a).Transplantation of healthy salivary glands stem ⁄ progenitor cells collected prior to irradiation and transplantation after treatment may reduce hyposalivation and xerostomia.

The first evidence of ductal-like SSPC functionality *in vivo* was reported from studies in which donor cells isolated from salisphere cultures were transplanted back into irradiated recipient murine glands (Lombaert *et al*., 2008a; Maria *et al*., 2012). Recovery of salivary gland function of 70% of the transplanted animals was achieved with as few as 300 c-Kit$^+$ SSPCs from primary salispheres. In serial transplantation experiments, only 100 c-Kit$^+$ donor-derived cells isolated from salispheres grown from primary recipient glands repopulated glands in a secondary transplant.Non-c-Kit-expressing cells were much less potent leading to 33% recovery following transplantation of 10,000–90,000 cells (Lombaert *et al*., 2008a). Importantly, and in contrast to studies involving transplanted BMCs, the transplanted c-Kit$^+$ SSPCs had functionally integrated within the recipient gland, expressed donor-derived markers, and displayed ductal and acinar cell-type morphologies (Lombaert *et al*., 2008a). Studies of the regenerative capacity of potential SSPCs expressing the CD24, CD49f, and CD133 ductal-associated marker proteins yielded similar exciting functional recovery, with effective cell numbers of approximately 5,000 CD24, CD29, or CD133-expressing cells (Maria *et al*.,2012). When side population (SP) cells, shown to be stem cell-like cells in other tissues, were isolated from salivary glands and immediately transplanted into irradiated recipient glands without a culture period, functional recovery was observed within 2months, however integrated acinar and ductal-like donor cells were not detected in the recipient salivary glands (Larderet *et al*.,2006; Passineau *et al*.,2010; Mishima *et al*.,2012). These data suggest that selection for donor ductal-like cells, both by inclusion of an *in vitro* cell culture phase, and/or employment of marker proteins enhances functional recovery *in vivo*.

Studies regarding hSSPCs are few in number as yet and crucial assays for the reliable assessment of hSSPC differentiation and proliferation capabilities are still lacking. Even if hSSPCs mirror the *in vivo* functional ability of murine SSPCs, they still represent by no means the only cell-based option for a xerostomia therapy. Since 1998, a huge effort has been directed toward the investigation of human embryonic stem cell (hESC) potential as a source of cells for therapeutic applications, based on their capability to turn into any cell type in the body and self-renew indefinitely (Thomson *et al*.,1998 Reubinoff *et al*., 2000). In 2001, the first clinical trial using hESC-derived cells began, as a therapy for spinal cord injury(Alper,2009). An hESC-based approach to xerostomia therapy has not yet been reported, and may be hazardous due to the vulnerable nature of post-radiotherapy patients, in combination with the inherent teratogenicity of hESCs and their tendency to acquire karyotypic abnormalities during *in vitro* culture, exclusive of the ethically contentious nature of hESC research (Baker *et al*.,2007;Hussein *et al*.,2011). Technically, the expertise required to generate hSSPCs from hESCs is currently still lacking, but it remains possible that hESC-derived hSSPCs may represent an interesting option for xerostomia therapy in the future. Transplantation of BMCs into numerous disease-like mouse models and the progression toward clinical trials using BMCs suggest also that existing adult human stem cells represent a simple source of cells for xerostomia therapy (Lombaert *et al.,* 2006; Lombaert *et al.,*2008a; Karamouzian *et al*., 2012; Bai *et al*.,2012). Although mobilized BMCs seem to have some ameliorating effect on hyposalivation in studies described above, this effect was most likely due to growth factor secretion. Transdifferentiation of BMCs into acinar cells was not observed, and functional recovery was attributed to stimulation of surviving endogenous SSPCs (Lombaert *et al*., 2006; Lombaert *et al*.,2008c; Couzin,2006; Bai *et al*.,2012). BMC-mediated hyposalivation rescue is therefore limited first by the requirement for surviving SSPCs and second by the lifespan of the growth factor-secreting BMCs. It is hypothesized that hSSPCs are likely to be preferable to hESCs and BMCs as therapeutic agents for hyposalivation, when considering the ability of murine SSPCs to differentiate appropriately into saliva-producing cells, integrate effectively into host tissue, and rescue hyposalivation. It has been speculated further that a long-term cell therapy for hyposalivation is feasible, through the employment of hSSPCs.

In the near future, these cells may have the potential to reduce radiotherapy-induced salivary gland dysfunction in patients.

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