Exploring the regeneration potential of salivary glands using organoids as a model

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CHAPTER 1

GENERAL INTRODUCTION & OUTLINE OF THE THESIS

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Salivary gland dysfunction occurs when a large number of acinar cells, the functional secretory units of the gland, have been irreversibly damaged. This may occur as consequence of radiotherapy treatment (RT) for head and neck cancer and can be further aggravated by radiation-induced alterations of the tissue environment, such as disruption of the parasympathetic innervation[1,2], senescence[3,4] changes in extracellular matrix composition[5-7] and a depletion of potential stem/progenitor cells[8]. The progressive decline of the salivary gland epithelium, due to the often unavoidable inclusion of the salivary gland within the irradiation field, leaves 40% of head and neck cancer survivors facing the challenges of the significant morbidities of radiation treatment, including irreversible hyposalivation, that will lead to the onset of xerostomia, which drastically decreases their quality of life[9].

Although several clinical studies have provided evidence that partial recovery of saliva production can occur in patients treated with intensity-modulated radiation therapy (IMRT)[10], indicative of the potential for endogenous tissue repair following RT, the mechanisms behind adult salivary gland regeneration potential are still unknown. Recent retrospective studies have shown that while healthy human acinar cells are able to divide and act as a cell source for the slow turnover maintenance of the secretory unit, radiation treatment drastically reduces this source of cell replacement[11], indicating that other cell types might be responsible for the regeneration of the damaged gland. The currently limited knowledge of the extrinsic and intrinsic factors that regulate salivary gland homeostasis and regeneration after damage has so far curtailed the application into the clinic of regenerative strategies, such as reactivation of endogenous repair programs or transplantation of stem/progenitor cells to replace the lost tissue. Therefore, the majority of currently available treatments are primarily palliative solutions, such as saliva substitutes, which aim to temporarily alleviate the discomfort of the symptoms[12].

Adult organs, including the salivary glands, maintain their function through the ability of resident adult stem/progenitor-like cells to preserve a correct balance between self-renewal and differentiation. While fast turnover tissues, such as intestine, skin and hair follicles, rely on the homeostatic activity of their resident stem cells, facilitating significant advances in understanding their renewal potential, slow turnover tissues, such as salivary glands, primarily rely on the activity of tissue resident stem-like cells upon injury. This makes the identification of the intrinsic and extrinsic factors involved in the regeneration processes within slow turnover tissues more challenging and leaving no consensus within the field as to the nature of the key players[13].

Over the past 10-15 years, the use of conditional and inducible lineage tracing mouse models opened up the possibility to temporally control the expression of putative molecular switches

within the salivary gland epithelium. This strategy allowed researchers to map cell fate as well as to compare cell expression patterns during homeostasis and regeneration. Recent work has defined that while lineage restricted progenitor cells seem to be the cellular source involved in the maintenance of the different salivary gland compartments during homeostasis[1,14,15], plasticity mechanisms, more than a dedicated stem cell population seems to be involved in salivary gland regeneration in a context-specific manner[16,17].

While mouse *in vivo* experiments have helped to elucidate cell dynamics during salivary gland homeostasis and regeneration, and shed light on several progenitor populations within the mouse salivary gland[1,14,15], their limited translational potential to humans[18] has raised the need for new approaches to study regeneration processes in human salivary glands. Therefore, to understand the dynamics of human salivary gland stem/progenitor cells during homeostasis and regeneration, it is necessary to use strategies that will allow characterization and test functionality of adult salivary gland stem cells, and eventually allow for the quantitative analysis of the contribution of each cell type to the regeneration of damaged tissue (and homeostasis).

The establishment of adult stem cell-based organoids has allowed the modelling of regenerative processes for several organs[19] including salivary glands[18,20,21]. Organoids are defined as 3D structures derived from adult tissues stem cells, iPSCs or ESCs capable of self-renewal and self-organization through cell sorting and spatially restricted lineage commitment, and have features resembling the counterpart *in vivo* tissue[22,23]. The ability to generate salivary gland organoids depends on the isolated population of cells and on the niche factors that allow on one hand to keep cells in their stem-like state, and on the other hand drive differentiation of progenitor-like cells into specialized salivary gland secretory cells.

The work described in this thesis uses organoid systems as a regenerative model to investigate mouse and human salivary gland stem/progenitor-like cells as well as the niche signaling pathways that might be involved in controlling cell fate and explores their potential use for autologous transplantation in patients suffering from RT-induced hyposalivation.

Chapter 2: reviews the literature focusing on how recent advances in stem cells biology and changes in adult stem cell definition have influenced salivary gland biology defining a new regenerative landscape.

Chapter 3: discusses the complexity of the salivary gland stem cell niche focusing on elucidating the effect of the Wnt/B catenin signaling pathway on the regenerative potential of salivary gland stem/progenitor cells. It also introduces the uses of organoids to dissect/manipulate the salivary gland niche, as well as new parameters to evaluate functionality of stem/progenitor cells and their potential use in regenerative therapies.

Chapter 4: provides evidence that Yes-Associated-Protein (YAP) acts as a sensor of tissue integrity during salivary gland regeneration thereby regulating stem cell self-renewal both in

mouse and human-derived salivary gland cells. It also introduces a potential YAP activation-based pharmacological strategy to improve the radiation response of human salivary gland-derived cells that if validated in an *in vivo* system could open up new treatment opportunities to rescue the radiation-induced hyposalivation phenotype.

Chapter 5: demonstrates that perturbation of salivary gland homeostasis leads to changes in the basal autophagy flux and it describes how the dynamic of these changes varies between the different compartments of the gland. Finally, it demonstrates that a constant fuel of the autophagy machinery is necessary to maintain the self-renewal ability of salivary gland-derived cells.

Chapter 6: describes the application of a gene network co-expression framework on stem-like cell-enriched and differentiated human salivary gland-derived organoid cultures to obtain a "holistic view" of the molecular mechanisms that might drive salivary gland regeneration. Probing the transcriptomic landscape of salivary gland organoids identified potential novel mechanisms of salivary gland renewal.

Chapter 7: describes the development of a GMP-compliant protocol that allows the isolation and expansion of human-derived salivary gland cells. Furthermore, it demonstrates the safety and feasibility of the first organoid-based cell therapy to potentially rescue radiation-induced hyposalivation in head and neck cancer patients.

Chapter 8: summarizes the findings of this thesis, puts them in a general perspective and discusses their potential impact in basic stem cell research and ultimately the translation towards the development of a therapeutic intervention for radiation-induced hyposalivation

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