Guardians of the oral cavity
van Dijk, I.A.

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CHAPTER 7

SUMMARY
The oral cavity is a hostile environment as it is subject to various forms of stress, such as bacterial toxins, mechanical assault and harmful compounds. Saliva protects the oral cavity against these threats in many ways. It acts like a lubricant that protects against mechanical stress, it forms a thin film on the oral mucosa that serves as a barrier against bacteria and noxious compounds, it contains antimicrobial peptides, and it functions as a buffer maintaining pH. Saliva is a complex liquid that, besides water and electrolytes, contains a plethora of proteins, peptides and lipids. The most common peptides in saliva are the histatins, with histatin 5 being the most studied, mainly because of its anti-Candida activity. Another histatin, highly present in human saliva, is histatin 1 (Hst1). Many functions have been suggested, including binding of tannins, protection of tooth enamel and a role in wound healing. The function of Hst1, in particular with respect to the oral tissues, is the subject of this thesis.

In chapter 2 we show that Hst1 enhances cell-substrate and cell-cell adhesion. Using phase-contrast time-lapse microscopy we found that cells attach and spread faster in the presence of Hst1. Epithelial cells and fibroblasts respond to Hst1 in a similar way. We show that the effect is specific for Hst1, as other histatins or a scrambled version of Hst1 did not improve cellular adhesion. The phosphoryl group in Hst1 proved to play a stimulating role in enhancing cell attachment, but it is not essential. Furthermore, using an intestinal cell line, we found that Hst1 improves the epithelial barrier function. This is a very interesting finding, because the mouth as well as the intestines are highly colonized by microorganisms and therefore a good barrier function is essential.

In chapter 3 we further explored the effect of Hst1 on cellular adhesion. We show that endothelial cells are also sensitive to Hst1, as in the presence of Hst1 endothelial cells attach and spread faster onto their substrate. Using a Transwell assay we observed that the passage of differently-sized fluorophores across a monolayer of endothelial cells is decreased if the cells are treated with Hst1, indicating that it improves the endothelial barrier. In epithelial cells we confirmed the improved barrier function, as the cell cultures treated with Hst1 show decreased translocation of the bacterium *Streptococcus suis*. Furthermore, upon incubation with Hst1, we observed activation of E-cadherin and upregulation of ZO-1, two junctional proteins. In addition, a marker for cell differentiation, apolipoprotein A-IV, is upregulated in cells treated with Hst1. Differentiation and barrier formation are similar processes that involve epithelial-to-mesenchymal transition (EMT) and its counterpart mesenchymal-to-epithelial transition (MET). Since Hst1 stimulates differentiation and barrier formation, we hypothesized that Hst1 is a MET-factor. To continue this line of research, we grew oral cancer cells into spheroids as a simple model for a tumor, plated them and added EMT-factors (EGF and TGFβ) to induce tumor outgrowth: the spheroid would lose its compact shape and cells migrate out of the spheroid. Spheroids that were grown in the presence of Hst1 remain more compact after being plated in a culture dish. This is another indication that Hst1 stimulates MET, as it counteracts the effect of EMT-factors, and it could possibly play a role in tumor outgrowth. A patent by Yaguchi and Kawakami from 2013 claims that Hst1 can be used as a marker for metastasis in certain forms of cancer. More research is needed to understand the connections between these findings and our results.
In chapter 4 we return to the oral cavity and propose an application of Hst1 in dentistry. Rapid cell-substrate adhesion is essential in implantology, as the bone cells from the jaw as well as the epithelial cells and fibroblasts from the soft tissues need to attach to the implant to anchor it in a process called implant integration. Based on its cellular adhesion promoting effect, we hypothesize that Hst1 could play a stimulating role in dental implantology. To monitor cell attachment to titanium we developed a simplified model to study cells on a titanium surface. Titanium was sputtered on glass, thereby creating a very thin translucent layer (4 – 8 nm thick). This facilitates the use of phase-contrast time-lapse microscopy to study the cell behavior in real-time without the risk of phototoxicity as fluorescent labeling is not needed anymore. We showed in *in vitro* experiments that also on titanium, cells attach and spread faster in the presence of Hst1, which forms a basis to establish the effect of Hst1 in *in vivo* studies.

In chapter 5 we describe a protocol to perform the scratch or wound healing assay, a commonly used assay. The protocol was adapted to study the outgrowth of tumors (chapter 3). In addition, we included hints and additional protocols to tackle pitfalls that are associated with the scratch assay. Custom-made software to analyze results is also included.

In chapter 6 we discuss our findings in a wider context and provide an overview of the effects of Hst1 on cell-substrate adhesion and barrier function, the possible working mechanisms behind these effects and the potential functions of Hst1 in health and disease. As described in this thesis we propose physiological functions of Hst1, namely the maintenance of epithelial integrity by improving cellular junctions and barrier function. Another function could be rapid re-attachment of cells to the teeth. Stress in the oral cavity can cause damage to the tissues, including the soft-tissues surrounding the teeth. If cells that connect to the teeth detach, re-attachment should be fast to prevent microorganisms from entering the space between tooth and tissue. Hst1 could promote this re-attachment as it enhances cell-substrate adhesion. Furthermore, the chapter discusses possible roles of Hst1 in different types of EMT/MET, in pathologies such as diabetes, oral mucositis, gingival overgrowth and oral cancer, and the potential therapeutic applications of Hst1.