## Discovery of the wound-healing capacity of salivary histatins

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**English summary**

Wounds in the oral cavity heal much faster than skin lesions, with similar wounds healing in seven days in the oral cavity compared to several weeks on the skin. Accelerated healing in the oral cavity has been attributed to various factors, including better microcirculation in oral tissue, a higher turnover rate for oral epithelium, and the presence of saliva. The exact component responsible remained elusive for a long time. This thesis describes the discovery of a new wound-healing promoting component in saliva: histatin. In addition to the discovery, a large part of this thesis is about the working mechanism utilized by histatin.

The 2nd chapter describes the discovery of histatin as wound-healing component of human saliva. We tried in the lab to imitate wound healing. An important aspect of wound healing is the so-called re-epithelialization, which is the closure of a wound by the adjacent healthy epithelium. The model we used in the lab is as follows: in a petridish a confluent layer of epithelial cells (the cells that are responsible for re-epithelialization) is grown. Subsequently, a scratch (the wound) is made that leaves a space in the middle of the petridish. Next, the speed by which these wound were closed was measured under different conditions. In this model we found that saliva-treated 'wounds' in petridishes closed much faster than those that were treated with a control medium. Thus, we were able to show enhanced wound healing by saliva, in the lab. The next question was obvious: what in saliva is responsible for the enhanced wound closure? We answered this question by fractionating saliva with liquid chromatography. Eventually we found that there was only one component active in saliva. We analyzed this fraction by mass spectrometry, and it turned out to be histatin. Apart from the discovery of histatin, we also established in this chapter that histatin-mediated wound closure, is dependent of a specific activating system called Erk1/2.

The 3rd chapter is a follow-up study of chapter 2. This chapter further evaluates some issues we couldn't address in the 2nd chapter. We show that also cells directly derived from the mouth are responsive to histatin. In the 2nd chapter we used a cell line, which with eternal growing abilities have advantages over primary cells, but as a consequence, they sometimes are less representative for the *in-vivo* situation. In addition, we studied the role of salivary mucins in wound healing. However, based on our findings, and those published by others, we could clarify whether mucins play a big role in oral wound healing.

The 4th chapter describes the effect of histatin in a complex wound model, made from artificial, tissue-engineered skin. Also in this, highly resembling the human skin, model histatin turned out to enhance wound healing. A next step would be to actually test it in humans or first animal models, something that hopefully will be realized in the near future. In addition, in this chapter we determine the minimal domain necessary for activation. And, we were able to make the normal linear molecule histatin cyclic. The cyclic histatin has a remarkable activity compared to the linear one. It is 1,000-fold more potent, or in other words, a concentration that is 1,000-fold less than the linear is sufficient to do the same job: enhance wound healing. This remarkable finding is ascribed to the possible higher affinity of cyclic peptides with the putative epithelial cellular receptor. Such a receptor basically is the switch that can turn cells 'on'. And, cells that are turned 'on' are more active in wound healing.

Histatin is not unique, there are more molecules that are similar to histatin and that have wound-healing capacities. In chapter 5 we compare histatin with LL-37, which is the most-studied molecule of the type that is similar to histatin. We found that histatin probably has a different mode of action in activating cells than LL-37. In contrast to LL-37, histatin is not cytotoxic, even at extremely high concentrations. In addition, histatin does not seem to influence inflammation reactions by cells, whereas LL-37 has a firm effect on inflammation. We thus conclude that histatin actually doesn't really meet the criteria of the type of molecules like LL-37.

The 6th chapter describes as specific part of regulation of histatin activity, the uptake by the epithelial cell. By taking up histatin, a cell can regulate activity, after all, once histatin is taken up it cannot activate the cell anymore. This a common manner of cells to regulate activation. An interesting finding is that the uptake amount of histatin does not correlate with the activation properties. So, a very active histatin variant is equally taken up by the cell when compared to an almost inactive histatin variant. We suspect that for uptake an initial interaction with the receptor is enough, whereas for activation a secondary interaction is required.

Upon our exceptional finding in chapter 4, that the cyclic histatin is a lot more active than the linear histatin, we would have loved to do follow-up studies. However, making cyclic histatin in the traditional manner is so complex and work-intensive, that follow-up studies were difficult to initiate. Therefore, we designed a new method to cyclisize histatins with the aid of a specific enzyme. This is described in chapter 7. This methods is not limited to histatin research, but can easilily be implemented in a wide

variety of peptide research. With this method in hands, we could accurately show that cyclic histatin has a higher binding affinity for the cellular receptor, and thus that this is probably the reason for the superior activity.

This thesis describes the discovery and characterization of histatin, a molecule exclusively present in saliva, and not in any other bodily secretion fluids such as sweat. In addition, we could not find any reason to assume that histatin has any negative side effects on wound healing, such as increased inflammation. And, we show that by changing the structure of the molecule (linear to cyclic) the molecule become a lot more effective.