On the relevance of PS exposure as a regulatory event for ADAM10-mediated substrate release

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Summary

The "*a disintegrin and metalloproteinase 10*" (ADAM10) mediates the release of the vast majority of transmembrane bound or GPI-anchored substrates, together with its close homologue ADAM17. The protease is vital for several physiological and pathophysiological processes. Although ADAM10 activity in these processes is comprehensively described, little is known about the mechanism controlling the protease shedding activity. Given that the cytoplasmic domain of ADAM10 is negligible for substrate shedding, the focus of current research has shifted to the extracellular domains. In this context, also the plasma membrane itself may play an essential role in the regulation of ADAM10 shedding activity. This novel concept of the plasma membrane acting as a regulator has been recently described to be the penultimate step controlling ADAM17 shedding activity. The exposure of the phospholipid phosphatidylserine (PS) and its direct interaction with ADAM17 was identified as the primary trigger for substrate release. Based on this newly acquired knowledge for ADAM17 and the common structural and functional similarities between ADAM17 and ADAM10, this thesis focuses on the question of whether PS exposure also regulates ADAM10-mediated substrate shedding.

In the context of this overall aim, the first question was whether PS exposure regulates ADAM10-mediated substrate shedding. A series of experiments with different cell types were carried out; these showed that stimulation with different activators of ADAM10 led to enhanced externalisation of PS with a concomitant increase in substrate release. The headgroup of PS (OPS) showed a competitive inhibitory effect on ADAM10-mediated substrate release, presumably by abolishing ADAM10 interaction with PS. This inhibitory effect of OPS on ADAM10-mediated substrate release was shown for multiple ADAM10 substrates and stimuli, indicating that PS exposure is in general essential for ADAM10 shedding activity. Finally, using a rabbit erythrocyte – pVCC model, PS exposure was highlighted as the primary trigger of ADAM10-mediated substrate shedding.

Furthermore, the question arose whether calcium-activated phospholipid scramblases (e.g. anoctamin 6, TMEM16F/ANO6) might contribute to PS exposure and thereby affect ADAM10-mediated substrate release. Overexpression of phospholipid scramblase TMEM16F/ANO6 was shown to contribute to PS exposure and related enhanced ADAM10-mediated substrate release. In an approach of gain - and loss of function of TMEM16F/ANO6 scrambling activity, PS exposure was shown to be directly connected to ADAM10 shedding activity. On the one hand, the human hyperactive TMEM16F/ANO6 mutant (human ANO6 D408G) showed enhanced PS exposure in conjunction with increased ADAM10-mediated substrate release, under constitutive conditions. On the other hand, B-cells from a patient with the Scott

syndrome showed TMEM16F/ANO6-related impaired PS scrambling, resulting in the absence of ADAM10 shedding activity.

The final aim was to identify the mechanism behind the interaction between ADAM10 and PS. For ADAM17, it was shown that the protease intercalates via an amphipathic helix into the lipid bilayer and interacts through a polycationic motif directly with PS. The stalk domain of ADAM10 combines these two attributes, by showing the characteristics of an amphipathic helix and containing a polycationic motif. Biophysical experiments with a peptide version of the ADAM10 stalk domain indicated a direct interaction of ADAM10 with PS and its intercalation into the phospholipid bilayer. Furthermore, the mutation of the positively charged amino acids in the stalk domain of ADAM10 significantly reduced the ability of the proteases to release its substrate betacellulin. These results highlight the importance of positively charged amino acids in the ADAM10 stalk domain for the interaction with PS and substrate shedding.

The results presented in this thesis show for the first time that the cell membrane plays an important role in the ADAM10-mediated substrate release; they also highlight PS exposure as a general regulatory mechanism for two of the most prominent proteases: ADAM10 and ADAM17.