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Dissertations-Kurzfassung

Investigation of a possible dominant negative effect of endoglin missense mutations in Morbus Osler / Hereditary Hemorrhagic Telangiectasia (HHT)

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The homodimeric transmembrane receptor endoglin (CD105) plays an important role in angiogenesis. This is highlighted by mutations in its gene, leading to receptor haploinsufficiency and causing the vascular disorder HHT1. The main role of endoglin function has been assigned to the modulation of transforming growth factor beta and bone morphogenetic protein signalling in endothelial cells. Nevertheless, other functions of endoglin have been revealed to be involved in different cellular functions and in other cell types than endothelial cells. Compared to the exploration of its natural function, little experimental data have been gathered about the mode of action of endoglin HHT mutations at the cellular level, especially missense mutations, and to what degree these might interfere with normal endoglin function.

This work investigated formation and cellular localisation of possible homo- and heterodimers composed of endoglin wild-type and endoglin missense mutant proteins by application of fluorescence-based microscopic techniques, such as bimolecular fluorescence complementation (BiFC), immunofluorescence staining with the endoglin specific monoclonal antibody SN6, and protein interaction studies by Forster Resonance Energy Transfer (FRET).

The results show that all of the investigated missense mutants dimerise with themselves, as well as with wild-type endoglin, and localise, depending on the position of the affected amino acid, either in the rough endoplasmic reticulum (rER) or in the plasma membrane of the cells. It is shown that the rER retained mutants reduce the amount of endogenous wild-type endoglin on the plasma membrane through interception in the rER when transiently or stably expressed in HMEC-1 endothelial cells. As a result of this, endoglin modulated TGF-beta-1 signal transduction is also abrogated, which is not due to TGF-beta receptor ER trafficking interference. Protein interaction analyses by FRET show that rER located endoglin missense mutants do not perturb protein processing of other membrane receptors, such as TbRII, ALK5 or ALK1. In summary it is shown that missense mutations act in a dominant negative way towards the wild type receptor.

To date no medical treatment of HHT is available, but one is on the search for substances that increase expression of endoglin receptors. As shown in this work, this might be problematic with patients carrying missense mutations as expression of these can be expected to be also increased which might contribute to an opposite effect than desired.