PhD-project

“Role of human Sec62 protein in precursor-selective gating of the Sec61 channel in protein entry into the ER of normal and cancer cells”

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In human cells, the endoplasmic reticulum (ER) has central roles in synthesis, folding, and sorting of proteins as well as in acting as a dynamic calcium reservoir. The Sec61/62 complex in the membrane of the ER is involved in the biogenesis of many proteins at the ER and in ER Ca\(^{2+}\) leakage. In its open conformation, the heterotrimeric Sec61 complex provides an aqueous path for the entry of polypeptides into the ER as well as for passive Ca\(^{2+}\) efflux from the ER. The Sec62 protein mediates gating of the dynamic Sec61 channel to the open state in a precursor-specific manner and to the closed state after Ca\(^{2+}\) has started to leak from the ER. For these distinct activities Sec62 comprises a ribosome binding site in its cytosolic amino-terminal domain and an EF hand in its cytosolic carboxy-terminal domain.

Sec62 can also have a pathological function since overexpression of the SEC62 gene is linked to prostate and lung cancer and was shown to be a cancer driver gene. This function appears to be related to two hallmarks of cancer, invasive potential and stress tolerance. That is, overexpression of the SEC62 gene in non-cancerous HEK293 cells leads to increased migratory potential and stress tolerance. Reciprocally, silencing of the SEC62 gene in prostate and lung cancer cells leads to loss of the migratory and invasive potential as well as to reduced stress tolerance.

In the PhD project, the physiological and pathophysiological functions of human Sec62 protein will be studied under our established conditions by gene silencing and over-expression, respectively, in a human model cell line in combination with structural biology, proteomic analysis and protein transport assays. Specifically, HeLa cells will be transfected with a non-silencing or two different SEC62 silencing siRNAs and the label-free proteome will be analyzed for three biological replicates in the lab of our collaboration partner at the MPI for Biochemistry in Martinsried (F. Förster). Recently, we successfully carried out such an analysis after SEC61A1 silencing and found almost 400 proteins of the secretory pathway to be negatively affected (unpublished results). For Sec62, this approach
will lead us to a list of precursor proteins, which depend on Sec62 for ER protein entry, and should allow us to informatically deduce the sequence features that are responsible for Sec62 dependence. Randomly selected precursors of this list will be evaluated by the PhD student in our in vitro and in vivo ER import assays and will allow us to evaluate the informatic analysis\(^4\). For the informatic analysis we will have help from our collaboration partner in Computational Biology at Saarland University (V. Helms)\(^1\). In parallel, isolated rough microsomes will be isolated from normal cells and after SEC62 silencing and over-expression, respectively, and characterized by cryoelectron tomography by our collaboration partner (F. Förster)\(^13\). In these structural studies the focus will be on the architecture of protein complexes, which are associated with ribosomes, synthesizing certain Sec62-dependent precursor polypeptides. Here, we will employ precursors (such as ERj3- and prion-protein), which we have previously identified\(^4\) and which were recently confirmed by others\(^14\). Ideally, the list of positively and negatively affected proteins and the structural analyses will also contribute to our insights into the pathophysiology of SEC62 gene over-expression.

Reference List


