Purification and crystallization of a human protein possibly involved in angiogenesis

Background:
Blood vessels are essential for the delivery of nutrients and oxygen to tissues, as well as for the removal of waste products. Patients with tumors, diabetes or with wounds all have active formation and remodeling of blood vessels, a process called angiogenesis.

In the search for "new" genes involved in angiogenesis we performed a microarray study based on the embryoid body (EB) model. In this model embryonic stem cells are aggregated into spheroids and stimulated with VEGFA to adopt a hematopoietic phenotype. If the EBs are embedded in a collagen gel they will start to sprout into the surrounding matrix. In the study the sprout fraction was separated from the core fraction and the gene expression was analyzed with the microarray technique. The results suggested that a lot of genes already implicated in angiogenesis were upregulated in the sprout fraction, indicating that significant data was indeed produced. The next step was then to identify new genes with similar expression patterns and investigate their role in blood vessel formation and maintenance.

One gene that showed a very interesting expression pattern caught our attention. This gene had been previously rather unstudied but has a structural similarity to a known family of proteins as predicted by http://swissmodel.expasy.org. Due to this similarity it is likely that the protein is somehow associated with intercellular and intracellular transport of vesicles but a totally different function can not be excluded.

We try to describe the protein in terms of function, interactions and expression pattern and we have found evidence that it is indeed involved in endothelial cell migration *in vitro* and that both VEGFA and Collagen-I are positive regulators of the gene. We have also obtained *in vivo* data suggesting that the homologues of the gene are up-regulated in the vasculature (PECAM positive cells) of both embryonic and adult mouse tissue as well as in developing zebrafish (*ISH*).

As mentioned above the structure of the protein can be predicted using the structures of related proteins as templates. However, this configuration is only a qualified guess and in order to find out for sure what the protein looks like we would like to use x-ray crystallography. Using this technique it is also possible to obtain co-crystals together with interactionpartners.

Outline:

Step1: **To clone the gene into a bacterial vector and express the protein in BL21 cells. Solubility is the key!**

The gene is already expressed in a HIS-tag vector (pRESETB), but the product did not turn out soluble despite the fact that the protein is not

predicted to be membrane bound. Addition of a HIS-tag, misfolding or occurrence of inclusion bodies might be the reason for the insolubility and a switch of expression vector might therefore be necessary.

Step2: **Purify the protein using the appropriate affinity column followed by a size exclusion chromatography.**

Depending on the tag different columns can be used for purification. Crystallization of the protein requires particularly pure samples and hence a second purification step using for example size exclusion might be needed.

Step3: **X-ray chromatography will be performed in collaboration with Pål Stenmark.**

Reference material:

Protein production and purification. Nat Methods. 2008 Feb;5(2):135-46.

Functional genomics and structural biology in the definition of gene function. Methods Mol Biol. 2009;513:199-227.

Contact:

If you are interested in this project please contact:

Irmeli Barkefors
Uppsala University
Dept. Medical Biochemistry and Microbiology
BMC
Husarg 3 Box 582
751 85 Uppsala, Sweden

Irmeli.Barkefors@imbim.uu.se
phone: +46-18-471 4276
http://www.imbim.uu.se/forskning/kreugerresearch.html