STSM-Report: October 11, 2010 to October 15, 2010 **Marieke Wottawa**

In the past years several hints for PHD isoform specific roles in cell adhesion and tumor metastasis have been found. However, the impact of PHD2 for tumor metastasis and the signaling pathways involve are not known so far. Therefore, different PHD2 knockdown cell lines were established in the department of Cardiovascular Physiology at the University of Göttingen. We used these cell lines in a RT-PCR assay studying extracellular matrix and adhesion molecules. Several genes appeared to be differently expressed in the PHD2 knockdown cell lines compared to wild type cells, which may be regulated by so far unknown PHD2 targets. Therefore, the STSM in the laboratory of Johanna Myllyharju, Oulu Center for Cell-Matrix Research, Institute of Biomedicine, University of Oulu in Finnland, was very helpful to learn the hydroxylation-coupled decarboxylation of 2-oxo[1-^{14}C]glutarate assay. The establishment of this assay in our laboratory will now allow us to study the hydroxylation of potential novel PHD2 targets and might explain by this how PHD2 is involved in regulating cell adhesion and tumor metastasis.

During the STSM I was acquainted with the protocol for the hydroxylation-coupled decarboxylation of 2-oxo[1-^{14}C]glutarate assay, which has been used earlier to characterize the hydroxylation activity of the Prolyl hydroxylase domain enzymes (PHDs) (Hirsilä et al., 2003). For an application to study potential novel PHD2 targets we additionally discussed possible modifications.

Because lacking a potential novel PHD2 target I performed the assay by using synthetic peptides of HIF-1α as substrates and the PHD isoforms 1 to 3 and the factor inhibiting HIF (FIH) as enzymes. These used enzymes were flag-taged recombinant proteins produced in insect cells. As a substrate for the prolyl hydroxylation by the PHDs the DLD19 peptide was used. This peptide harbors the N-terminal hydroxylation site of HIF-1α (Pro^{564}) which is hydroxylated by the PHDs and was published by Mirsilä et al. 2003. To study the asparagenyl hydroxylation by FIH as a substrate the DES35 peptide was used which harbors the asparagenyl residue 803 of HIF-1α which is hydroxylated by FIH (Koivunen et al., 2004).

Additionally, the effect of two histone demethylase inhibitors on the PHD hydroxylation activity was measured. Histone demethylases are like the PHDs 2-oxoglutarate-Fe(II)-dependent dioxygenases that hydroxylate the methyl groups of the histone on lysine and argenine residues. Methylation on arginine residues correlates with transcriptional activation, while methylation on lysine residues leads to either activation or repression (Kouzarides, 2002). The inhibitory effect on the proly- and asparagenyl-hydroxylase activity was measured by using a cell permeable and a modified histone demethylase inhibitor. For the three PHD isoforms and FIH a strong inhibitory effect of the histone demethylase inhibitors was only

observed at higher concentrations about 5 mM and inhibitory effect of the unmodified, cell permeable histone demethylase inhibitor was better at lower concentrations compared to the modified substance (exemplarily shown for PHD1, Fig. 1).

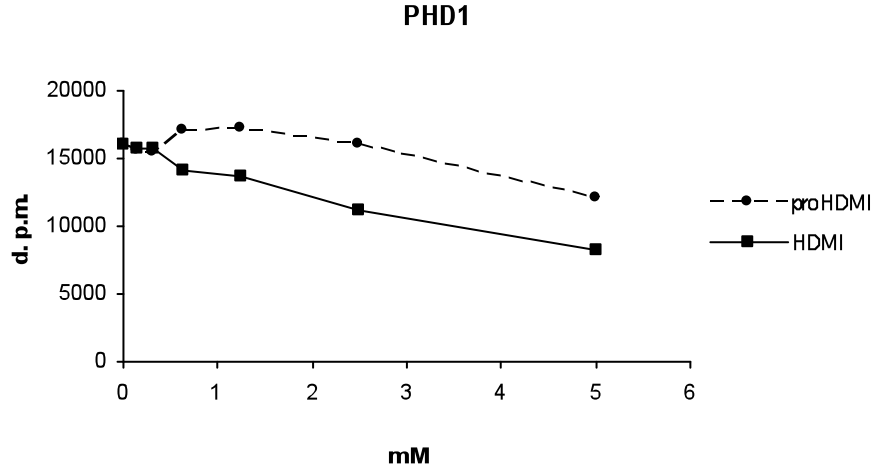

Fig. 1: decarboxylation of 2-oxo[1-^{14}C]glutarate assay using recombinat PHD1 as enzyme and the DLD19 peptide as substrat. A cell permeable histone demethylase inhibitor (HDMi) and a modified histone demethylase inhibitor (proHDMi) was added in increasing amounts from 0,156 to 5 mM.

After the STSM in the laboratory of Johanna Myllyharju I am now able to establish the hydroxylation-coupled decarboxylation of 2-oxo[1-^{14}C]glutarate assay in our laboratory to study the potential hydroxylation of novel PHD targets.

Additionally, the STSM gave me the opportunity to discuss the results of my current project focusing on the involvement of PHD2 in tumor metastasis with the members of the laboratory of Johanna Myllyharju. Furthermore I got a deeper insight in the projects currently worked on in the hosting institute. Therefore, the STMS was very beneficial to strengthen the collaboration of our laboratories.
By visiting lectures and seminars I got a deeper insight into research at a Finnish university and the possibilities for young postdocs in Finland.
Therefore, I am very grateful that I got the chance to go to the laboratory of Johanna Myllyharju during a STSM and I would like to thank Johanna Myllyharju for her generous hospitality.

Sincerely,

Marieke Wottawa

Hirsilä M, Koivunen P, Günzler V, Kivirikko KI, Myllyharju J., Characterization of the human prolyl 4-hydroxylases that modify the hypoxia-inducible factor, J Biol Chem., 278(33):30772-80, 2003
Koivunen P, Hirsilä M, Günzler V, Kivirikko KI, Myllyharju J., Catalytic properties of the asparaginyl hydroxylase (FIH) in the oxygen sensing pathway are distinct from those of its prolyl 4-hydroxylases, J Biol Chem, 279(11):9899-904, 2004
Kouzarides T, Histone methylation in transcriptional control, Curr Opin Genet Dev, 12: 198−209, 2002