Cyagen Animal Model Award (CAMA)

Sample Application

Summary of the proposed study:

Deciphering the role of Cy1 and Cy2 in pulmonary hypertension.

Pulmonary hypertension (PH) is a severe life-threatening disease characterized by progressive increase of pulmonary vascular resistance leading to right ventricle (RV) hypertrophy (RVH) and ultimately heart failure. The pathophysiology of PH involves dysfunction of endothelial cells (ECs) and smooth muscle cells (SMCs), however the exact molecular mechanisms contributing to progression of PH are still poorly understood.

Mutations in TGF-β superfamily receptors cause PH. It is known that TGF-β receptors play an essential role in endothelial cell function since mice lacking these receptors die during early development due to severe vascualar defects. Cy1 and Cy2 were recently shown to bind to Rx1 implicating a role of this Cy1/Cy2/Rx1 pathway in vascular development and maintenance of vascular quiescence. However, it is not known whether Cy1 and Cy2 are important for vascular remodeling during PH or play a pathophysiological relevant role for this disease.

We aim to decipher the role of Cy1 and Cy2 in the development of PH. Specifically, we will establish new and novel mouse models to elucidate the precise (and synergistic) role of Cy1 and Cy2 during vascular remodeling in PH. Using a combination of in vivo and in vitro approaches we will investigate how Cy1/Cy2/Rx1 signaling functions in the maintenance of pulmonary and cardiac vascular homeostasis and during disease. Specifically, we will investigate and monitor changes in the expression of identified downstream targets and microRNAs in mutant mice. We will also cross our newly generated Cy1 and Cy2 mutant mice to generate tissue-specific mutants to understand the function of Cy1 and Cy2 in different cells of the vasculature.

Description of the animal models and proposed experiments:

We will use CRISPR to generate constitutive Cy1 KO($Cy1^{-/-}$) and ES cell targeting to generate conditional Cy2 knockout ($Cy2^{cKO}$) mice. To obtain tissue-specific Cy2 KO mice we will cross $Cy2^{cKO}$ mice with several endothelial and smooth muscle cell-specific Cre lines. In addition we will cross Cy1 KO mice with $Cy2^{cKO}$ to generate Cy1/Cy2 double KO (DKO) mice.

1. Characterisation of Cy1 and Cy2 mutant mice

We will perform a comprehensive morphological characterization of the vasculature of Cy1 and Cy2 mutant mice. To understand the role of Cy1 and Cy2 during PH we will induce PH by hypoxia. After induction of PH we will analyze pulmonary hemodynamics, RV function and investigate the pulmonary vasculature using MRI, echocardiography, and right heart catherterization. Subsequently, tissue will be frozen for further analysis by quantitative RT-PCR (qRT-PCR), immunohistochemistry and immunostaining. In addition, we will perform µ-CT on hearts and lungs from single and Cy1/Cy2 mutant mice to generate high-resolution 3D images of the microvasculature.

2. Dissection of Cy1/Cy2-dependent signaling pathways in the vasculature.

To identify Cy1/Cy2-dependent signaling pathways relevant for vascular remodeling, we will perform gene expression, miRNA and proteomic profiling of RV and lung tissue, including laser-assisted microdissection of vessels. Lungs and hearts from normoxic and hypoxic controls as well as from single and double Cy1 and Cy2 mutant mice will analysed. To identify direct target genes of Cy1/Cy2 signaling we will perform chromatin immunoprecipitation (ChIP) experiments combined with massive parallel DNA sequencing (ChIP-Seq).

3. Characterization of downstream targets of Cy1 and Cy2.

Interestingly, we have shown recently that the loss of Cy1 and Cy2 in endothelial cells results in increased expression of FGF signalling due to altered expression of several miRs. FGF signaling can promote hyperproliferation of ECs and SMCs, hallmarks of aberrant vascular remodeling and PH progression. Therefore, we will study the role of FGF signalling downstream of Cy1/Cy2. We will cross our Cy1/Cy2 mutant mice with FGF reporter mice to determine in vivo the pattern of FGF activation under basal conditions and in response to PH.

4. Identification of Cy1 and Cy2 as targets for therapeutic intervention.

We will attempt to reverse adverse effects resulting from the loss of Cy1 and Cy2 in PH by treating our mice with existing agonists/antagonists. Depending on the outcome of our experiments we plan to manipulate Cy1/Cy2 levels in vivo in preclinical models using non-genetic means to rescue a potential phenotype and/or to ameliorate PH.

Expected outcome and its potential impact on the field:

At present it is not known whether Cy1 and Cy2 play a role in the progression of PH. By generating Cy1 KO and Cy2 cKO mutant mice we will for the first time determine how the loss of Cy1 and Cy2 impacts on the progression of PH. These new mouse models will also allow us to determine whether altered TGF-β signaling resulting from the loss of Cy1 and Cy2 leads to changes in downstream targets and aberrant vascular remodeling. Analysis of signaling pathways combined with ChIP-Seq, transcriptional and proteomic profiling and use of specific agonists/antagonists will identify relevant pathways and Cy1/Cy2 target genes responsible for vascular remodeling. Ultimately, the generation of these new mouse models will significantly improve our understanding of the molecular mechanisms responsible for PH.