Masters project in Caroline Hill's laboratory, Cancer Research UK London Research Institute

The generation and analysis of AAV-mediated tagged knock-ins of the TGF-β receptors

Our laboratory at the Cancer Research UK London Research Institute focuses on the transforming growth factor β (TGF-β) superfamily of ligands which comprises the TGF-βs, Activin, Nodals, BMPs and GDFs. These ligands are known to control many key aspects of embryonic development and adult tissue homeostasis, and TGF-β, BMP and Nodal signalling have been strongly implicated in cancer. The best understood downstream signalling pathway activated by these ligands is the Smad pathway. Ligands bind to a type II TGF-β receptor, which complexes with and transphosphorylates a type I receptor. This activated form of the type I receptor is then competent to phosphorylate the receptor-regulated Smads (R-Smads), the effectors of the pathway, at their C-terminus. The Smads accumulate in the nucleus where they can regulate gene transcription in conjunction with other DNA-binding transcription factors (for a review, see (Schmierer and Hill, 2007)).

In our lab we want to understand how these pathways function both *in vitro* and *in vivo* using model the powerful combination of model tissue culture systems and vertebrate developmental systems, especially early zebrafish and *Xenopus* embryos. These systems have given us insights into the mechanisms and dynamics of TGF-β signalling, and how its deregulation can lead to tumourigenesis.

We have recently published an analysis of the dynamics of canonical TGF-β signalling, which signals through the type I receptor ALK5 and the type II receptor TβRII to phosphorylate Smad2 (Vizan et al., 2013). We showed that regulation of receptor localisation was critical for controlling the dynamics of Smad2 phosphorylation. More specifically, rapid internalisation of receptors upon ligand binding left cells in a refractory state where they were unresponsive to further acute stimulation. We are currently trying to determine the mechanism by which receptors are trafficked to the cell surface, internalised and targeted for degradation.

This project will focus on understanding whether similar principles underlie signalling through other TGF-β superfamily members, specifically the BMPs, Activin and Nodal. We currently lack good reagents to visualise the receptors for these ligands, so a key component of the project will be the use of novel adeno-associated virus (AAV) technology (Khan et al., 2011) to generate endogenous knock-ins of tagged receptors. The student will knock in fluorescent tags and short epitope tags in the endogenous BMP type I receptor ALK2 and in the endogenous Activin/Nodal type I receptor ALK4. For ALK2, this will be done in the MDA-MB-231 breast cancer line, that responds well to BMPs. For ALK4, we propose to use the P19 embryonic carcinoma cell line that we are currently using in the lab to understand Activin and Nodal signalling dynamics and function.

Cell lines with these tagged receptors will then be used to compare and contrast signalling through BMP, Activin, Nodal and TGF-β. The student will be able to track the internalisation of the receptors through immunofluorescence and the use of an

assay to specifically label cell surface protein populations. It may also be possible to identify factors that interact with the receptors via immunoprecipitation, something that is extremely difficult to do with the endogenous untagged proteins. We anticipate that a detailed knowledge of the dynamics of signalling from the different ligands will give us a greater understanding of how TGF-β superfamily signalling is regulated in both normal and pathological situations.

References

Khan, I.F., Hirata, R.K., and Russell, D.W. (2011). AAV-mediated gene targeting methods for human cells. Nat Protoc *6*, 482-501.

Schmierer, B., and Hill, C.S. (2007). TGFβ-SMAD signal transduction: molecular specificity and functional flexibility. Nat Rev Mol Cell Biol *8*, 970-982.

Vizan, P., Miller, D.S., Gori, I., Das, D., Schmierer, B., and Hill, C.S. (2013). Controlling Long-Term Signaling: Receptor Dynamics Determine Attenuation and Refractory Behavior of the TGF-β Pathway. Sci Signal *6*, ra106.

Practical information:

Caroline's lab is part of CRUK's London Research Institutes (LRI), located at Lincolns' Inn Fields, in the heart of the London City Centre. The institute is world renowned for its basic research related to Cancer and is extremely international. More information about the institute and the lab can be found here: http://www.london-research-institute.org.uk/ .

Although the internship is unpaid and we cannot offer accommodation, all consumables and benchfees are covered by CRUK. A Dutch master student who is currently working here has managed to get stipends from several sources such as the Nederlands Kanker Instituut (NKI) the Erasmus program. If needed, we could assist with finding a place to live.

On a day to day basis the student(s) will be working closely together with either a post doc or PhD student but they will be directly supervised by Caroline. Our lab consists of ten people in total who get along very well and also get together outside of work hours. There are two Dutch speaking lab members that may be able to help out with general questions with respect to translating administrative matters.

Motivated students who are interested can contact Caroline directly by email (caroline.hill@cancer.org.uk) with a CV and names of two referees.

Questions can also be addressed to:

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