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

Exploring a non-canonical Smad pathway downstream of the TGF-β receptors

The Hill laboratory at the Cancer Research Institute London Research Institute focuses on the transforming growth factor β (TGF-β) superfamily of ligands, comprising TGF-βs, Activin/Nodals and BMPs/GDFs. These ligands control key biological processes in embryonic development and adult organisms through their ability to regulate cell proliferation, migration, adhesion, differentiation and survival. Moreover, aberrant TGF-β, Nodal and BMP signalling has been strongly implicated in cancer. The best understood signalling pathway downstream of these ligands is the Smad pathway. Ligand binding activates a receptor complex comprising type I and type II receptors. The activated type I receptor then phosphorylates receptor-regulated Smads (R-Smads) at their extreme C-terminus, enabling them to form complexes with Smad4. These Smad complexes accumulate in the nucleus where they regulate the transcription of target genes in conjunction with other DNA-binding transcription factors (for a review, see (Schmierer and Hill, 2007).

In my lab we want to understand how these pathways function in normal physiological contexts, such as early embryonic development and untransformed adult cells, and how deregulation of these signalling pathways contributes to tumorigenesis. To do this we use the powerful combination of *in vitro* and tissue culture systems with early vertebrate development, in particular, zebrafish.

In 2008 we made the unexpected discovery that as well as inducing phosphorylation of the R-Smads, Smad2 and 3, TGF-β also induces phosphorylation of Smad1/5 in the majority of epithelial cells, cancer cell lines and fibroblasts that we tested (Daly et al., 2008). We are currently trying to understand the mechanism underlying this phosphorylation and we want to establish the function of this branch of TGF-β/Smad signalling, particularly understanding its importance in tumour development and dissemination.

We have shown that two classes of type I receptor are required, ALK5 and ALK2/3, together with the TGF-β type II receptor (Daly et al., 2008). However, we know nothing about the nature of the receptor complex. This will be the focus of the Masters Student's project. Using cell lines that we have generated that express epitope-tagged versions of these receptors, the student will determine whether these type I and type II receptors form a complex in response to ligand, using both a co-immunoprecipitation approach and also an antibody-based two-site TR-FRET assay using Cy3 and Cy5-labelled antibodies (Calleja et al., 2012). In addition, we are generating an ALK2 reporter construct that fluoresces when

ALK2 is activated. This will also be used to investigate activation of ALK2 in response to TGF-β. Furthermore, we have shown that the kinase activity of ALK5 is absolutely required for Smad1 phosphorylation in response to TGF-β, but we do not understand why this is the case (Daly et al., 2008). One hypothesis is that it is required to activate ALK2 and we have some preliminary evidence for this. This will be investigated further using *in vitro* kinase assays, where the student will determine whether ALK5 can phosphorylate ALK2 *in vitro*, and if so on what sites and with what consequence for ALK2 activity. To do this, we have obtained from our collaborator, Alex Bullock (University of Oxford), recombinant coiled-coil ALK2 and ALK5 fusions that can form complexes and thus mimic receptor association upon ligand binding.

In addition, if time, the student will take a proteomic approach to discover additional components that promote activation of these non-canonical receptor complexes. Using an MDA-MB-231 cell line that stably expresses FLAG-ALK2, the student will screen for interactors of ALK2 in the absence and presence of TGF-β signalling. Large scale immunoprecipitations will be performed, followed by Mass Spectrometry analysis at the LRI Mass Spectrometry facility. We will focus particularly on identifying proteins that associate with ALK2 in a ligand-dependent manner in the membrane fraction.

References

Calleja, V., Leboucher, P., and Larijani, B. (2012). Protein activation dynamics in cells and tumor micro arrays assessed by time resolved Forster resonance energy transfer. Methods in enzymology *506*, 225-246.

Daly, A.C., Randall, R.A., and Hill, C.S. (2008). Transforming growth factor β-induced Smad1/5 phosphorylation in epithelial cells is mediated by novel receptor complexes and is essential for anchorage-independent growth. Molecular and cellular biology *28*, 6889-6902.

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

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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