Project outline for an M. Sc. student

STAT3 regulation of Cystic Fibrosis Transmembrane Regulator (CFTR) in Cystic fibrosis

Introduction

A major challenge in human genetics is to understand how regulatory single polymorphisms in non-coding region of the human genome functions and modifies human diseases outcome. A transcription factor is a protein that binds with a specific DNA sequence and thus controls transcription of DNA genetic information to messenger RNA. STAT3 is a transcription factor which belongs to STAT group of transcriptional regulators. In particular, STAT3 is involved in regulation of many cellular processes like regulating differentiation of T helper cell 17, apoptosis and stemness of cells. Labenski et al. (2011) demonstrated that STAT3 is a modifier of cystic fibrosis disease manifestation in patients. Moreover, we know that the intragenic background of the disease causing gene CFTR determines cystic fibrosis disease severity (Mekus et al. 2003). This project bridges both observations and aims to find out which of the variants within the CFTR gene alter STAT3-mediated gene regulation of CFTR.

Project proposal

The group is currently sequencing the CFTR gene comparing patients with mild and severe course of the disease. At the start of the project, we will contribute a list of variable positions within the CFTR gene determined by next generation sequencing. The student will determine biochemically how these SNPs of the CFTR gene interact with STAT3. First, the student will screen for STAT3 binding sites on the CFTR gene using *in silico* technologies. STAT3 functions by using it´s binding motifs in different cellular scenarios to modulate gene expression of targets cells. Initial bioinformatic analysis of STAT3 binding sites on the CFTR gene have identified general STAT3 binding sites and cell type specific binding sites.

After this phase when promising candidate SNPs have been identified by the *in silico* techniques, the project will use tools to examine DNA-protein interactions in order to test how STAT3 interacts with CFTR SNPs. Electrophoretic mobility shift assay (EMSA) and supershift assay both with nuclear protein extracts and recombinant proteins will be used. Subsequently, we will further prove the identified interaction by Co-IP, western blotting and protein mass spectrometry.

Timeline: Earliest start for this project is the end of 2015 / beginning of 2016 as the NGS data needs to be completed first.

Month 1: Computational genome in-silico analysis and design of DNA probes
Month 2-4: Experimental prove for in-silico findings (EMSA, Co-IP, MassSpec)
Month 4-6: Write-up
We are flexible and will adjust accordingly to accommodate exigencies.

Reagents: All techniques to be used are all already established in the lab and cell lines to be used are already available.

Skills student will gain: Computational analysis of gene regulation, EMSA, Western blotting, Co-IP, Mass Spectrometry, Genetic association study skills.

Whom we are looking for: The student must be fluent in English and comfortable to work in an international team.

Senior Supervisor: PD Dr Frauke Stanke
Junior supervisor: Chidiebere U Awah MD M.Sc PhD student-

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