Thesis Abstract

Name: Md Mesbah Uddin Talukder

E-mail: mesbah77777@gmail.com, mmut2@cam.ac.uk

Home Institution: Department of Pharmacy
University of Asia Pacific
Block # A, House # 73/C, Road # 5A
Dhanmondi R/A, Dhaka 1209
Bangladesh

Host Institution: Department of Pharmacology
University of Cambridge
Tennis Court Road
Cambridge, CB2 1PD
UK

Title: Analysis of the structure and molecular interactions of seipin, using atomic force microscopy

Summary: In humans, disruption of the gene BSCL2, encoding the protein seipin, causes congenital generalized lipodystrophy (CGL), with severe insulin resistance and dyslipidaemia. While the causative gene has been known for over a decade, the molecular functions of seipin are only now being uncovered. In this study, I used atomic force microscopy (AFM) to examine the structure of seipin and the nature of its interactions with potential binding partners.

Most pathogenic mutations in BSCL2 represent substantial disruptions, including significant deletions and frameshifts. However, several more subtle mutations have been reported that introduce single amino acid substitutions or premature stop codons. I tested the effects on seipin structure of three mutations that cause single amino acid substitutions (T78A, L91P, A212P) and three that result in premature truncation (E113X, R138X, Q391X), in order to gain insight into how they may cause CGL. I show that wild-type human seipin forms oligomers of twelve subunits in a circular configuration, but that four of the mutants do not, the exceptions being T78A and Q391X. Hence, a failure to assemble appropriately likely represents a key pathogenic mechanism for seipin mutants. The Q391X mutant does not interact with lipin 1, a seipin-interacting protein and a critical regulator of adipogenesis, potentially

accounting for the ability of this mutation to cause CGL. The mechanisms underlying the pathogenic effect of the T78A mutation remains unclear.

I hypothesized that seipin functions as a scaffold protein, bringing into close proximity proteins acting in both the adipogenic and lipolytic pathways. In support of this hypothesis, I show that seipin interacts separately with the enzymes glycerol-3-phosphate acyltransferase 3 (GPAT3), 1-acylglycerol-3-phosphate-O-acyltransferase 2 (AGPAT2) and lipin 1, and also forms a tripartite complex with AGPAT2 and lipin 1. Since GPAT3, AGPAT2 and lipin 1 operate sequentially in the lipid biosynthetic pathway, this apparent scaffolding effect of seipin may increase the efficiency of the pathway as a whole. I also show that seipin interacts with the lipid-droplet protein perilipin and the lipolytic enzyme ATGL, two critical regulators of lipolysis. This finding suggests that seipin may also structurally organize proteins operating in the lipolytic pathway.