

Screening Approaches to Understand Cellular Lipid Homeostasis

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Lipids serve multiple functions in cells including: establishing the membrane bilayer, a source of precursor molecules for signalling factors such as hormones, and a general metabolic role in energy storage. In keeping with these critical functions, cells employ intricate and strictly regulated homeostatic mechanics to maintain cellular lipid balance. To interrogate these systems, we have employed several phenotypic screening approaches, using both chemical and siRNA libraries, to identify regulators of lipid transport and biosynthesis in mammalian cell culture models [1-2]. Cellular changes were monitored by automated imaging, and image processing algorithms were used to identify screening hits, with a goal of linking compounds to their respective target proteins and pathways. These screens revealed the Wnt pathway as an unappreciated, and potent, regulator of both cholesterol homeostasis and an activator of lipid droplet accumulation in cells [1].

Lipid droplets are the primary storage organelle for neutral lipids, like triglycerides and sterol esters, in cells. While our screening efforts could conclusively establish the initial part of the canonical Wnt signaling pathway as modulating lipid droplet accumulation, we were unable to identify the signaling mediators that controlled the proximal transcriptional regulators of this organelle suggesting an unknown branch of the pathway was involved. Therefore, we performed *in silico* promoter analyses and RNAseq to identify changes in gene expression linked to the phenotype of lipid droplet accumulation. Together, this examination of transcriptional regulation lead to identification of several candidate factors that may serve as part of the “master” regulatory network controlling lipid droplet biogenesis. We found gene silencing diminished the number of lipid droplets in response to Wnt stimulation, while overexpression of these genes was sufficient to induce lipid droplet accumulation in cells, confirming the role of this transcriptional network in directing lipid droplet biogenesis.

[1] Scott, C.C., *et al.*, *EMBO Rep.*, **2015**, 16(6), 741-752.

[2] Moreau, D, Scott, C, and J. Gruenberg, *Chimia*, **2011**, 65(11), 846-848.