IMMUNOFLUORESCENT CONFOCAL ANALYSIS REVEALS TARGETING OF SND P102 TO LIPID DROPLETS

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The liver plays central roles in the organism, including the orchestration of lipid homeostasis. Lipid droplets (LD), the intracellular lipid stores, are dynamic organelles composed by a triglyceride and cholesteryl ester core with a surrounding monolayer of phospholipid and cholesterol. We now know that many proteins can associate to this monolayer. Some of them are constitutively attached (i.e. adipophilin) while others (i.e. TIP-47) translocate from other subcellular locations to LD surface under different stimuli. SND p102 is a rat protein homologue to the human p100 coactivator, whose main subcellular location is the endoplasmic reticulum. Nevertheless, its corresponding cow homologue locates also on lipid droplets of mammary gland secretory cells. The aim of this work was to analyze the targeting, if any, of SND p102 to lipid droplets in hepatocytes cultured in basal or prosteatotic conditions.

Primary cultures of rat hepatocytes were treated with 0.6 mM oleic acid to induce lipid droplet growth and proliferation. After 16h, cells were fixed and stained for immunocytochemistry. Neutral lipids were stained with Bodipy 493/503; adipophilin (also called ADRP, adipocyte differentiation related protein) and SND p102 were visualized with fluorescent antibodies. Micrographies were acquired with a laser scanning confocal microscope (Olympus Fluoview FV500).

Neither in basal nor in steatotic conditions was colocalization detected by the naked eye. However, in steatotic hepatocytes there was correspondence of fluorescence linear profiles (Olympus FV500 Viewer) for SND p102 and ADRP in some LD and, supporting this observation, SND p102 fluorescence intensity peaked next to Bodipy’s maximum intensity points. Colocalization analysis (ImageJ) [1] of SND p102 and adipophilin was performed and Pearson’s R and Manders’ coefficients were calculated according to Costes et al [2]. Low R values were encountered for the distribution of ADRP and SND p102 both in oleate-supplemented and non-supplemented cultures. Nonetheless, Manders’ coefficients values indicated positive colocalization, it being higher in lipid loaded hepatocytes. Adipophilin amount in oleate-treated cells, as calculated by western blotting, was up to six fold that one found in untreated hepatocytes. As a consequence, the total amount of ADRP colocalizing with SND p102 is even much higher than estimated if Manders’ coefficients are only considered.

We conclude that ADRP and SND p102 share location partially when LD growth is promoted by extracellular fatty acids.