

CARF expression-based screening of steatosis-modulating compounds

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CARF expression-based screening of steatosis-modulating compounds

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Introduction: Steatosis, excessive accumulation of fat in liver, is a stressed state of liver caused by various factors such as obesity, diabetes, alcohol consumption, certain medications and non-alcoholic fatty liver disease. While it is generally considered a benign condition, prolonged steatosis often progresses to serious liver diseases including liver fibrosis, nonalcoholic steatohepatitis and hepatocarcinoma. Whereas drug development is expensive and long process, anti-steatosis natural compounds are anticipated to be useful for management of this condition and prevent its progress to complicated lethal pathologies. CARF protein has been shown to play a key role in regulating the cellular response to stress. It has been shown to control the fate of cells to apoptosis, senescence and malignant transformation by its low, high and super-high levels, respectively. Most recently it has been shown that CARF expression may serve as a quantitative marker for stress response [1,2].

Methods: We used CARF expression screening in liver-derived cells (HepG2) as an assay to select compounds with steatosis-modulating activity. Cells were treated with Free Fatty Acid (FFA) and analyzed for expression of CARF by Western blotting and immunocytostaining by specific antibodies raised in our laboratory. In parallel assays, cells were subjected to Nile Red (NR) staining. We also used an additional marker Mortalin that has been shown to regulate liver fibrosis, HCC and its recurrence.

Results: HepG2 cells treated with non toxic concentration of FFA showed downregulation of CARF suggesting its role in lipid metabolism in line with a recent report [3]. We investigated if this phenomenon could be used as an assay system to screen anti-steatosis compounds. FFA-treated HepG2 cells were subjected 30 small molecules. Expression analysis of CARF revealed modulation of CARF expression with ~18 out of 30 compounds. Parallel analyses of cells for accumulation of FFA by NR staining showed its decrease cells treated with ~14/18 compounds. Several of these compounds showed similar structure and belonged to withanolide class of phytochemicals. Furthermore, crude extracts from Ashwagandha containing mixture of these withanolides showed remarkable response suggesting use of CARF expression as a reliable reporter assay for anti-steatosis compound screening. Such compounds may offer a convenient and economic way to manage steatosis and related liver pathologies.

Conclusion: CARF-expression based screening of a small number of natural compounds led to identification of candidate steatosis-modulating compounds and warrant further molecular analyses.

1. Kalra *et al.* *Cell Stress Chaperones* **2020**, *25*, 481-494.
2. Kalra *et al.* *Exp Cell Res* **2023**, 429-442.
3. Hasan *et al.* *Cells* **2023**, 12-23.

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