

NICOTINIC ACID RECEPTOR GPR109A IS DOWN-REGULATED IN HUMAN MACROPHAGE-DERIVED FOAM CELLS

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Introduction Nicotinic acid (NA) has been known to exert favourable effects on plasma lipoproteins. It regresses atherosclerosis in human imaging studies and reduces atherosclerosis in mice, mediated by its receptor GPR109a on myeloid cells, independent of its lipoprotein effect. Since GPR109a is expressed by human monocytes, we hypothesized that NA may drive cholesterol efflux from human macrophage-derived foam cells.

Methods THP-1 cells were induced into foam cells by acetylated LDL. After treatment with NA, GW1929 (a PPAR γ agonist) and vehicle controls, cholesterol efflux was assessed using HDL3 and apo-AI as cholesterol acceptors. qRT-PCR was performed using primers for genes responsible for inflammation and reverse cholesterol transport (RCT). ELISA's for PPAR γ activity and cAMP response were performed to investigate NA's putative cellular mechanisms of action. Fluorescence immunohistochemistry on ex-vivo human carotid atherosclerotic plaques was carried out against CD68, adipophilin (a foam cells marker), as well as GPR109a. Immediately adjacent sections were stained with Oil-red-O to visualize lipid content and distribution.

Results In basal THP-1 cells, NA suppressed LPS-induced mRNA transcription of MCP-1 by $76.6 \pm 12.2\%$ ($P < 0.01$) and TNF- α by $56.1 \pm 11.5\%$ ($P < 0.01$), yet restored LPS-induced suppression of PPAR γ transcription by $536.5 \pm 46.4\%$ ($P < 0.001$) and its downstream effector CD36 by $116.8 \pm 19.8\%$ ($P < 0.01$). Whilst direct PPAR γ -agonism promoted cholesterol efflux from THP-1 derived foam cells by $37.7 \pm 3.1\%$ ($P < 0.01$) and stimulated transcription of LXR α by $87.9 \pm 9.5\%$ ($P < 0.001$) and ABCG1 by $101.2 \pm 15.5\%$ ($P < 0.01$), NA showed no effect in foam cells on either cholesterol

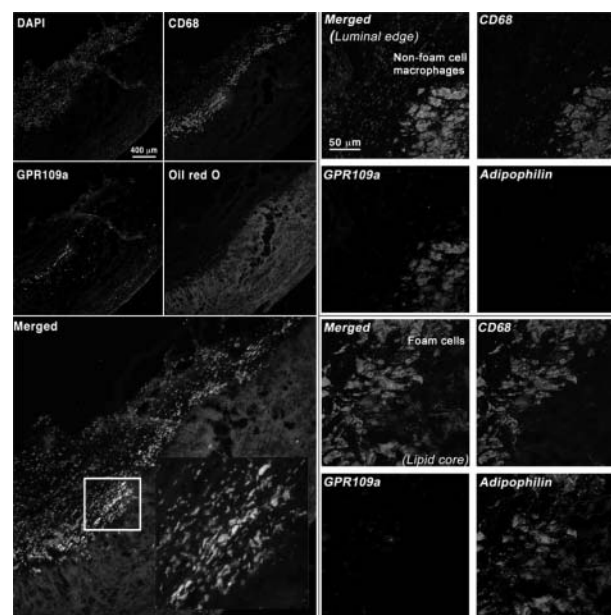


Figure 2

efflux or key RCT genes transcription. NA was found to activate PPAR γ pathway and suppress cAMP response in basal macrophages; but these effects were lost in foam cells, since its receptor, GPR109a, was down-regulated by foam cell transformation. This observation was confirmed in explanted human carotid plaques. Plaque CD68 positive macrophages were found clustered at the interface between the acellular lipid pool (LP) and the overlying fibrous cap. GPR109a co-expression was observed in CD68-positive cells outside the LP, whereas CD68-positive cells within the LP, which are highly likely to represent macrophage-derived foam cells, do not co-express GPR109a. This co-expression finding was further confirmed using antibody raised against a specific lipid-droplet associated protein, adipophilin.

Conclusions This study shows that despite NA's anti-inflammatory effect on human macrophages, it does not appear to enhance cholesterol efflux in foam cells, which is at least partially ascribed to GPR109a down-regulation upon foam cell transformation. This implies that direct NA-promotion of cholesterol efflux from foam cells may not contribute to atherosclerosis regression, which has been demonstrated with this drug.

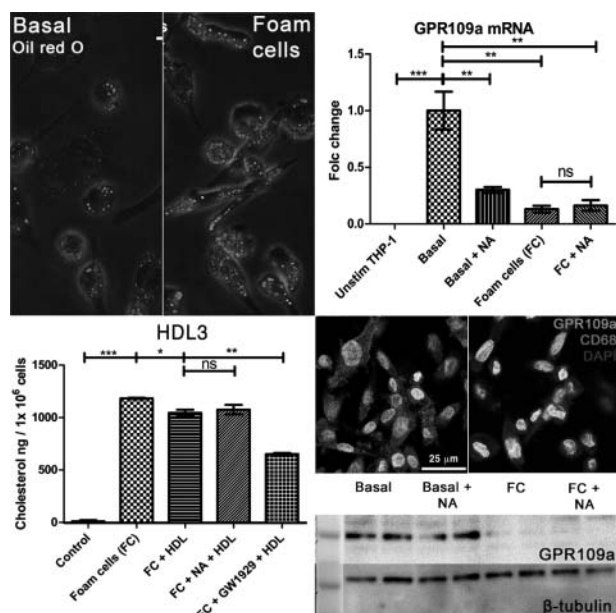


Figure 1