

Characterizing adipocyte cell free dna and its contribution to immune activation

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Abstract

Background: Adipose tissue regulates endocrine and immune homeostasis through its secretome. Growing evidence suggests that adipose tissue cell-free DNA (cfDNA) triggers the activity of recipient cells, mainly tissue resident macrophages, contributing to chronic inflammation, which characterizes obesity. CfDNA exists in various forms: protein-bound, fully naked, or encapsulated within vesicles. The biological characterization of adipocyte cfDNA and enfolded its activity to recipient cells can provide valuable information of its release mechanism and role in intercellular communication, contributing to pathogenic phenotypes.

Objective: This study aims to characterize cfDNA during adipocyte differentiation in order to understand its origin and its contribution to immune activation through effects on macrophages.

Methods: 3T3-L1 cells were differentiated into adipocytes in vitro. CfDNA was quantified in the culture supernatant of cells with varying lipid accumulation rates—normal, retarded, or accelerated—by using the standard differentiation cocktail or supplemented with DMSO or Bisphenol A (BPA), respectively. Confocal microscopy was used to examine nuclei shape, surface area, and micronuclei during adipocyte differentiation. CfDNA was isolated and analyzed by capillary electrophoresis. Then, cfDNA was administered to the murine macrophage cell line RAW 264.7 and confocal microscopy was used to demonstrate its internalization. The activation of recipient cells was estimated through expression levels of inflammation-related genes, secretion levels of proteins and antigen presentation via qPCR, ELISA and FACS analysis. TLR-9 receptor was tested as the major contributor in the macrophage activation.

Results: Our findings show that cfDNA release during adipocytes differentiation correlated with the lipid accumulation rate. Confocal images revealed smaller nuclei with blebbing and irregular border dents, as well as changes in micronuclei numbers, suggesting a connection to cfDNA release. The cfDNA's average length is around 2,000 bp, indicating an active release process. Tagged cfDNA has been shown to enter the cytoplasm of macrophages. Macrophages exposed for 6 and 24h to cfDNA demonstrated increased antigen presentation activity by elevated expression of MHCII and CD11c and expressed higher amounts of pro-inflammatory genes such as MCP-1, iNOS and CD11c as compared to untreated cells. Finally, TLR-9 gene expression levels were shown to be altered in treated macrophages.

Conclusion: Our data indicate that adipocyte differentiation is related to cfDNA active release, contributing to macrophage activation and potentially triggering a systemic inflammatory phenotype observed in obesity and related pathology.

Do you have any conflicts of interest?

No, I do not have a conflict of interest.