Disruption of adipogenesis associated to overexpression of caveolar protein, Polymerase I and Transcript Release Factor (PTRF)

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I. BACKGROUND

Adipose tissue is an endocrine organ with a paramount importance in energy homeostasis. Impaired adipogenesis renders an adipose tissue unable to expand to accommodate energy surplus, and this failure to sequester lipotoxic fatty acids has been proposed to lead ultimately to conditions such as diabetes and cardiovascular disease. While factors important for adipogenesis have been studied extensively, the factors that set the limits to adipose tissue expansion have not been determined.

Caveolae are multifunctional organelle in the plasmatic membrane very abundant and important in adipose tissue. It is proposed that the machinery for the lipid metabolism is located in caveolae. Alterations of caveolae play a role in many metabolic diseases.

II. OBJECTIVE

investigate the biogenesis of the adipocyte, to determine the factors that set the limits of its expansion

III. METHODOLOGY

Cell culture: Low passage *3T3-L1* fibroblasts were cultured with 10% FBS-DMEM medium. Two days after confluence, cells were differentiated using a hormonal cocktail with Rosiglitazone. Mature adipocytes (8 days post-differentiation) were used for the experiments

PTRF overexpression: 1) *3T3-L1* fibroblasts were transduced with lentiviral particles encoding the murine PTRF tagged by green fluorescent protein (EGFP). Cells expressing the transgene were selected and differentiated into adipocytes as described above. 2) Pharmacological induction of PTRF was carried out with 10uM dexamethasone added to mature adipocytes during 8 days.

Gene expression: RNA was isolated using Trizol and retrotranscripted to cDNA. qPCR determination was done with SYBR green

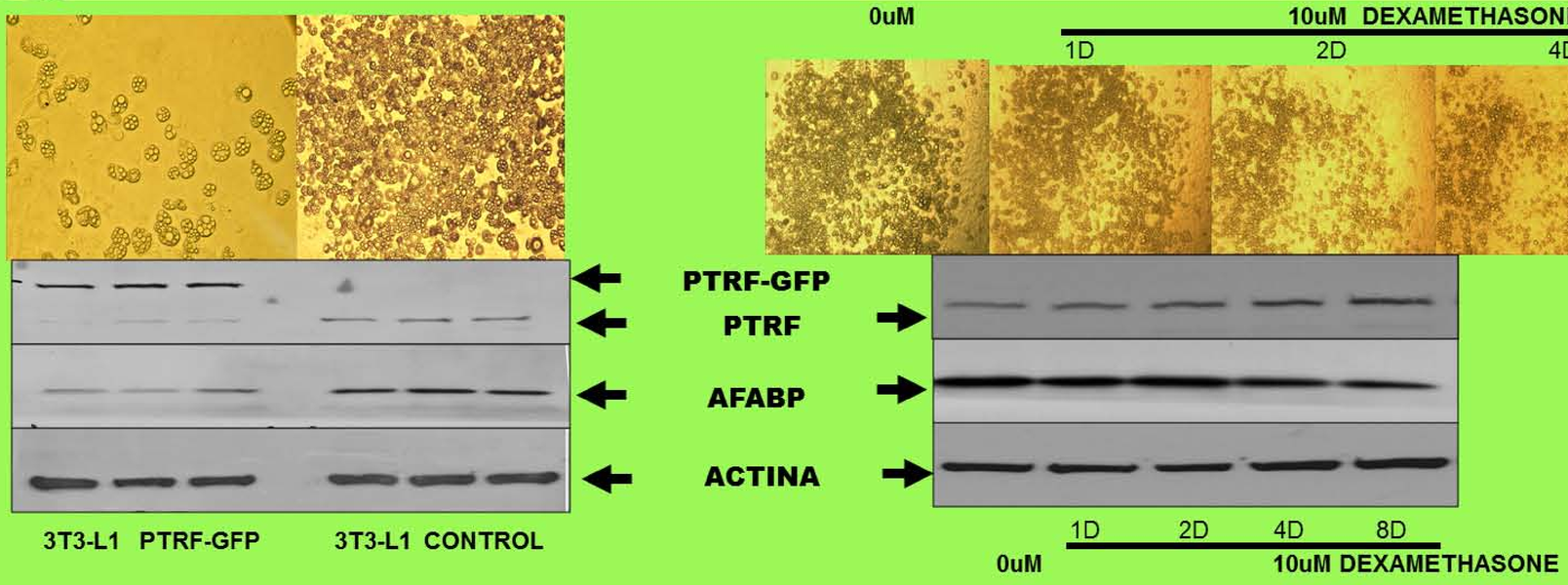
Western blot: Cells were lysed with RIPA and spun down. The supernatant was separated in 10% SDS-PAGE gel and transferred onto a PVDF membrane for inmunobloting with the appropiate antibodies .

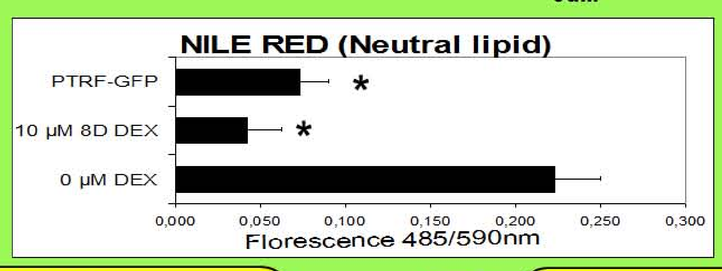
Lipid load. 1) Cells were resuspended with accutase. Nile red was use to stain neutral lipids and their quantification was performed in a BD FACSaria cytometer 2)Mature adipocytres were fixed with formaline and stained with Oil Red O.

Radiactive assays: *Lipogenesis*: the cells were loaded overnight with 3H-acetate. Later, lipids were extracted with 100% isopropanol and measured in a scintillation counter. *Lipolysis*: After overnight loading with 3H-palmitate lypolysis was induced with 10 uM isoproterenol and the released palmitate was determined in a scillation counter.

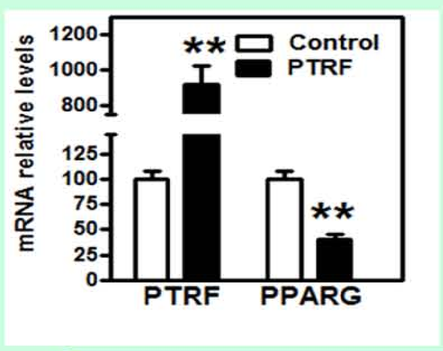
IV. RESULTS

1 **Lentiviral as well as pharmacological PTRF overexpression reduces** adipocyte fatty acid binding protein (*AFABP*), marker of **adipocyte differentiation, and lipid accumulation**, measured with Nile Red stain, in 3T3-L1 cell culture.



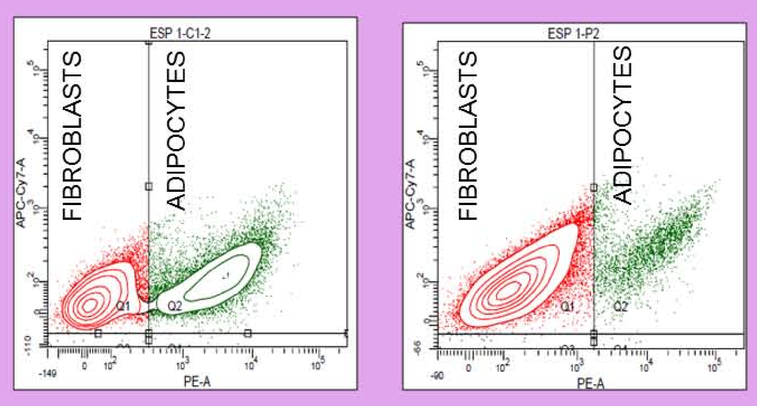


2 **Treatment with a PPAR gamma agonist**, does not rescue PTRF-overexpressing 3T3-l1 from impaired adipogenesis.

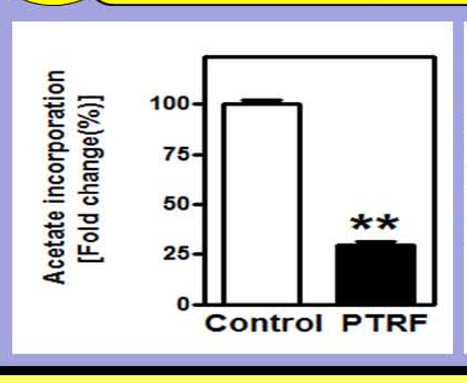


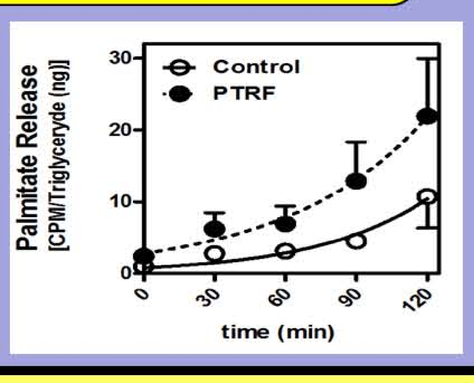
PPARγ levels are significantly lower in the line PTRF.

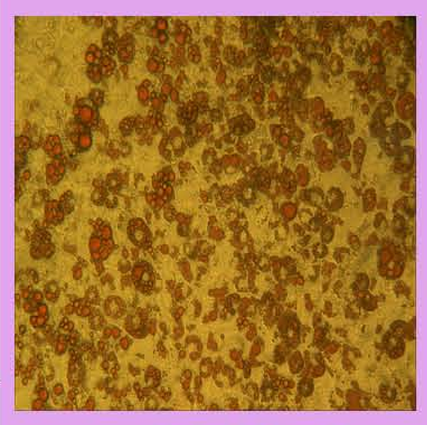
3 **Flow cytometry** and **Oil Red** lipid staining, corroborate loss of mature adipocyte in culture.

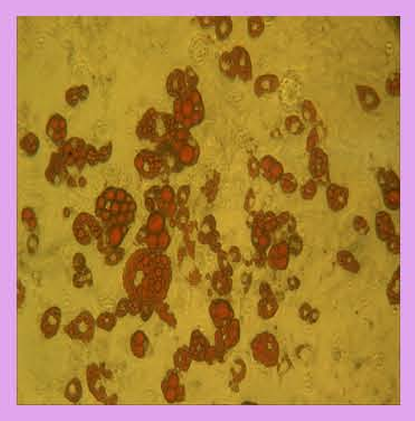


4 Lentiviral overexpression of PTRF decreased *de novo* lipogenesis and increased lipolysis in 3T3-L1 adipocytes.



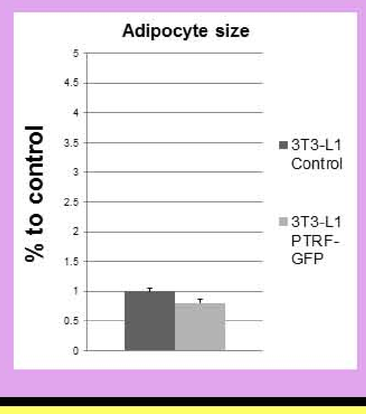


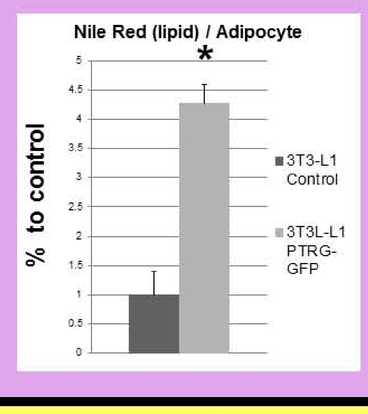




3T3-L1 CONTROL

3T3-L1 PTRF-GFP





V. CONCLUSION

Overexpression of PTRF, a caveolar protein, reduces ability to produce new adipocytes and hence limits the capacity to expand adipose tissues.

PTRF is a suitable adipocyte marker to predict pathological obesity in clinical management.