The Role Played By Alkaline phosphatases In Lipid Droplet Accumulation In Different Lipid-Storing Cell Types.

ABSTRACT

Alkaline phosphatases (ALPs) are a group of membrane-bound glycoproteins that occur in many species of animals and have a wide tissue distribution. ALPs have been shown to play a role in cell differentiation and organogenesis. In humans, the physiological role of ALP in skeletal mineralization is well documented. In routine clinical practice, ALP measurement is frequently used in the differential diagnosis of liver and bone diseases.

Studies have shown the presence of tissue non-specific ALP (*TNSALP*) activity in rat adipocytes, human preadipocytes and in a murine preadipocytic cell line, 3T3-L1. ALP has also been shown to play a role in adipogenesis in 3T3-L1 cells and human preadipocytes. The purpose of the present study was to determine whether the ALP that is expressed in a human hepatocarcinoma cell line, HepG2 has a role in intracellular lipid accumulation.

Intracellular lipid droplet accumulation in HepG2 cells was induced by addition of oleic acid coupled to albumin (Sigma-Aldrich, UK) to culture medium (Earle's Minimum Essential Medium [EMEM]) and used at a final concentration of 400μM. Tissue non-specific ALP inhibitors (levamisole and histidine) inhibited ALP activity and intracellular lipid accumulation in both the 3T3-L1 and HepG2 cells. Post-transcriptional silencing of the tissue non-specific alkaline phosphatase (*TNSALP*) gene using siRNA oligos inhibited intracellular lipid accumulation in both 3T3-L1 and HepG2 cells.

In both cell lines, the ALP mRNA levels decreased in cells transfected with the anti-ALP siRNA compared to untransfected cells. This decrease in gene expression was mirrored by a corresponding fall in ALP activity in both cell lines.

Quantification of the expression levels of the peroxisome proliferator activated receptor gamma (*PPARγ*) gene (an important regulator of adipogenesis) using real-time quantitative polymerase chain reaction (real-time qPCR) showed an upward regulation of its expression four days after induction of intracellular lipid droplet accumulation in both cell types after which the levels declined. Neither levamisole nor histidine affected the expression of *PPARγ*.

Immunostaining of HepG2 cells with monoclonal antibodies against adipophilin and staining for ALP using the ELF 97 kit (Molecular Probes, Holland) demonstrated that ALP activity was localized to the surface of the lipid droplet membrane.

A previous investigation has shown that ALP activity is higher in preadipocytes isolated from black compared to white females. Investigation of single nucleotide polymorphisms in the promoter region of the human *TNSALP* gene shows that genetic variation in the ALP promoter is not responsible for the ethnic differences in ALP activity observed in black and white South Africans.

In conclusion, the close association of ALP activity with the lipid droplet membrane in HepG2 and 3T3-L1 cells and the ability to block intracellular lipid accumulation using sequence specific oligonucleotides for ALP and pharmacological agents (histidine & levamisole) strongly indicates that ALP is involved in intracellular lipid accumulation in HepG2 cells and 3T3-L1 cells. This study also shows that *PPARγ*

gene expression increases during lipid accumulation in HepG2 cells but that inhibition of ALP with histidine or levamisole does not affect the expression of this gene. Thus, ALP must act downstream of *PPARγ* during intra-cellular lipid accumulation.