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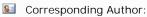
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Original Article

Overexpression of Protein Tyrosine Phosphatase 1B in HepG2 Cells Ameliorates Insulin-mediated Suppression of Apolipoprotein B mRNA Translation Via Its Untranslated Regions

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Abstract:

Background: The hepatic secretion of apolipoprotein B (apoB), containing lipoproteins, is known to be regulated by insulin, and the overproduction of these atherogenic lipoproteins occurs in insulin-resistant states. Protein tyrosine phosphatase 1B (PTP-1B) is a key regulator of hepatic insulin signaling and is also upregulated in insulin resistance. We aimed to investigate the role of PTP-1B in regulating apoB mRNA translational efficiency mediated by 5'/3' untranslated regions (UTRs) under conditions of insulin stimulation.

Methods: Human hepatoma HepG2 cells were transfected with a vector carrying the firefly luciferase reporter gene and either a chimeric apoB mRNA encoding the 5'/3' untranslated region (5'LUC3'-pGL3) or a null sequence of length equivalent to apoB 5' UTR (LUC-pGL3). The transfected cells were then infected with adenovirus carrying the mouse PTP-1B gene (AdPTP1B) in the absence or presence of insulin, and the cellular luciferase activity was determined. The RNA extracts from cells were transfected with constructs carrying 5'/3' apoB UTR, or a null sequence was also translated in vitro in a rabbit reticulocyte translation system.

Results: The luciferase activity of the cells transfected with constructs containing the apoB UTR sequences (5'LUC3') was significantly higher than that of the control constructs carrying a null sequence (p<0.01, n=12). Similar results were observed following in vitro translation studies showing a significantly higher expression of the 5'/3' UTR constructs (p<0.001, n=6). Treatment with 100 nM insulin led to a significant reduction in the luciferase activity of the constructs carrying apoB 5'/3' UTR (p<0.0001, n=12). The down regulation of the apoB mRNA translation mediated by insulin was mediated by the apoB 5'/3' UTR sequences since insulin did not affect the control constructs containing a null sequence. The infection of HepG2 cells expressing 5'LUC3' or control constructs with AdPTP-1B attenuated the inhibitory effect of insulin and led to higher levels of luciferase activity compared to the Adb $\,$ -gal infected control cells (p< 0.05, n=12). However, the activity was lower than that in the control cells infected with 5'LUC3'-pGL3 but not treated with insulin (p<0.05, n=12).

Conclusion: Our data suggest that PTP-1B can potentially modulate apoB synthesis by blocking insulin-mediated inhibition of the apoB mRNA translation via its 5'/3' UTR sequences. We hypothesize that the PTP-1B-mediated attenuation of the insulin action can lead to the upregulation of the apoB mRNA translation and contribute to a lipoprotein overproduction in conditions such as insulin resistance.

Keywords:

Protein tyrosine phosphatase 1B . Untranslated region . In vitro translation assay

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