

WNK1 Inhibition Reduces H3K9ac Expression in Hepatic Stellate Cells and Precision Cut Liver Slices

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BACKGROUND

Liver fibrosis is a leading cause of mortality worldwide, with no known cures. Fibrosis arises when chronic liver injury activates hepatic stellate cells (HSCs), signaled by growth factors such as platelet derived growth factor (PDGF). HSC activation increases proliferation, migration, and collagen secretion. To meet their increased energy demands activated HSCs upregulate glycolysis via Lysine-Deficient Kinase 1 (WNK1) signaling. This increases histone 3 lysine 9 acetylation (H3K9ac), which drives pro-fibrogenic gene expression. Whether WNK1 and H3K9ac interact directly, and the mechanism of possible interactions, is unknown. Decreased H3K9ac in response to WNK1 inhibition with WNK-IN-11 would provide evidence of a functional relationship between the two.

OBJECTIVE

The aim of this study is to determine a possible link between H3K9ac and WNK1 in HSC activation and liver fibrosis, specifically whether WNK1 inhibition with WNK-IN-11 leads to decreased H3K9ac expression in PDGF-activated HSCs and PDGF-fibrosis induced Precision Cut Liver Slices (PCLS).

METHODS

HSCs were treated for 4 hours in 10 μ M WNK-IN-11 in serum starved media, and 2 hours in 20 ng/ml PDGF. PCLS were treated for 120 hours under the same concentrations. Cells or PCLS were fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton, and blocked with either 5% BSA or 10% FBS in PBS for 1 hour. Samples were incubated with primary antibodies overnight at 4° C, followed by fluorophore-conjugated secondary antibodies and DAPI for nuclear staining. Staining was performed for H3K9ac, pWNK1, and Desmin, an intermediate filament protein which marks HSCs in tissue stains.

Results

Pharmacological inhibition of WNK1 attenuated expression of H3K9ac in response to PDGF treatment. Compared to the DMSO controls, WNK-IN-11 treatment resulted in a reduced

fold change in the intensity of H3K9ac expression in isolated HSCs as well as in Desmin-positive cells in PCLS.

CONCLUSIONS

We found inhibition of WNK-1 with WNK-IN-11 attenuated expression of H3K9ac in response to PDGF treatment in both *in vitro* and *ex vivo* models. How H3K9ac and WNK1 interact is still unclear and further research is needed.