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Ethanol-HIV Stimulates Macrophage-derived Extracellular Vesicles to Promote a Profibrotic Phenotype in Hepatic Stellate Cells

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INTRODUCTION

- Alcohol modulates the profibrotic liver microenvironment leading to advanced fibrosis/cirrhosis in people living with HIV
- Extracellular vesicles (EVs) that are released from macrophages under stress communicate with nearby hepatic cells and exacerbate liver disease progression

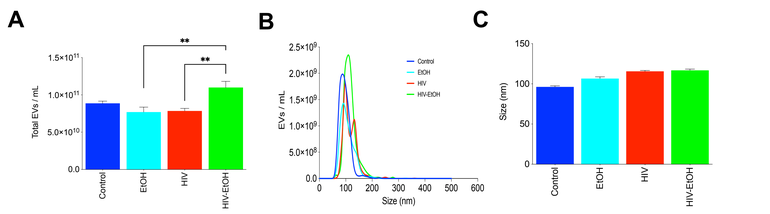
RESEARCH QUESTION

How does ethanol (EtOH) affect EV release in HIV-infected macrophages and regulate the profibrotic phenotype in hepatic stellate cells?

METHODS

- **Cells**: Human monocyte-derived macrophages (MDM), THP-1 monocytes, and hepatic stellate cells (LX-2).
- **Treatments**: Macrophages were infected with HIV (MOI 0.1) and exposed to 50mM EtOH. THP-1 cells were differentiated with PMA (5ng/mL).
- **EVs isolation and characterization**: Conditioned medium was collected from cultured macrophages for EVs isolation using ultracentrifugation. Quantification of EVs was performed using Nanoparticle tracking analysis, NanoSight NS300.
- **Transcriptional** assay by qPCR

RESULTS



RESULTS

Figure 1- EtOH-HIV treatment stimulates EV release from monocyte-derived macrophages (MDM). (**A**) EtOH and HIV-treatment increases EV secretion from HIV-infected MDM as compared to EtOH and HIV alone. (**B**) Size distribution of MDM-derived EVs. The majority of EVs released from the MDMs were in the size range of small EVs (50-200 nm). (**C**) There was no significant difference in the size of EVs within the treatment groups (**A-C**) Data were derived from NanoSight NS300. Statistical significance between the groups is indicated by asterisk(s) and determined by one-way ANOVA (** $p < 0.01$).

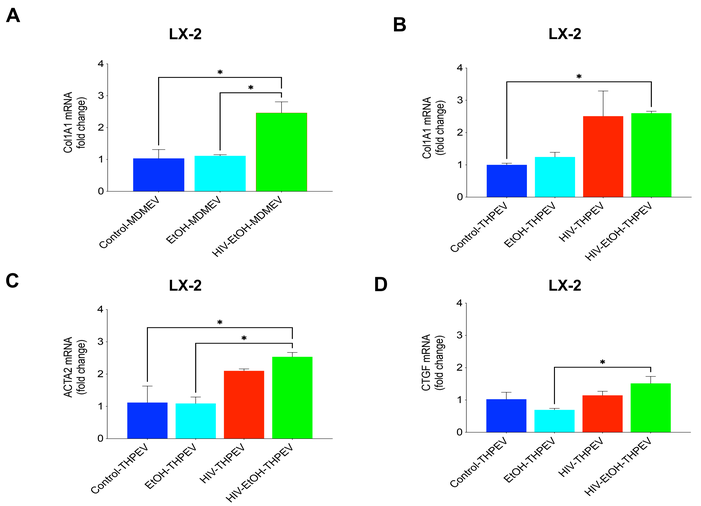


Figure 2- Profibrotic gene activation of hepatic stellate cells LX-2 by internalization of EtOH-HIV-induced macrophage-derived EVs. Transcriptional expression of profibrotic markers (**A**) Col1A1 expression in LX-2 cells exposed to MDMEV; (**B**) Col1A1 **(C)** ACTA2 and (**D**) CTGF mRNA expression in LX-2 cells exposed to THPEVs. Results represent the mean SEM. Statistical significance between the groups is indicated by asterisk(s) and determined by one-way ANOVA (* $p < 0.05$).

RESULTS

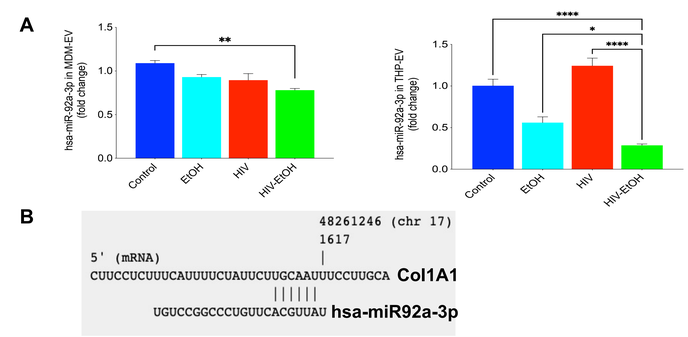
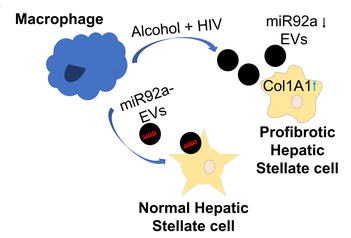


Figure 3- EtOH-HIV treatment downregulates hsa-miR92a-3p expression in macrophage-derived EVs and Col1A1 is a putative target of miR92a. Transcriptional expression of hsa-miR92a-3p in (**A**) MDMEV and THPEV; (**B**) Col1A1 is predicted as a putative target of miR92a-3p by searching miRmap database. Statistical significance between the groups is indicated by asterisk(s) and determined by one-way ANOVA (* $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$).

CONCLUSION

We conclude that a combination of ethanol and HIV stimulates macrophage-derived EVs with decreased expression of miR92a that activates the profibrotic phenotype in hepatic stellate cells and contributes to liver disease progression.



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