Delivery of extracellular vesicles-encapsulated microRNA-125b produced in genetically modified mesenchymal stromal cells inhibits hepatocellular carcinoma cell growth

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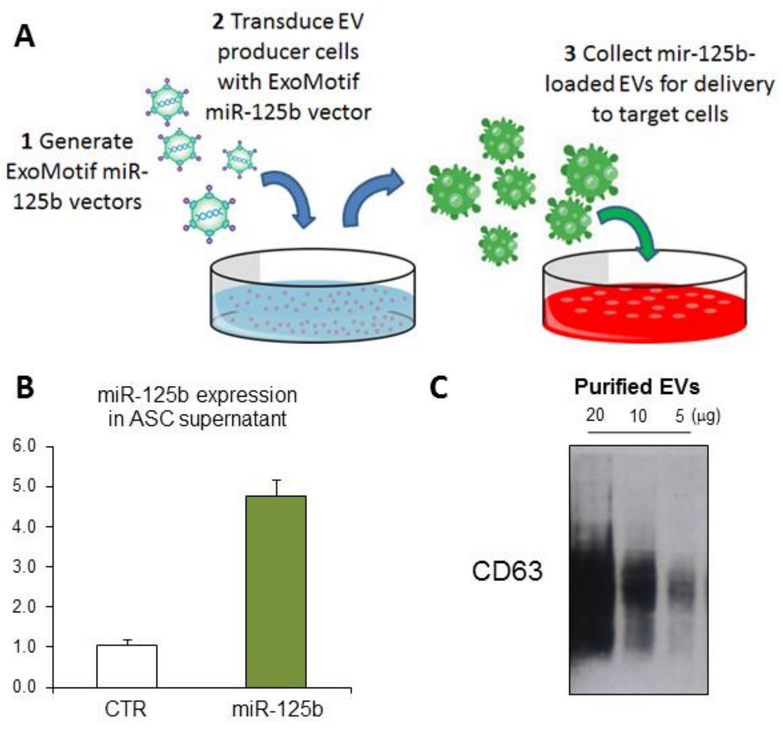
BACKGROUND

- Extracellular vesicles (EVs) are membranous vesicles originating from several cells and released in body fluids
- Delivery of therapeutic molecules such as miRNAs, through EVs may be an innovative avenue for cancer therapy.
- Over-expression of miRNA-125b in hepatocellular carcinoma cells (HCC) induces cell cycle arrest, inhibits proliferation, migration and invasion.
- We are currently evaluating a miRNA-125b replacement strategy for the treatment of HCC via delivery of EVs-containing miR-125b produced in engineered human stromal cells isolated from adipose tissue (ASCs).

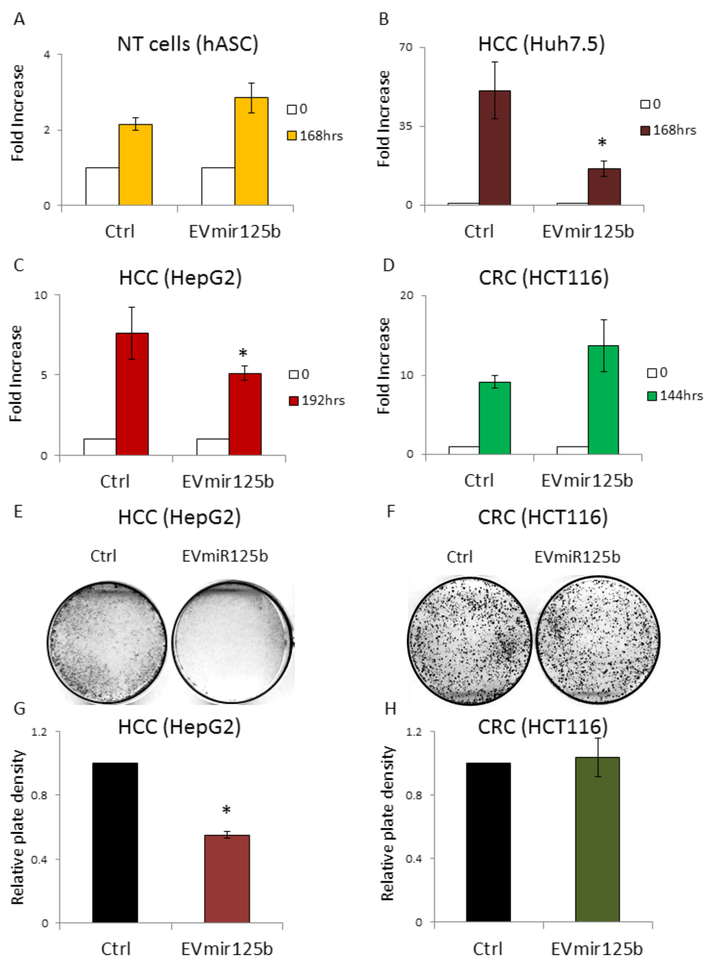
RESULTS

Adipose tissue-derived cells is a proficient source of extracellular vesicles-encapsulated microRNA-125b (miR-125b)



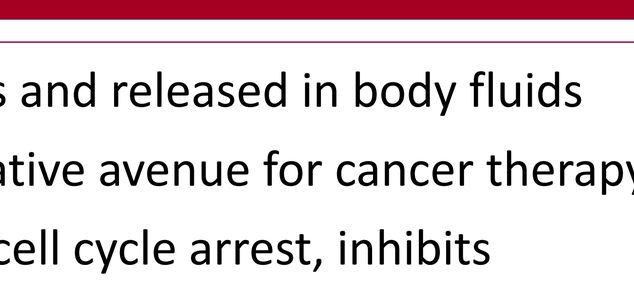
(**A**) Scheme of miR loading procedure into EVs. (**B**) ASC were transduced with lentiviral vectors expressing ExoMotif tagged miR-125b. After 48 hours conditioned medium was collected and EVs isolated. RT-PCR analysis of miR-125b expression in cell supernatant. (**C**) Immunoblot analysis of the EV marker CD63 in purified EV preparations.

ExoMotif tagged miR-125b selectively affects human hepatocarcinoma cells growth



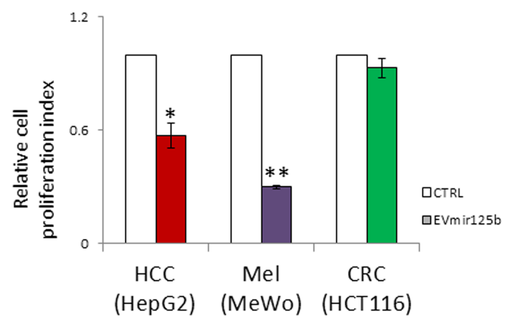
(**A-D**) Cell proliferation and (**E-H**) clonogenic assays performed on human non tumorigenic (NT) adipose tissue derived stromal cells (hASC), human hepatocellular carcinoma (HCC), and colorectal cancer (CRC) cells overexpressing EVmiR125b.

EVmiR-125b overexpression in HCC cells modulates MCL-1



EVmiR-125b gene transfer into HCC cells. (**A**) Fluorescence analysis. (**B**) Immunoblot analysis of the miR-125b target MCL1, an anti-apoptotic member of the Bcl-2 family.

EV-mediated delivery of miR-125b reduces hepatocarcinoma and melanoma cells growth



Clonogenic assay quantification performed on HCC, melanoma (Mel) and CRC cells exposed to EV-containing miR-125b purified from genetically-modified hASC.

Preliminary Conclusion & Future Direction

- It is possible to produce and purify EVs containing selected tumor suppressor miRNAs from engineered ASC.
- Future studied will focus on the possibility to exploit the tumor-homing capacity of ASC genetically-modified to secrete EVs containing miR-125b in 3D and *in vivo* models of HCC.