Studying the implication of the presence of master waiver of the genome in extracellular vesicles secreted by the ovarian tumor cell line A2780 when received by normal epithelial ovarian cells

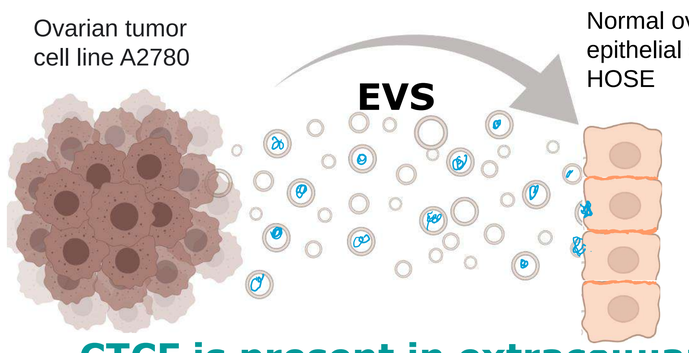
Facultad de Medicina
Clínica Alemana - Universidad del Desarrollo
Centro de Medicina Regenerativa

mdelcaampo@gmail.com
LLobos @udd.cl

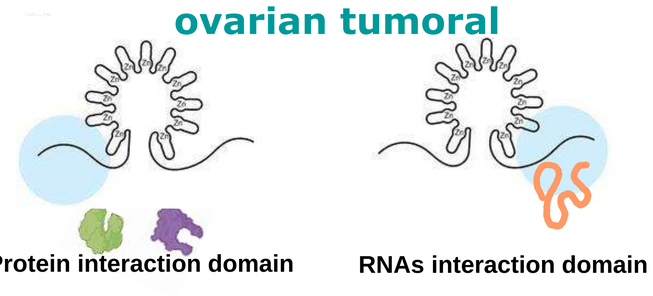
Matías del Campo-Smith, Eduardo Duran, Sebastián Urrejola, Carmen Romero Osses , Lorena Lobos-González

The master waiver of the genome CTCF modulates the differentiation and heterogeneity of eukaryotic cells through its interaction with the genome. Its functions include the structuring of the genome in loops to regulate the physical interaction between enhancer and promoter sequences, direct promotion and inhibition of gene expression, and even modulates nucleosome positioning. For this reason, the study of its role in cancer becomes crucial for understanding the progression of this disease. One of the fundamental mechanisms for cancer development is the control of the tumor microenvironment, and in this category extracellular vesicles (EVs) are crucial. To date, its presence and role in EVs have not been described. In this study we characterize the presence of this protein and its transcript in EVs secreted by A2780 ovarian cancer cells. EVs were characterized through western blot, NTA and electron microscopy CTCF protein was detected by western blot. It has recently been discovered that CTCF has a region capable of binding RNAs, which makes us suspect that this protein may be capable of sequestering RNAs and incorporating them into micro vesicles. Our bioinformatics studies showed that the RNAs with the highest probability of being sequestered in patients with ovarian cancer are MALAT1, NEAT1, PLEC, CPNE1, PNN, HGS and ADRM1. The next steps are to study the capacity of these CTCF+ EVs in modulating crucial tumor-promoting o cancer-related properties/characteristics such as invasion (Wound and transwell assays, clone, and spheroids) in the normal ovarian epithelium cells.

Experimental Set-up

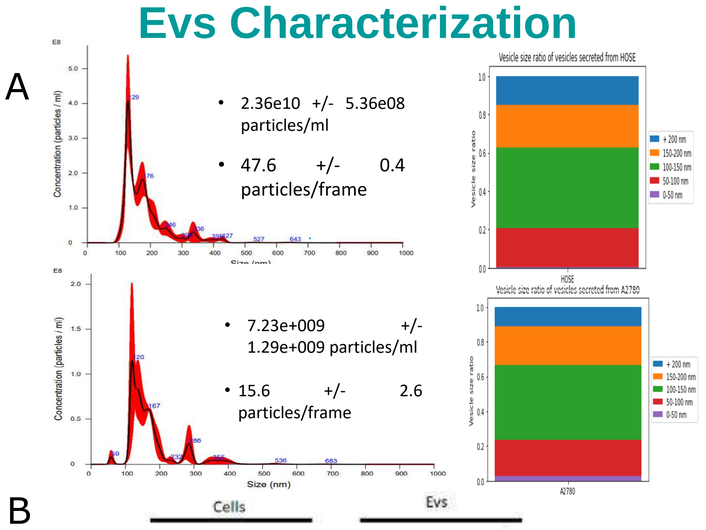


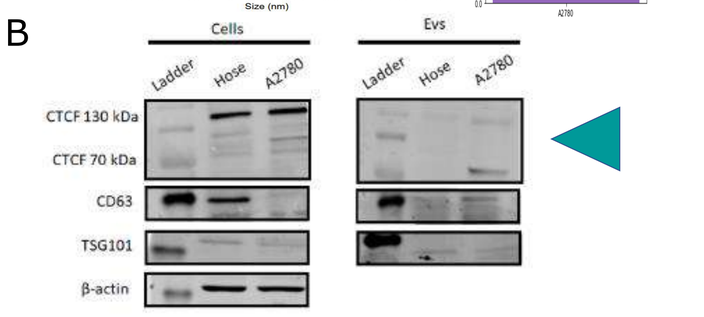
CTCF is present in extracellular vesicles secreted by A2780 ovarian tumoral



Could CTCF sequester proteins and/or RNAs to the EVs?

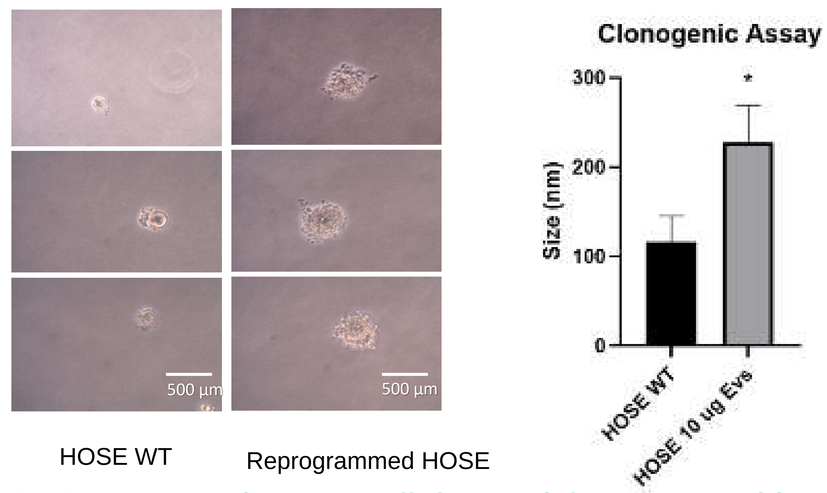
Evs Characterization





Evs characterization figure:
A) Left: NTA characterization for Evs secreted from HOSE (top) and A2780 (bottom), Right: Stacked bar plot for vesicle size ratio. **B)** Western blot of cells and their secreted EVs.

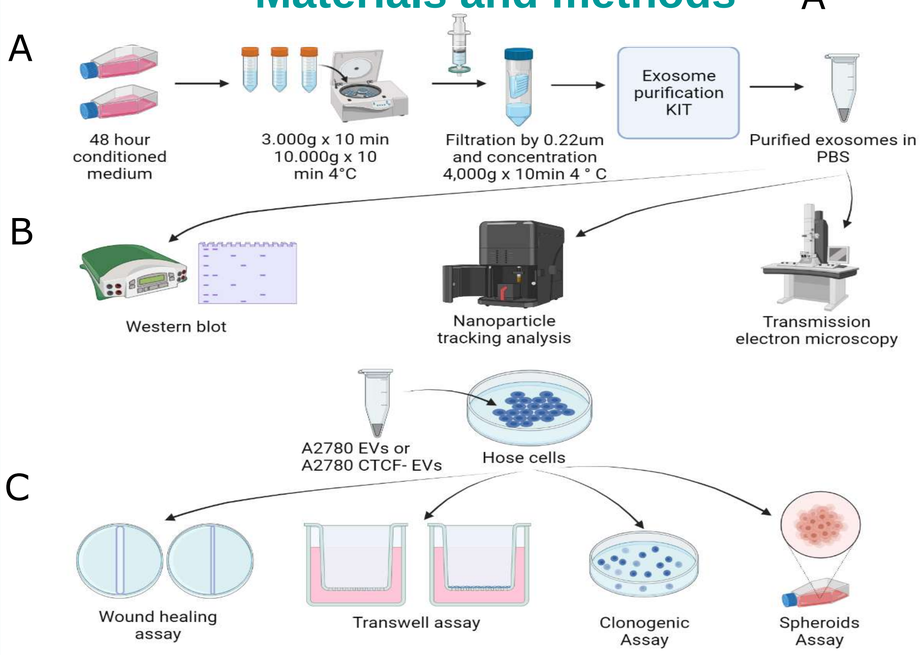
Functional Results *In Vitro*



CTCF present in extracellular vesicles secreted by A2780 ovarian tumor cells could contribute to the increase in anchor - independent growth of HOSE receptor cells.

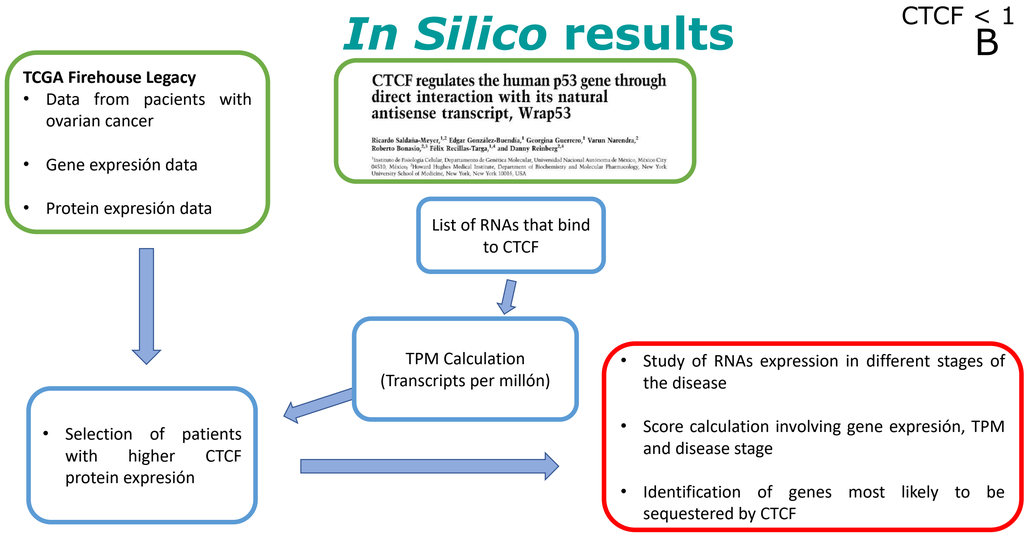
Functional results in vitro figure:
Hose cells were reprogrammed for 48 hours with EVs secreted by a2780 cells. Then, the cells were seeded on a soft agar for 15 days. **A)** Left: Clones of A2780 WT and reprogrammed A2780. Right: Quantification of clone sizes, the experiment was done in triplicate and the statistics used was a unpaired t-test.

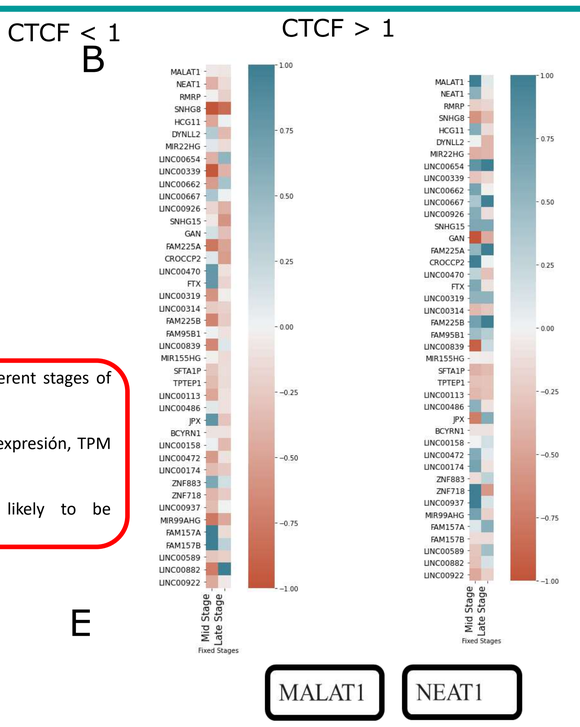
Materials and methods

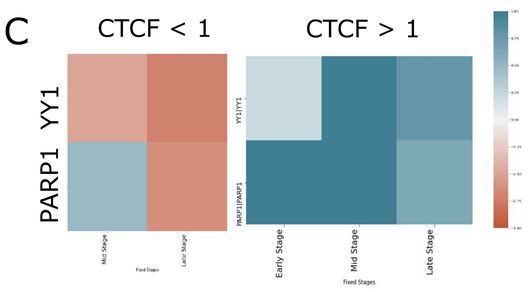


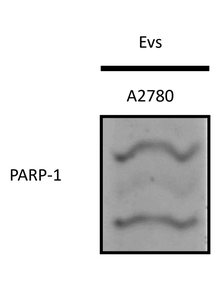
Materials and methods figure:
A) Experimental workflow for obtaining exosomes of the HOSE, A2780 and A2780 CTCF- cell lines. **B)** EVs characterization via western blot, NTA and sTEM. **C)** Hose cells will be stimulated with exosomes secreted by the A2780 or CTCF-A2780 cell line. Then, functional tests will be performed to evaluate migration and invasion (wound healing and transwell assay), anchorage-free growth (Clonogenic assay) and% of cancer stem cells (Spheroids assay). Figure generated by BioRender.com

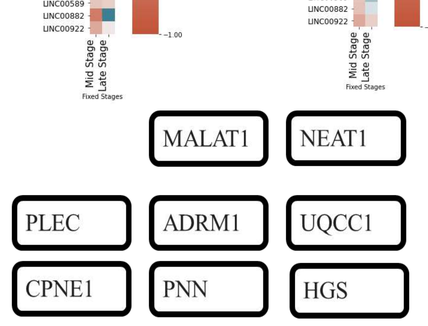
In Silico results











In silico results:
A) Experimental workflow diagram for obtaining proteins and RNAs candidates. **B)** Expression of CTCF binding LncRNAs in pacients with low and high CTCF protein expression. **C)** Expression of CTCF binding proteins in pacients with low and high CTCF protein expression. **D)** Western blot of PARP-1 of EVs secreted from A2780 cells. **E)** Selected RNAs most likely to be sequestered by CTCF.

Conclusions

- CTCF is present as a cargo in EVs secreted by the a2780 cell line.
- CTCF is a promising agent in promoting metastasis when loaded to evs due to the large number of functions in the regulation of genetic expression

Discusions

Since functional experiments have not yet been carried out, it remains to be demonstrated what is the contribution of CTCF as cargo in EVs is in increasing the metastatic properties of receptor cells.

Acknowledgment

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