Molecular Mechanisms By Which HPV Negative Cervical Cancer Cell Cycle and Cause an Increased Cell Growth

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Introduction

Exosomes are nanovesicles that are released at higher concentrations from cancer cells[1]. They have the ability to induce micro-environmental changes that could leas to the formation of tumors, turning off anti-tumor responses and it can attach its self so that it can start metastatic growth[1].

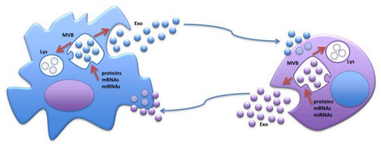


Figure 1: Exosome Communication[2]

There is evidence that demonstrates how exosomes play a role as mediators of extracellular signaling[1]. This is important because the cell cycle depends on both intracellular and extracellular responses in order to successfully proliferate.

To fully determine the molecular mechanism that is causing HPV negative cervical cancer (HNCC), it is important to understand if and how the exosomes are having an effect on the normal epithelial cell growth.

Materials and Methods

Cell Cycle Analysis

- Normal epithelial cells were fixated with ethanol for 1-24 hour periods
- Flow cytometry cell cycle analysis was completed using a Propidium Iodide (PI) stain
- The normal epithelial cells that were exposed to HNCC exosomes were examined using flow cytometry to determine which phase the cell cycle arrest occurred in

Exosome Isolation

- Conditioned media was collected from DOTC 4510 HPV negative cervical cancer cell line
- Exosomes were isolated by placing the conditioned media through 100kda MWCO Amicon filters followed by pelleting at 10,000 x g

Results

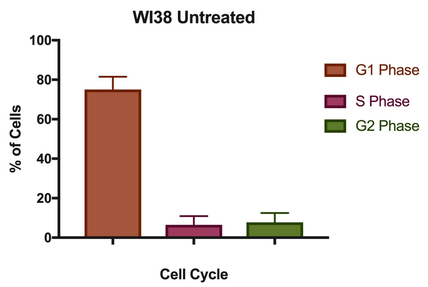


Figure 1: Cell cycle analysis of WI38 cells untreated with exosomes

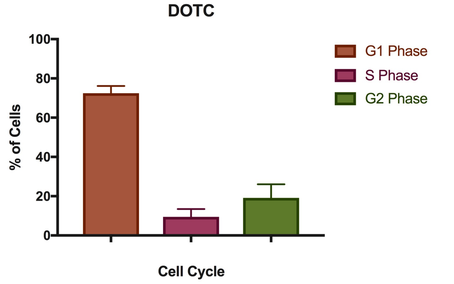


Figure 2: Cell cycle analysis of DOTC 4510 cells prior to exosome isolation

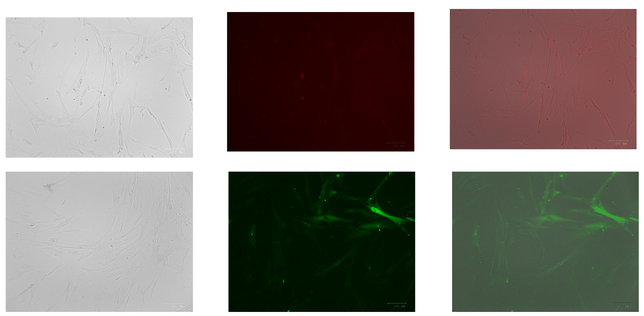
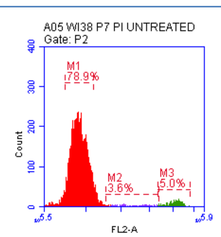


Figure 3: Isolated exosomes labeled with Exo-Glow Kit



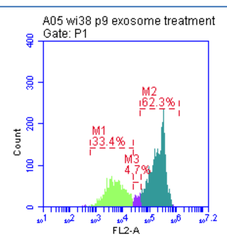


Figure 4 (left): WI38 cell cycle analysis prior to exosome treatment

Figure 5 (right): Wi38 cells after a 48 hour exosome treatment

Conclusions

- Exosomes were successfully isolated from DOTC Cervical Cancer Cells
- We do see uptake of HNCC Exosomes by WI-38 cells
- Normal WI-38 cells show a normal cell cycle distribution pattern.
- Treatment with HNCC exosomes causes WI-38 cells to be blocked in the G2 phase of the cell cycle.

Future studies:

- Try to determine which cell cycle pathways are being affected in the WI-38 cells
  - Look at the p53 checkpoint

References

1. Tickner, Jacob A., Aaron J Urquhart, Sally-Anne Stephenson, Derek J. Richard, and Kenneth J. O'Byrne. Functions and Therapeutic Roles of Exosomes in Cancer. *Oncol. Frontiers in Oncology.* 2014. Web.
2. Neviani, P., & Fabbri, M. (2015). Exosomic microRNAs in the Tumor Microenvironment. Frontiers in Medicine, 2, 47.

Acknowledgements

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