INVESTIGATING THE EFFECTS OF MUCINS ON CANCER METASTASIS

Introduction:

- Exosomes are extracellular vesicle (30 to 100 nm) derived from intraluminal endosomal vesicles. They are involved in paracrine communication between cells and organ acting as molecular shuttle of virtually all type of biomolecule [1].
- Exosomes from non-malignant tumour cells help forming “tumour niches”, in other words they create a favourable tumour microenvironment (TM) that promotes metastasis and induces tumoral behaviour in the components of the ECM (extracellular matrix) [2].
- Mucins are transmembrane glycoproteins with multiple functions, that normally work as physical barriers against bacteria, but have been observed to be overexpressed in many epithelial cancers [3].
- Integrins are heterodimeric membrane proteins that possess an active and inactive form depending on their configuration and are involved in cell attachment to the ECM and cell migration [4].

Project brief & Aim:

Investigating the processes involved in metastasis regulation and cell migration using siRNA knockdown and qPCR.

AIM: To confirm that exosomes carrying mucins from tumoral cells, will induce changes in the expression of integrins in the metastatic target tissue.

Results:

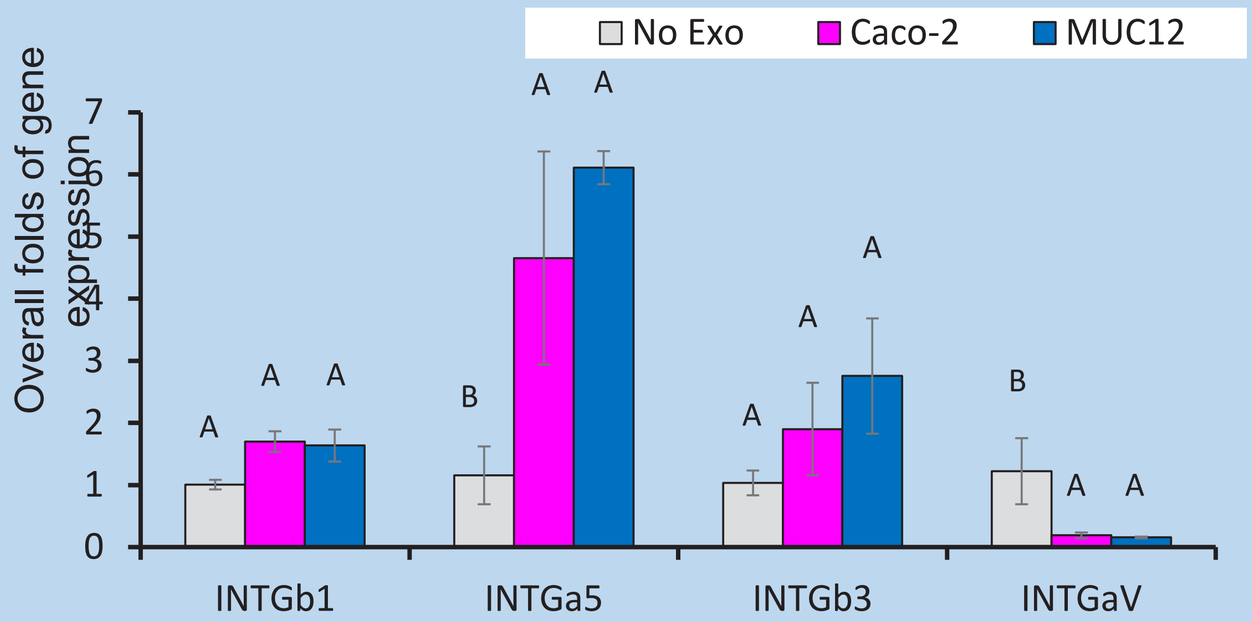


Fig 1. Integrin expression (2^ΔΔCt ±SE) after treatment with different exosomes. Shared letters mean the One-way ANOVA and Tukey test was not statistically significant (p>0.05).

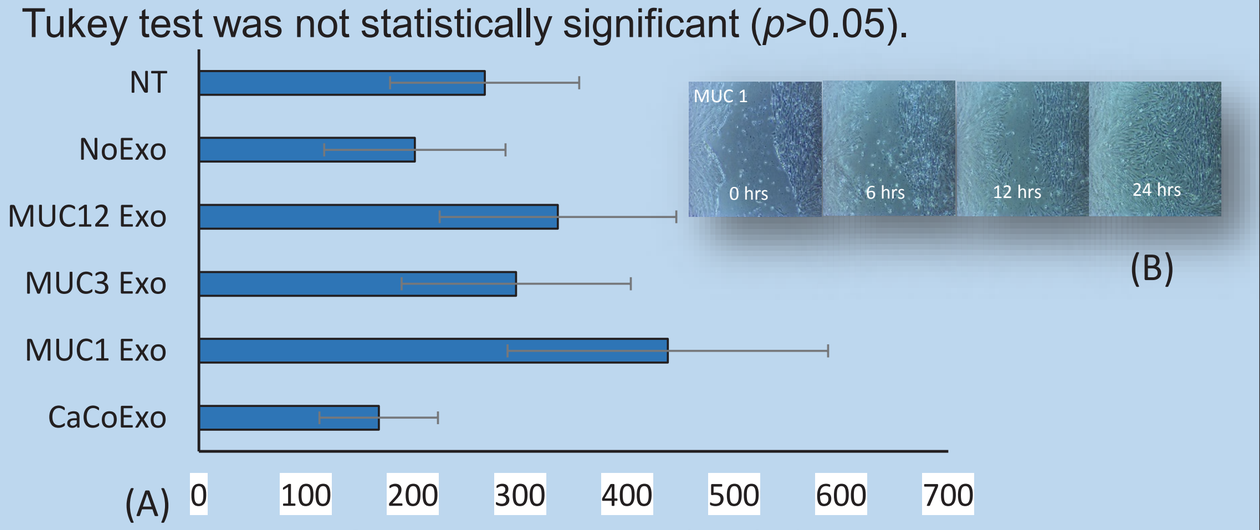
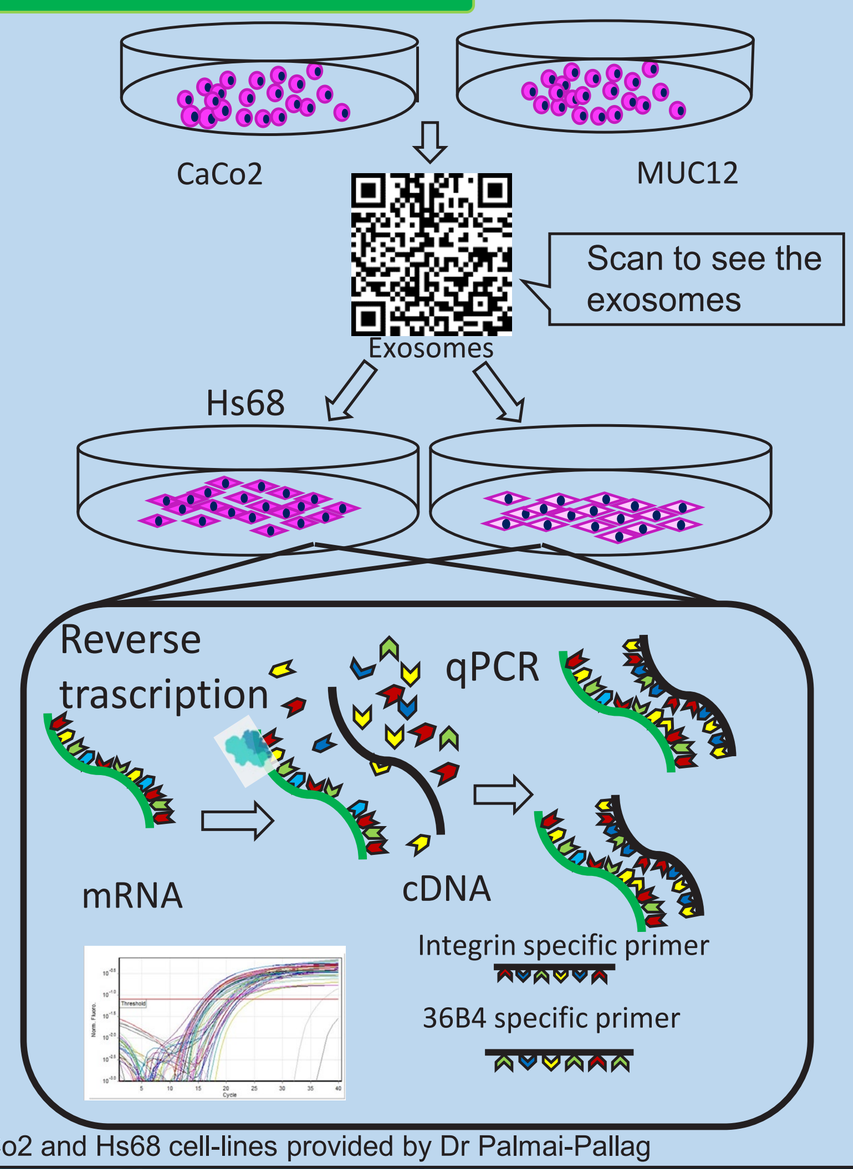


Fig 2. (A) Migration assay of Hs68 cells treated with different exosomes (±SE) pictures taken every 3 hrs for 24 hrs. Kruskall-Wallis was not statistically significant (p> 0.05). (B) Pictures of cells treated with MUC1 and scratched.

Methods:



CaCo2 and Hs68 cell-lines provided by Dr Palmai-Pallag

Ongoing research:

The next step is to knock down Mucin expression and repeat the same experiments. Preliminary data suggest that knock down of MUC12 caused a decrease of INTa5 from 5.26 folds to 2.72 relative folds of gene expression.

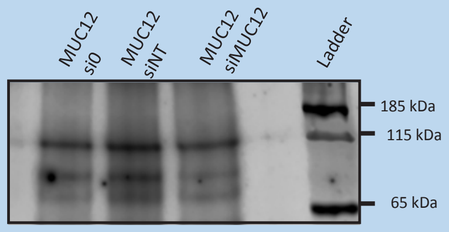


Fig 3. Western blot showing siRNA mediated MUC12 depletion in Caco2 cells

Discussion:

Previous studies showed that Integrin α5β1 (primary receptor for fibronectin) is expressed in leukemia, prostate and breast cancer cells and facilitates interaction with the bone stromal cells [4]. Our study showed that MUC12 significantly increased gene expression of α5 subunits (Fig.1). Although cell proliferation assay did not show significant changes between treatments (Fig. 2)

Moreover our results shed light into findings by Williams et al. (1999) who discovered decreased levels of expression of MUC12 in colorectal cancer. But explained it just as the reduction of MUC12 and its EGF-like motif which led to loss of normal cell signalling. We provided evidences that lower levels of MUC12 have the downstream effect of increasing INTaV and might promote bone metastasis, as αvβ3 (Osteopontin) enhanced osteoclasts’ recruitment and migration [3]. However, further experiments with siRNA are needed for validation.

References:

1. Siveen, K.S., Raza, A., Ahmed, E.I., Khan, A.Q., Prabhu, K.S., Kuttikrishnan, S., Mateo, J.M., Zayed, H., Rasul, K., Azizi, F., Dermime, S., Steinhoff, M. and Uddin, S. (2019) The Role of Extracellular Vesicles as Modulators of the Tumor Microenvironment, Metastasis and Drug Resistance in Colorectal Cancer. *Cancers,* 11(6).
2. Zhao, H., Achreja, A., Iessi, E., Logozzi, M., Mizzoni, D., Di Raimo, R., Nagrath, D. and Fais, S. (2018) The key role of extracellular vesicles in the metastatic process. *Biochimica Et Biophysica Acta-Reviews on Cancer,* 1869(1), 64-77.
3. Nath, S. and Mukherjee, P. (2014) MUC1: a multifaceted oncoprotein with a key role in cancer progression. *Trends in Molecular Medicine,* 20(6), 332-342
4. Schneider, J.G., Amend, S.R. and Weilbaecher, K.N. (2011) Integrins and bone metastasis: Integrating tumor cell and stromal cell interactions. *Bone,* 48(1), 54-65.
5. Williams, S.J., McGuckin, M.A., Gotley, D.C., Eyre, H.J., Sutherland, G.R. and Antalis, T.M. (1999) Two novel mucin genes down-regulated in colorectal cancer identified by differential display. *Cancer Research,* 59(16), 4083-4089.

Acknowledgments:
Immense gratitude for the help received by: Dr. Csanad Bachrati, Dr Enrico Ferrari, Choa ping Ng, Kristian Boasman and James Bennet.

Student: Jin Ke Michele Pan

Supervisor: Dr Timea Palmai-Pallag