DECIPHERING THE ROLE OF GLYCOSYLATED EXTRACELLULAR VESICLES ON RECIPIENT CELL BEHAVIOUR

Introduction

Alterations in the glycosylation pathway, including increased expression of the truncated O-glycan sialyl-Tn (STn), are a common feature of gastric cancer cells that correlates with patient poor survival [1,2]. Interestingly, our group has showed the presence of the gastric cancer-associated glycan STn in extracellular vesicles (EVs) [3]. EVs are small nanoparticles secreted by all cell types, including tumor cells, which have the capacity to communicate and reprogram distant cells [4].

Hypothesis: EVs released by cancer cells with more aggressive features could influence the migration capacity of the recipient cells.

Aim

Explore the impact of STn positive EVs on the modulation of the migration capacity of recipient cells.

Methodology

A genetically modified gastric cancer cell line, MKN45 SimpleCell (SC), to homogeneously synthesize the gastric cancer-associated O-glycan STn [2,5] and its wild type version, MKN45 WT, were used.

The presence of STn in the human gastric cancer cell lines MKN45 WT and MKN45 SC was evaluated by immunofluorescence.

EVs were isolated by ultracentrifugation, at 100,000g, and characterized by transmission electron microscopy (TEM), nanoparticle tracking analyses (NTA) and western blotting, where the presence of specific EV markers as well as the STn antigen was assessed.

The role of STn positive EVs in reprogramming the migration capacity of recipient cells was evaluated by a wound-healing assay. Briefly, cells were plated and left to adhere for 24h after which 5µg of EVs were added. Cells were monitored during 115h and the free space between cells was measured at different time-points. Results are shown as average ± SEM and two-way ANOVA was used for statistical analysis.

Results

Figure 1: Detection of the STn glycan in the MKN45 WT and SC cell lines. MKN45 SC cells homogeneously expressed high levels of STn, while its detection was absent in the MKN45 WT cells.

Figure 2: Characterization of the EVs isolated from MKN45 WT and MKN45 SC cell lines. (a) Presence of the EVs was evaluated by TEM. (b) Both cell lines secreted EVs with similar size, although (c) more particles were released by MKN45 SC, as assessed by NTA. (d) Specific EV markers were detected by WB in EVs isolated from both MKN45 WT and MKN45 SC cells. STn was only detected in MKN45 SC cells and EVs and an enrichment of STn antigen was found in SC EVs when compared to the parental cells.

Figure 3: Migration capacity of MKN45 WT and MKN45 SC cell lines. Premature truncation of O-glycans promoted an increased migration capacity in MKN45 SimpleCell line.

Figure 4: Migration capacity of MKN45 SC cells untreated and treated with 5µg MKN45 WT-derived EVs. The migration capacity of the MKN45 SC cell line was not affected by the treatment with WT EVs.

Figure 5: Migration capacity of MKN45 WT cells untreated and treated with 5µg MKN45 SC-derived EVs. The migration capacity of the MKN45 WT cells significantly increased when in co-culture with STn positive EVs.

Conclusions

• MKN45 SC cells secreted more EVs than WT cells.
• STn expression highly detected in the MKN45 SC cell line but not in the MKN45 WT cells or WT EVs.
• Enrichment of the STn antigen observed in MKN45 SC EVs when compared to the parental cells.
• MKN45 WT cells showed an increased migration capacity when treated with MKN45 SC-derived EVs when compared with the untreated ones.

References