Differential EGFR glycosylation in α2,3-sialyltransferases silenced pancreatic cancer cell lines.

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Introduction and objectives

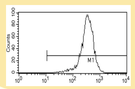
- Pancreatic ductal adenocarcinoma (PDA) is the third leading cause of cancer mortality in developed countries, with a 5-year survival rate of only 8%. This unfortunate prognosis is mainly due to the absence of symptoms and consequent delayed diagnosis as well as to the high aggressiveness and resistance of PDA to current therapies [1].
- Protein glycosylation is one of the most common post translational modifications. Aberrant glycosylation is a main feature in oncogenic transformation. N-glycans and O-glycans have a key role in tumour cell proliferation, adhesion, invasion and metastasis [2]. α2,3-sialyltransferases (STs) ST3GAL3 and ST3GAL4 are involved in the biosynthesis of the tumour associated carbohydrate antigen Sialyl-Lewis x, which plays a relevant role in the metastatic process.
- Epidermal growth factor receptor (EGFR) is a cell membrane glycoprotein overexpressed in different cancer types and it has an important role in the regulation of cancer progression. EGFR is a carrier of glycosylation changes associated to tumorigenesis, which can alter its activity and signalling [3].
- The goal of the present study is to analyse the EGFR glycosylation pattern in ST3GAL3 and ST3GAL4 silenced and wild type PDA cell lines, and to determine the influence of such changes in the activation of the EGFR and its signalling.

Experimental approach

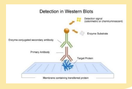
Silencing of α2,3-STs ST3GAL3 and ST3GAL4 in PDA BxPC-3 and Capan-1 cells by transduction with specific shRNAs, shRNA9 and shRNA1 respectively.

Analyses of silenced and control (scramble) cell lines EGFR levels by:

Flow cytometry



Western blotting



EGFR **Immunoprecipitation**

Cell lysates were immunopurified using EGFR monoclonal-Ab and Protein-A sepharose.

EGFR activation of cell lines

24h starving→ 10' EGF stimulation → cell lysates

EGFR glycosylation pattern analyses: WB with specific lectin against α2,6-sialic acid and SLeX antibody

Analysis of phospho-tyrosine total levels and of activated specific phospho-tyrosine/serine EGFR residues levels

Analyses of phosphorylation levels of MAPK and AKT proteins

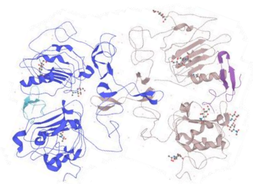


Figure 1. *EGFR model projection [4].*

Proliferation signalling Y1173 and Y1068

Internalization of the receptor S1046/47 and Y1045

Results

EGFR expression of silenced and control PDA cell lines

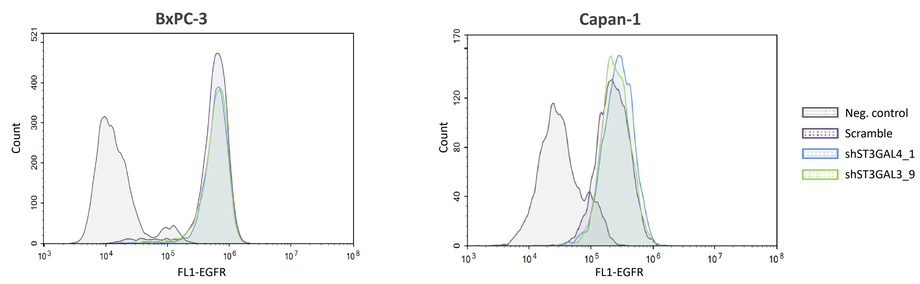


Figure 2. *EGFR expression levels of STs silenced and scramble cell lines by flow cytometry for BxPC-3 (left) and Capan-1 (right).*

Flow cytometry results showed similar EGFR expression levels of both BxPC-3 and Capan-1 ST3GAL3 and ST3GAL4 silenced cell lines compared to the parental ones, results also confirmed by Western Blot.

EGFR glycosylation pattern analysis

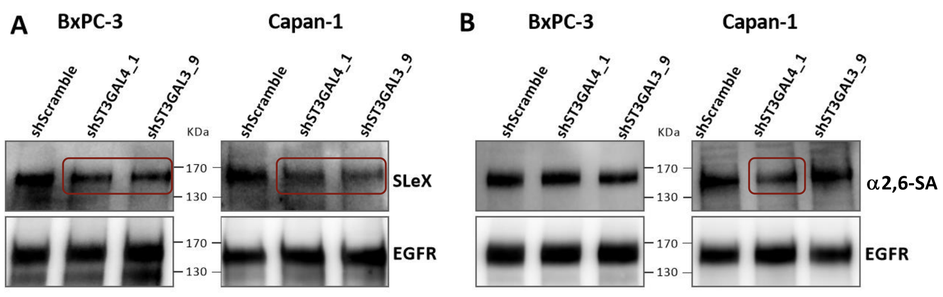


Figure 3. *α2,6-sialic acid and SLeX detection on immunopurified cell lysates from STs silenced and scramble cell lines for **A.** SLeX and **B.** α2,6-sialic acid (upper images) and EGFR detection after stripping of the membrane (lower images).*

BxPC-3 and Capan-1 silenced PDA cell lines showed a tendency to decrease SLeX expression and slight changes in the levels of α2,6-sialic acid.

Conclusions and future perspectives

- We have observed a decreasing trend on EGFR SLeX expression in silenced cell lines compared to the control ones and slight changes in the levels of EGFR α-2,6 sialic acid.
- Silencing of α-2,3 sialyltransferases ST3GAL3 and ST3GAL4 in PDA cell lines alters the phosphorylation pattern of EGFR which regulates either cell proliferation or EGFR internalization.
- Altered EGFR glycosylation affects EGFR downstream signalling pathway, an increase in AKT activation is observed in the silenced cell lines after EGF stimulation.
- Further studies are being performed to determine the influence of these alterations in the EGF induced proliferation and dimerization of the receptor.

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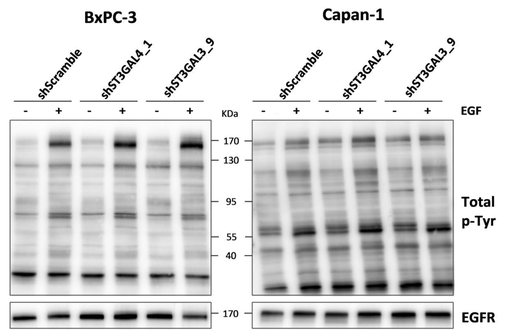
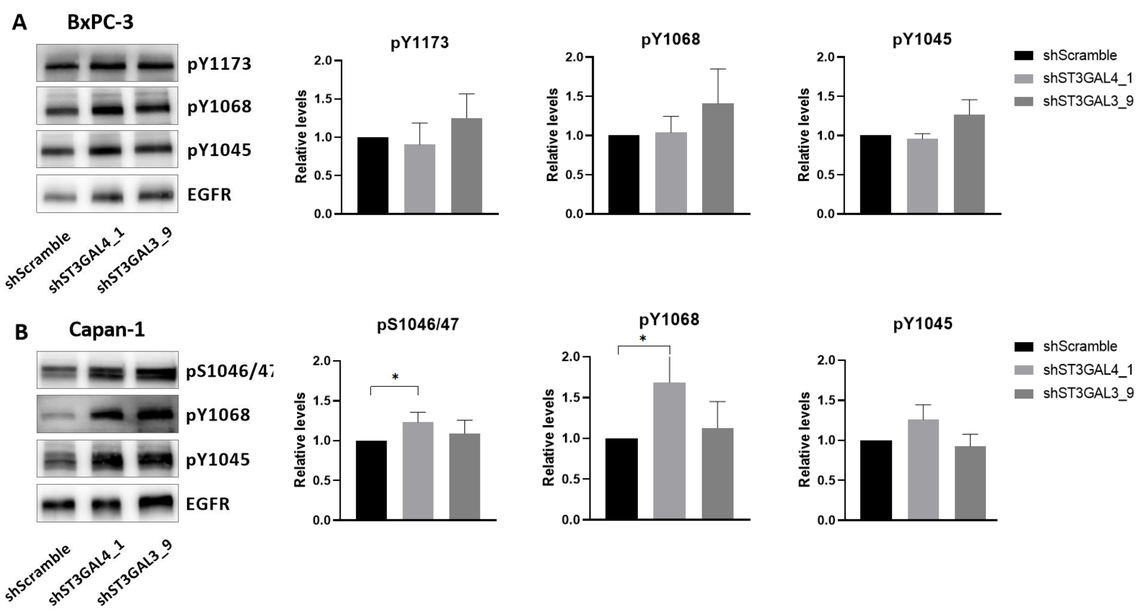
Effects of silencing STs in the activation of EGFR



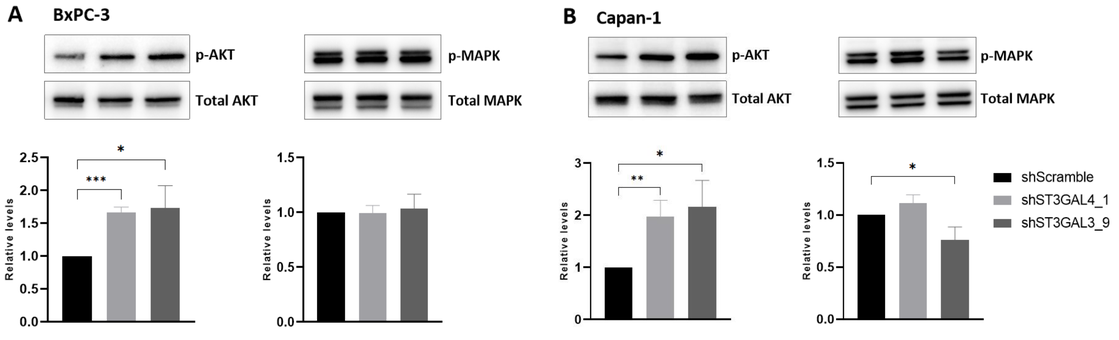
STs silenced cell lines showed higher levels of EGFR activation after EGF stimulation both in BxPC-3 and Capan-1.

Figure 4. *Analysis of the expression levels of total-phosphotyrosine residues and EGFR of BxPC-3 (left) and Capan-1 (right) EGF stimulated and control whole cell lysates.*



BxPC-3 and Capan-1 STs silenced cell lines showed a phosphorylation increase (about 30%) in the residues involved in proliferation signalling and internalization of the receptor.

Figure 5. *Analysis of the phosphorylation of the C-terminal residues of EGFR involved in the internalization of the receptor (S1046/47 and Y1045) and the proliferation signalling pathway (Y1173 and Y1068). BxPC-3 **A.** and Capan-1 **B.** representative images of WB against Y1045 , S1046/47, Y1173 and Y1068 and graphs presenting mean ± SD. The signal level of each phosphoresidue was determined by densitometry and normalized versus total EGFR protein. Densitometry values are presented relative to scramble.*



BxPC-3 and Capan-1 STs silenced cell lines showed a phosphorylation increase of AKT protein. Capan-1 ST3Gal3 silenced cell line showed decreased MAPK phosphorylation.

Figure 6. *Analysis of the phosphorylation level of MAPK and AKT proteins of **A. BxPC-3** and **B. Capan-1** EGF stimulated whole cell lysates. Representative images of WB against phospho and total levels of AKT and MAPK and graphs presenting mean ± SD. Three different blots from independently generated cell lysates were analysed by densitometry, values were normalized to AKT or MAPK total levels and are presented relative to scramble.*

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