Heparan Sulphate in Breast Cancer
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ABSTRACT

Breast cancer is the most common cancer in women worldwide. Breast cancer deaths are caused largely by the metastasis of tumour cells to the other parts of the body. The sulphation status in heparan sulphate plays roles on transforming a normal cell into a malignant and invasive cell. Targeting heparan sulphate in breast cancer is one of the challenges currently in the field of breast cancer research.

Recent studies have shown that silencing the HS3ST3A1 gene in MCF-12A breast epithelial cell lines leads decreased cell proliferation. Microarray analysis revealed that two genes, SESN2 and CDC6, are involved in the decreased in cell proliferation. The up regulation of SESN2 and down regulation of CDC6 are further analysed at protein level which is consistent to the gene expression level.

Hypothetical pathways were drawn out to show the interactions between the three genes, HS3ST3A1, CDC6 and SESN2. This pathway analysis suggests that SESN2 may act independently of p53 and CDC6 deactivated due to the severed interactions of the HS chains. These proteins are potential biomarkers for early stages of breast cancer.

INTRODUCTION

MATERIALS AND METHODS

Small Interference RNA (siRNA) Transfection

MCF-12A cells grown at 70-80% confluency were transfected with HS3ST3A1 siRNA and non targeting control siRNA, utilizing siPORT Amine Transfection Agent (Ambion) in Opti-MEM (Invitrogen). RNA was extracted from the cells 48 hours post transfection.

RNA Extraction and Quantification

Total RNA was extracted and purified. RNA samples were quantified with NanoDRop spectrophotometer (Thermo Scientific). The concentration of RNA was determined by the absorbance at 260 nm. Purity of RNA was determined by the 260nm/280nm ratio.

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Reverse Transcription Polymerase Chain Reaction

cDNA was synthesized using the SuperScript III First-Strand Synthesis for RT-PCR according to the manufacturer’s recommendations.

Quantitative Real Time Polymerase Chain Reaction (qRT-PCR)

qRT-PCR was performed to observe the relative fold change of treated and control groups. A melting curve was performed to ensure the products are well amplified. Fold changes for genes of interest were performed after normalization with GAPDH.

Western Blot

Total protein was extracted 72 hours post transfection. Western blot analysis was performed to analyse the protein expression. Proteins were loaded and run in a SDS-PAGE and gel was transferred to a PVDF membrane. Membrane was blocked in 5% milk in TBST and incubated with primary and secondary antibodies. ECL substrate was added and exposed at different times on photographic X-ray films.

RESULTS AND DISCUSSION

Silencing Efficiency of HS3ST3A1 in MCF-12A cells

![Graphs showing silencing efficiency of HS3ST3A1 and GAPDH](image)

Figure 1.1: a: Silencing effect of HS3ST3A1 siRNA targeting HS3ST3A1 mRNA in MCF-12A cells. b: Silencing effect of GAPDH siRNA targeting GAPDH mRNA in MCF-12A cells.

Western Blot Analysis of SESN2 and CDC6

![Western blot images](image)

Figure 1.3 Band size of 54 kDa was observed which is the size of SESN2 protein.
Figure 1.4 Band size of 63 kDa was observed which is the size of CDC6 protein.

The present study suggests that the down regulation of *HS3ST3A1* leads to an up regulation of *SESN2* and down regulation of *CDC6*. The consistent protein expressions with the gene expression revealed from microarray analysis suggest possible pathways on how *HS3ST3A1* may lead to the activities of *SESN2* and *CDC6*.

*SESN2* is induced by p53 in the response of DNA damage. In the pathway listed, *SESN2*’s up regulation may be independent of p53 and may be directly activated by *HS3ST3A1*. *HS3ST3A1* may induce a DNA damage mechanism and thereby activates *SESN2*. *SESN2* arrests cell proliferation by inhibiting mTORC, which is a cell cycle activator.

*CDC6* may be triggered due to the arrest of cell cycle as it is a protein involved in the formation of pre-RC. *CDC6* is capable of recruiting pre-RC proteins to bind to the ORC to initiate DNA replication. The down regulation of *CDC6* may be a response of cell cycle arrest induced by *HS3ST3A1*.

Another seemingly pathway for is the impaired HS functions which lead to a severed interactions with EGF receptors, which in turn bring about the down regulation of *CDC6* and freeze the cell cycle progression. EGF receptors are essential for several signalling cascades in
cell cycle progression (Rexer et al. 2009). Currently there were no studies found a direct interaction between EGFR and CDC6, and thus the proposed pathway.

Figure 1.5 Proposed pathways of SESN2 and CDC6 when HS3ST3A1 is down regulated.

HS3ST3A1, CDC6 and SESN2 may serve as potential biomarkers for early stages of breast cancers. SESN2 can be used as a biomarker to detect the presence of increased ROS activity which indirectly causes cancers with prolonged exposures (Hay 2008).

Coupled with CDC6, both SESN2 and CDC6 may act as dual biomarkers for the early detection of breast cancers. As mentioned, CDC6 may not be a good biomarker due to the several characteristics of a tumour cell including invasion and migration capabilities. However with the addition of SESN2, the levels of SESN2 and CDC6 may give indication of a change in expression of a normal epithelial cell which the development of cancerous cell from a normal one may be identified and treated before metastasis occurs.

CONCLUSION

Recent studies show that the down regulation of CDC6 and up regulation of SESN2 are affected by the down regulation of HS3ST3A1. The present study took a step further to analyse the proteins expression levels and the directions of regulation agree with the gene expression level. This study enables us to draw out hypothetical pathways on the interactions between HS3ST3A1, CDC6 and SESN2. Novel biomarkers of these 3 genes can be used to detect early stages of breast cancer.
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REFERENCES
