Abrogation of Taxol® Chemo-resistance via Epigenetic Immune Checkpoints Regulation in Triple Negative Breast Cancer

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Background and Aim
Chemotherapy is the typical therapy of Triple Negative Breast Cancer (TNBC). This “monotherapy” is the main cause beyond TNBC chemo-resistant cases. MicroRNAs (miRNAs) and long non-coding RNAs (lncRNAs) have been revealed as eminent players in human pathophysiological processes. For example, our previous studies have characterized LncRNA-XIST as a potent tumor suppressor in TNBC cell lines. Moreover, miR-34a, a tumor suppressor in TNBC, has been showed to be overexpressed in TNBC tissues and cell line. Immunotherapy, which elicits non-BC, long-lived anti-tumor activity, showed to be a potential cue for TNBC. However, regulation of programmed-cell-death-ligand-1(PD-L1) and Mesothelin (MSLN) targeted therapies are yet understudied in TNBC. In addition, among the crucial proteins which have dual role in cancer and immunity and has not been studied in TNBC is suppressor of cytokine-signaling (SOCS-3) protein and phosphatidylinositol N-acetyl-glucosaminyltransferase subunit C (PIG-C). This study aims to investigate a possible relation between Taxol® chemoresistance, expression of miR-34a and Lnc XIST and the expression of MSLN, SOCS-C, PIG-C and PD-L1 in triple negative breast cancer tissues.

Subjects, Materials and Methods

**Subjects:** 20 pairs of BC tissues and adjacent non-BC tissues were collected from patients undergoing tumor resection surgery

**Materials:** MDA-MB-231 triple negative breast cancer cell line was used, cultured and maintained for transfection and manipulation of gene expression purposes. For MTT assay, MDA-MB-231 cells were treated with 100µM Taxol®

**Methods:** MDA-MB-231 cells were cultured in 10 centimeter plate with 10 milliliters full DMEM. Total RNA was extracted from BC tissues, adjacent non-BC tissues and MDA-MB-231 cells using Biozol followed by reverse transcription cDNA synthesis, amplification and quantification using quantitative-real-time PCR.

Statistical Methods: Analysis was performed using the GraphPad Prism 7.02 software. All data were expressed in Relative quantitation (RQ). Data were expressed as mean ± standard error of the mean (SEM). A p-value < 0.05 were considered statistically significant***P<0.0001, **P<0.001, *P<0.01, =P<0.05.

**Results**

**Impact of Paclitaxel on Transfected and Co-Transfected MDA-MB 231 Cell line**
MTT assay showed that the percentage of cell viability decreased, the most, significantly when cells treated with Taxol® were co-transfected with miR-34a mimics and siTSIX (p<0.0001) compared to untreated, untransfected cells.

**Impact of miR-34a on the 4 potential genes**
MIIR-34a mimics significantly decreased MSLN expression (p<0.0001), PIGC expression (p<0.0001) and expression of PD-L1 expression (p<0.0013) while transient expression of SOCS-3 (p<0.0192) compared all to mock cells.

**Impact of knocking down in-XIST on the 4 potential genes**
Knocking down in-XIST significantly increased MSLN expression (p<0.0250), PIGC expression (p<0.0243) and expression of PD-L1 expression (p<0.0008) but the expression of SOCS-3 increased (p<0.0002) in comparison to mock cells.

**Impact of combined miR-34a overexpression and in-XIST knocking down on the 4 potential genes**
The combination between STX and miR-34a mimics significantly downregulated MSLN expression (p<0.0001), PIG-C expression (p<0.0001) and PD-L1 expression (p<0.0037), compared to mock cells, while SOCS-3 expression was upregulated (p<0.0001) in comparison to mock cells.

**Expression Profiling of MSLN and PIG-C in Breast Cancer Patients**
Triple Negative Breast Cancer tissues showed significant overexpression of both mRNA MSLN (p<0.0001) and mRNA PIG-C (p<0.0001) compared to healthy breast tissue.

**Conclusion**
This study reveals that Taxol® chemo-resistance is augmented by the overexpression of MSLN, PIG-C and PD-L1 and the downregulation of SOCS-3 which are all related to the overexpression of miR-34a and Lnc XIST in TNBC patients.

**References**

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