Abstract

Breast cancer is one of the most common cancers in women, and the second leading cause of cancer death in women. The majority of deaths occur due to metastasis. Inflammatory breast cancer (IBC) is a highly aggressive and rare form of breast cancer with a high propensity to metastasize. IBC is also known to have worse prognosis than other types of breast cancer [1]. The epithelial to mesenchymal transition (EMT), where the epithelial features in carcinoma cells are converted to mesenchymal phenotype, increases cells motility and invasion thereby confer metastatic properties upon cancer cells [2]. Various markers and regulators associated with EMT have been reported in breast cancer patients [3]. The purpose of this study is to determine the role of soluble E-cadherin (sEcad), an 80-kDa extracellular proteolytic fragment of full-length E-cadherin, in regulating EMT. We found that sEcad overexpression affects the expression of some of the known EMT markers such as Vimentin, Slug, Twist and E-cadherin.

Introduction

• Inflammatory breast cancer (IBC) is a form of breast cancer that is characterized by rapid proliferation and metastasis.

• One of the therapeutic targets identified is E-cadherin however much research hasn’t been done to understand the role of E-cadherin and its soluble fragments in IBC.

• The epithelial to mesenchymal transition (EMT) is thought to be one of the most important steps in cancer metastasis [4].

• EMT is initiated by EMT inducing signals like HGF, EGF, PDGF which activates transcription factors such as Snail, Slug, ZEB1, Twist and so on. Some of the major EMT regulators are Snail, Zeb and Twist families. These transcriptional factors regulate EMT and cancer metastasis [4].

• The role of soluble E-cadherin, an 80-kDa extracellular proteolytic fragment of full-length E-cadherin, in EMT is unknown.

Research Question

Does Soluble E-Cadherin regulate Epithelial to Mesenchymal transition (EMT)?

Methods

Real-Time PCR:

• The Total RNA was extracted from SUM149 control and sEcad overexpression cells using the TRIzol reagent.

• The cDNA were obtained by using the High Capacity cDNA Reverse Transcription Kit.

• Real-time PCR primers were designed using PRIMER 3 software for epithelial markers (E-cadherin-Ecad), mesenchymal marker (Vimentin-Vim, Fibronectin FN1- Fib, Twist, Snail and Slug) and other EMT regulators (NNMT, Serpine2, HDAC1, RHOA, PLOD1).

• qRT-PCR analysis was conducted using a SYBR Green Supermix kit. The fold change in expression was calculated using the 2–ΔΔCT method with the GAPDH mRNA as an internal control. Experiments for each sample were performed in triplicate.

Western Blot

• Immunoblotting was used to evaluate protein expression of Vim, Fib, Ecad, Twist and Slug in control and sEcad overexpressing SUM149 and SUM 190 cell lines.

Results

Figure 1. GSEA Hallmark analysis of RNA Seq in the SUM149 control and sEcad overexpression group shows the enrichment of EMT pathways in the sEcad overexpression group. (Hu, Xiao Ding)

Figure 2. Real-time PCR was performed to analyze expression of different genes. EMT regulators (NNMT, Serpine2, HDAC1, RHOA, PLOD1), epithelial marker (Ecad), and mesenchymal markers (Vim, FN1, Twist, Snail and Slug) were used to compare between Control and sEcad overexpressing SUM149 cells.

Results (Cont’d)

Figure 4. Western blot results show that the protein expression level of EMT markers such as Vim, Twist, Fib and Ecad were upregulated, while Slug was downregulated in sEcad overexpressing SUM190 cells.

Conclusions

• EMT pathways were enriched in the sEcad overexpression versus control group by GSEA hallmark analysis.

• By PCR, higher gene expression of Vim, Snail, and Ecad were observed in sEcad-expressing cells. However, FN1 and Twist showed lower expression in sEcad overexpressing SUM149 cells compare with control.

• By western blot, we observed that EMT markers such as Vim, Twist and sEcad were upregulated in sEcad overexpressing SUM149 and SUM190 cells, with the downregulation of FN1 in SUM190 cells.

• Additional experiments are warranted to evaluate how sEcad regulates the expression and function of EMT inducing transcription factors.

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References