Bioinformatics-Based Prediction of FUT8 as a Therapeutic Target in Estrogen Receptor-Positive Breast Cancer

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Abstract

Introduction: Estrogen receptor-positive (ER-positive) breast cancer is a subgroup of breast tumors that is more likely to respond to hormone therapy. ER-positive and ER-negative breast cancers tend to show different patterns of metastasis because of different signaling cascade and genes that are activated by estrogen response. Genetic factors can contribute to high rates of metastasis in ER-positive breast cancer. Fucosyltransferase 8 (FUT8) is a member of fucosyltransferases family and plays an important role in α-1,6 linkage to the first GlcNAc residue of N-glycans chain. In this study, for the first time, we predicted FUT8 by bioinformatics tools as a novel therapeutic target for ER-positive breast cancer.

Methods: Microarray gene expression data of 9 patients with ER+ve and 10 individuals with ER-ve breast cancer was extracted from Geodatasets. Gene expression of two ER+ and ER- patients was compared with logfc and then sorted by their p-values. Moreover, the most related pathway, protein interaction, and function of this gene were identified with GeneCard and DAVID databases.

Results: FUT8 was highly expressed in patients with ER+ve breast cancer that may be associated with the metastasis. FUT8 encodes an enzyme that belongs to fucosyltransferases family. The expression of this gene may contribute to the malignancy features of cancer cells and their invasive and metastatic capabilities.

Conclusions: Having in mind FUT8 hyperexpression, its function in malignancy, and its pathways, it can be concluded that FUT8 can be used as a therapeutic target in ER+ve breast cancer.

INTRODUCTION

Breast cancer is one of the most prevalent malignancies in women with 4 major types according to the presence of sex hormone receptors on the cancer cell. Endocrine receptor-positive (estrogen or progesterone receptors), HER2-positive, triple positive (these tumors are positive for estrogen receptors, progesterone receptors, and HER2), and triple negative (these tumors are not positive for estrogen receptors, progesterone receptors, and HER2) tumors are the main categories of breast cancer [1-3]. About 80% of all breast cancers are estrogen receptor-positive (ER-positive) that may

influence the growth of cancer cells in response to the estrogen [4-6]. About 65% of breast tumors are progesterone receptor-positive (PR-positive) which grow in response to another sex hormone called progesterone [7-9]. These hormones especially estrogen, play an important role in cell cycle and tumor progression [4, 10, 11]. Estrogens are considered to play a major role in promoting the proliferation of normal and neoplastic breast epithelial cells. Three major mechanisms are suggested to be involved in estrogen carcinogenic effects: 1. Stimulation of cellular proliferation through estrogens receptor-mediated hormonal activity. Actually, when hormones bind to the cell surface receptors, hormone-responsive genes and signaling cascades including MAPK signaling and PI3K signaling pathways, are turned on for DNA synthesis and cell proliferation. 2. Increasing mutation level through a cytochrome P450-mediated metabolic activation. Cytochrome P-450 catalyzes the oxidative metabolism of estrogen and estradiol to a 2-hydroxycatechol estrogen that can induce genetic mutations. 3. Induction of aneuploidy including loss of 9p11-13 and 4p15.3-16 deletion that have been introduced to be tumorigenic in several studies [12-14]. In addition, genetic factors play an important role in breast cancer initiation and progression. Among genetics element, somatic mutations happen more frequently in breast cells [15]. However, some patients show a germline mutation in BRCA1 and BRCA2 genes. These genes are used in many prediction tests for breast cancer in patients with a familial background [16-18]. Some of the important genes are expressed only after activating the estrogen receptor and affect both normal and cancer cells. Therefore, identification of molecular keys in breast cancer can result in better decision making about breast cancer treatment. In this article, Microarray gene expression data of breast cancer patients were studied from Geodatabase to identify critical and different genes between ER-positive and ER-negative breast cancer.

METHODS

Gene Profile Extraction

Microarray gene expression data of 9 cases with ER+ve and 10 patients with ER-ve breast cancer (GSE32394) were extracted from NCBI Geodatasets [19]. This profile included samples of ER+ve and ER-ve patients that were submitted in September 2011 and updated in April 2017.

Gene Expression Profile Analysis

This profile was analyzed; using limma package in R program. Limma is an R/Bioconductor package and a powerful differential expression analyzing tool for RNA-sequencing and microarray studies [20, 21]. Generated unnormalized data were normalized by robust multiarray averaging (RMA) method. To determine data value and their normalization, the distribution of the samples was performed. In this step, genes were compared based on their log fold change (logfc) and their level of expression between ER+ve and ER-ve breast cancer patients. They were then sorted into hyperexpressed genes in ER+ve patients and hypoexressed genes in ER-ve breast cancer individuals according to their p-value and vice versa to determine specific gene in ER+ve breast cancer. Moreover, the expression level of a gene of interest was determined among all 19 samples through the profile graph in NCBI Geodatasets. Finally, a gene that was hyperexpressed and had the lowest p-value in ER+ve breast cancer patients was chosen for further analysis. The most related pathway (Gene Ontology) and function of the gene of interest were identified by the GeneCard and DAVID databases for understanding its effect in the pathogenesis of ER+ breast tumor.
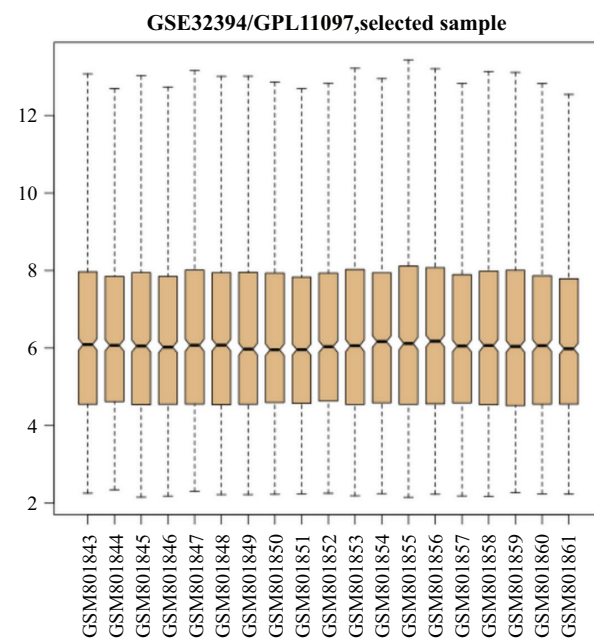
Figure 1: Distribution of Data Value: Distribution Can Be Viewed by Box Plot to Indicate Data Value and Normalization

GSE32394/NM_178155.1_psr1_a_at/FUT8
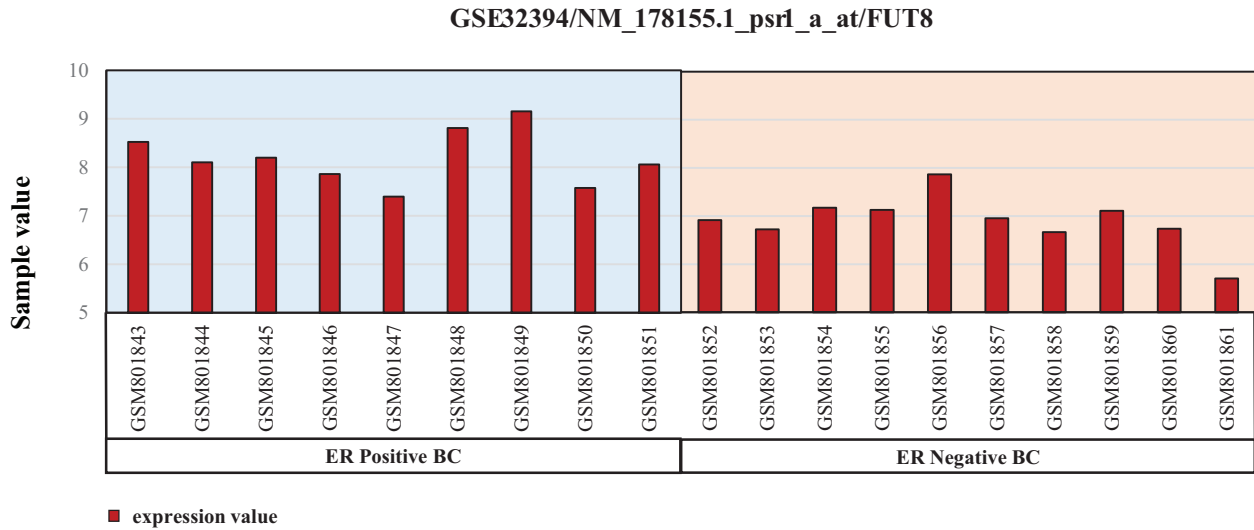
Figure 2: Different Expression Level of FUT8 Was Observed Among ER+ and ER-ve Breast Cancer (GSM Indicates Samples).

GSE32394/GPL11097/NM_178155.1_psr1_a_at
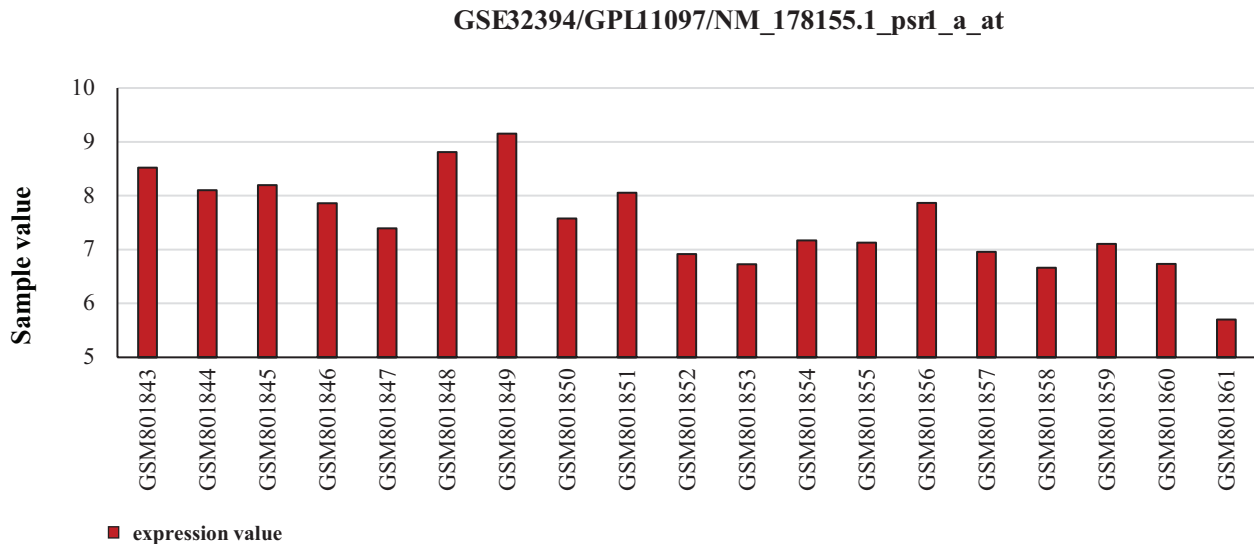
Figure 3: FUT8 Expression Among All Samples

The interaction of the protein product of this gene with other proteins was also identified to confirm its activity through STRING databases.
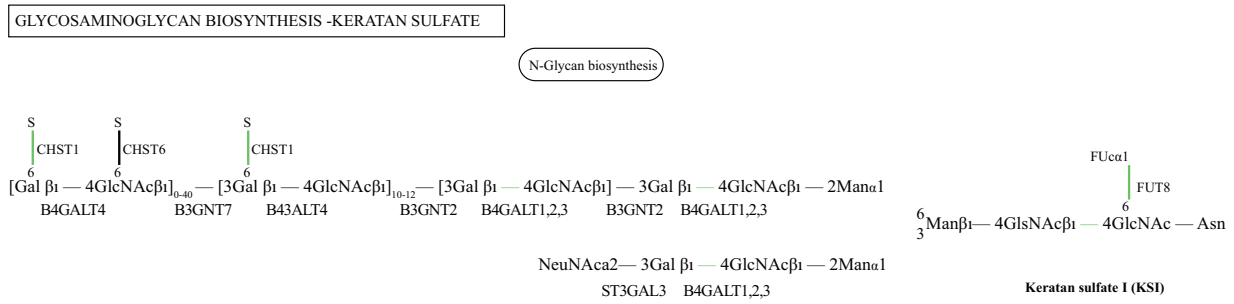
RESULTS

According to the analysis of distributed data value and its box plot, it was concluded that it can be a valuable microarray data for gene expression analysis (Figure 1). FUT8 was hyperexpressed with 2.2064 logfc in ER+ve breast cancer patients compared to ER-ve breast cancer patients through gene expression profile analysis (Figure 2). This gene is located on 14q23.3 and encodes a protein with 575 aa and has SH3_9 SH3_1 SKI NodZ motifs.

FUT8 expression was identified among 19 samples to recognize its overexpression in all samples (Figure 3). According to this figure, it's determined that FUT8 was hyperexpressed in nearly all of the samples.

Important pathways of FUT8 included N-glycan synthesis pathway, biosynthesis of keratan sulfate, and transcriptional misregulation based on GeneCard and DAVID databases (Figure 4). N-glycan synthesis pathway plays an important role in cancer invasion and metastasis. N-glycan, which is synthesized by FUT8, exhibits in a number of glycoproteins such as EGFR, IGFR, and FGFR. Recently, N-glycan synthesis pathway and biosynthesis of keratan sulfate are considered as therapeutic targets for cancer.
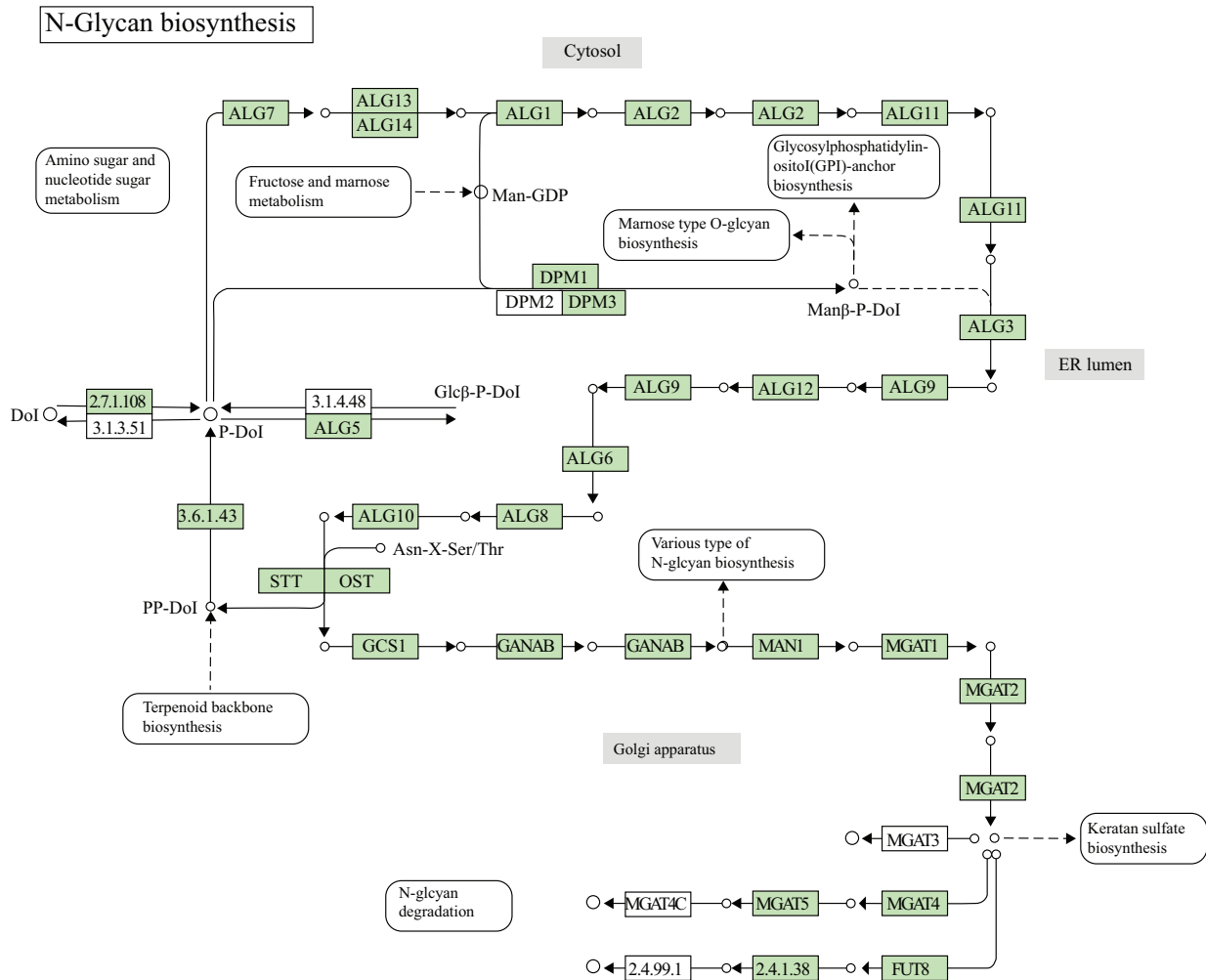
A

GLYCOSAMINOGLYCAN BIOSYNTHESIS -KERATAN SULFATE

N-Glycan biosynthesis

$$\begin{array}{c} S \\ | \text{ CHST1} \\ 6 \end{array} \quad \begin{array}{c} S \\ | \text{ CHST6} \\ 6 \end{array} \quad \begin{array}{c} S \\ | \text{ CHST1} \\ 6 \end{array}$$

$[\text{Gal }\beta_1 - 4\text{GlcNAc}\beta_1]_{0-40} - [3\text{Gal }\beta_1 - 4\text{GlcNAc}\beta_1]_{10-12} - [3\text{Gal }\beta_1 - 4\text{GlcNAc}\beta_1] - 3\text{Gal }\beta_1 - 4\text{GlcNAc}\beta_1 - 2\text{Man}\alpha_1$

B4GALT4   B3GNT7   B43ALT4   B3GNT2   B4GALT1,2,3   B3GNT2   B4GALT1,2,3

$\text{NeuNAca2} - 3\text{Gal }\beta_1 - 4\text{GlcNAc}\beta_1 - 2\text{Man}\alpha_1$

ST3GAL3   B4GALT1,2,3

$$\begin{array}{c} \text{FUc}\alpha_1 \\ | \text{ FUT8} \\ 6 \end{array}$$

$^{6}_{3}\text{Man}\beta_1 - 4\text{GlsNAc}\beta_1 - 4\text{GlcNAc} - \text{Asn}$

Keratan sulfate I (KSI)

B

N-Glycan biosynthesis

Figure 4: A) Keratan Sulfate Biosynthesis; B) N-Glycan Biosynthesis Pathway

FUT8 protein product interaction with apoptotic chromatin condensation inducer 1 (ACIN1) and DEAH-box helicase 15 (DHX15) indicates its role in tumorigenesis, according to STRING database (Figure 5). A nuclear protein is encoded by ACIN1 that induces apoptotic chromatin condensation

after being activated by caspase-3, without induction of DNA fragmentation [22]. Having in mind this pathway, ACIN1 plays a crucial role in breast neoplasms [23]. DHX15 is involved in pre-mRNA processing and helicase activity and has a critical function in breast carcinoma, glioma, and prostate cancer [24, 25]. According to this protein interaction, it can be expected that FUT8 upregulation may play a critical role in ER+ve breast cancer.
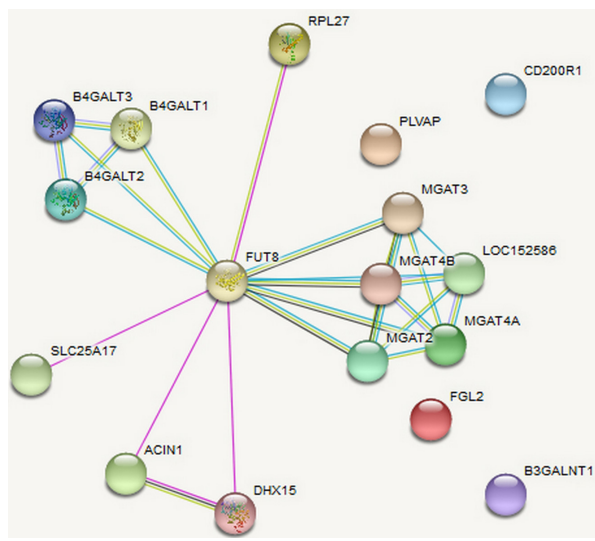
Figure 5: Experimentally Approved FUT8 Interaction With Other Protein Such As ACIN1 and DHX15

DISCUSSION

Breast cancer is one of the most prevalent types of cancer in women involving patients aged 40-50 years old [26]. So far, about 13 FUTs have been recognized, while only FUT8 is involved in core fucosylation (adding fucose in α-1,6-linkage to the innermost N-acetyl glucosamine of N-glycans). The regulatory function of adhesion molecules and growth factor receptors, such as α3β1 integrin, epidermal growth factor receptor, and hepatocyte growth factor receptor is core fucosylation of glycoproteins has important. Fucosyltransferase 8 (FUT8) belongs to fucosyltransferases family [27, 28]. Transfer of fucose from GDP-fucose to N-linked type complex glycopeptides on the cell surface is catalyzed by the product of this gene. FUT8 is one of the most important genes; playing a key role in malignancy and metastasis since fucosylation is essential for EGF receptor-mediated biological functions. Furthermore, fucosylation of glycoproteins regulates several biological functions of adhesion molecules

and growth factor receptors. FUT8 is upregulated in several types of cancers including, nonsmall cell lung cancer, hepatocarcinoma, ovarian cancer, lung cancer, colorectal cancer, melanoma and skin cancer [29, 30]. This is the first bioinformatics study that evaluates the FUT8 gene expression in ER+ve breast cancer; suggesting that the FUT8 gene expression is related to the incidence of ER+ve breast tumors. A significantly increased expression of FUT8 was observed in ER+ve breast cancer. These findings suggest that upregulated expression of FUT8 in ER+ breast tumors may mimic phenotypic characteristics of malignant tumor cells against estrogen that may be correlated to the high rate of metastasis in ER+ve breast cancer. Moreover, we found that the addition of N-glycan fucosylation to EGFR can occur by FUT8 according to the FUT8 role in N-glycan synthesis. Several studies have shown that increased fucosylation of EGFR, promoted a significant EGF-mediated cellular growth and proliferation in cancers such as melanoma, colorectal, and prostate cancer; highlighting FUT8 role in ER+ve breast cancer [28, 31, 32]. Generally, glycosylation of cell surface receptors including EGFR is important in cancer biology processes such as cellular proliferation and metastasis. Therefore, aberrant expression of FUT8 in these types of tumors would change the level of fucose in EGFR which can lead to high cell proliferation and metastasis in ER+ve breast cancer cell. In addition, having in mind the FUT8 interaction with ACIN1 and DHX15 with major effect on breast cancer, it is expected that FUT8 can have an effect on breast cancer as well [33]. These findings showed the critical role of FUT8 in ER+ breast cancer. However, experimental studies are required to approve the role of FUT8 in ER+ve breast cancer.

To the best of our knowledge, this was the first study that described FUT8 gene expression in ER+ breast cancer for the first time. Our results demonstrated that increased expression of FUT8 in ER+ breast cancer may be important in the acquisition of an aggressive phenotype and tumor metastasis that is more typically found in ER+ breast cancer rather than ER- breast cancer. In addition, our study introduced FUT8 expression as a therapeutic modality for target therapies including, CRISPR/Cas9 technique, genetic engineering, and gene therapy.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

ETHICS APPROVAL

This study is a bioinformatic study and is approved by SBU university.

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