RESEARCH ARTICLE

Cancer/Testis OIP5 and TAF7L Genes are Up-Regulated in Breast Cancer

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Abstract

  Breast cancer still remains as the most frequent cancer with second mortality rate in women worldwide. There are no validated biomarkers for detection of the disease in early stages with effective power in diagnosis and therapeutic approaches. Cancer/testis antigens are recently promising tumor antigens and suitable candidates for targeted therapies and generating cancer vaccines. We conducted the present study to analyze transcript changes of two cancer/testis antigens, OIP5 and TAF7L, in breast tumors and cell lines in comparison with normal breast tissues by quantitative real time RT-PCR for the first time. Significant over-expression of OIP5 was observed in breast tumors and three out of six cell lines including MDA-MB-468, T47D and SKBR3. Not significant expression of TAF7L was evident in breast tumors but significant increase was noted in three out of six cell lines including MDA-MB-231, BT474 and T47D. OIP5 has ssignificant role in chromatin organization and cell cycle control during cell cycle exit and normal chromosome segregation during mitosis and TAF7L is a component of the transcription factor IID, which is involved in transcription initiation of most protein coding genes. TAF7Lis located at X chromosome and belongs to the CT-X gene family of cancer/testis antigens which contains about 50% of CT antigens, including those which have been used in cancer immunotherapy.

  Keywords: Breast cancer biomarkers - targeted therapy - cancer/testis genes - OIP5 - TAF7L

Introduction

Breast cancer is the most frequent cancer worldwide with second mortality rate among women (Siegel et al., 2012). Current treatments including, partial or whole mastectomy, radiation therapy, chemotherapy and endocrine therapy have generated small improvements in clinical outcomes. Despite successful treatment of the primary malignancy, relapse and subsequent metastasis which usually ends to death, occur in most cases of breast cancer patients by distant spread to other organs through the bloodstream or lymphatic channels. Since breast cancer is a heterogeneous disease there is no gold standard therapy suitable for all breast tumors. Targeted therapy have received particular attentions in current researches and efforts are directed toward specific prognostic and predictive targets signatures that can guide targeted individualized therapeutics and screening approaches of the disease. Multiple tumor biomarkers have been reported in breast cancer including CA 15-3, MUC1 and CEA (Saini et al., 2013). However, none of them have been implicated in clinical practice because of false-positive rate in normal populations, and low diagnostic sensitivity and specificity (Harris et al., 2007).

Cancer/Testis (C/T) antigens are a group of tumor antigens and novel subjects for developing cancer vaccine and immunotherapy approaches. They aberrantly express in tumors with highest normal expression in testis as an immune privileged organ, and limited or no expression in normal tissues (Simpson et al., 2005; Ghafouri-Fard, Modarressi, 2009).

The blood-testis barrier, maintained by the tight junctions between the Sertoli cells, prevents communication between germ cells and the immune cells circulating within blood vessels, prevents them from eliciting an immune response. Evidences indicate that exposure to germ cell proteins can cause resistance against cancer (Eisenberg et al., 2013).

There are important similarities between the processes of germ-cell and cancer cell development (Simpson et al., 2005). Genetic alterations in cancer can result in the reactivation of normally silent germline expression programs, which might confer some of the characteristics of malignancy. The similarities between gametogenesis and cancer strengthen the possibility that cancer commandeers parts of the gametogenic programs in the process of cancer cell development (Simpson et al., 2005). There is a growing list of CT antigens; some of them are in clinical trial phase in cancers such as lung cancer (Kelly et al., 2011)

We designed this study to evaluate expression of two testis specific genes, Opa Interacting Protein 5 (OIP5) and

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Transcription Factor IID (TAF7L), in breast cancer patient and cell lines. In our previous study (Yazarloo et al. 2013) we selected these genes for expression analysis in Acute Myeloid Leukemia (AML), based on appropriate criteria, as almost restricted expression in normal testis, their critical function in the specific local tissues and abnormal elevated expression in various cancers. The results showed a gender dependent expression pattern in AML patients.

OIP5 is located at 15q15.1. It has significant role in chromatin organization and cell cycle control during cell cycle exit and normal chromosome segregation during mitosis, so it is attractive to be investigated in cancer research (Fujita et al., 2007). OIP5 expression is restricted to testis and in a lower level in bone marrow, thymus and colon. Over expression of OIP5 has been reported in glioblastoma (Freitas et al. 2013), colorectal (Chunet et al., 2010) and gastric (Nakamura et al., 2007) cancers.

Transcription Factor IID (TAF7L), located at Xq22.1, is a general transcription factor and essential compartment for recruitment of RNA polymerase II to initiate transcription of most protein coding genes.

It is a complex consisting of TATA box binding protein and TBP-associated factors. Its encoded protein could be a spermatogenesis-specific component of the DNA-binding general transcription factor complex TFIID.

TAF7L has been shown highly over expression in HPV positive head and neck squamous cell carcinoma (Slebos et al., 2006). In colorectal cancers frameshift mutations inTAF7L gene is reported as well (Oh et al., 2014)

We selected six breast cancer cell lines as the representative models for different breast cancer subtypes. Cell lines were selected based on a combination of their main characteristics covering the all five histological subtypes (Luminal A, Luminal B, Basal, Claudin-low and Her2-enriched) (Deborah et al., 2011) of breast cancer.

Materials and Methods

Patients and normal samples

Eight confirmed normal breast epithelial cells which were collected from the surgical removals of the mamoplastic surgeries were enrolled in the study. Normal testis was obtained from the orchidectomic surgeries. Fifty fresh frozen breast cancer tumors were purchased from the National Tumor Bank of Cancer Institute of Tehran University of Medical Sciences. All tissues were stored in -70°C until experiments.

Cell Lines

The six selected breast cancer cell lines were as follow: BT-474 (ATCC No.: HTB-20D), MDA-MB-468 (ATCC No.: HTB-132), MDA-MB-231(ATCC No.: HTB-26), T47D (ATCC No.: HTB-133), SKBR3 (ATCC No.: HTB-30) and MCF7 (ATCC No.: HTB-22) respectively. Cells

were cultured according to ATCC instructions in RPMI-1640 or DMEM media by adding 10% fetal calf serum (Invitrogen) and 1% penicillin/streptomycin (Invitrogen). Cells were incubated at 37°C in a humidified incubator with 5% CO_2. The flasks with about 2×10^6 cell counts were separated for RNA extraction.

RNA isolation and cDNA synthesis

Total RNA was isolated from the cell lines, fifty micrograms of tumors and normal tissues using Tripure reagent (Roche, Mannheim, Germany) following the manufacturer's protocol. Quality and quantity of extracted RNA were determined by NanoDrop spectrophotometer (Nano- Drop® Technologies) and running the RNAs in agarose gel. One microgram of total RNA was converted to cDNA using Takara cDNA synthesis kit (Takara, Japan) with a mix of random hexamers and oliogo dT as the reaction primers in a final volume of 20 μl according to the manufacture protocol with minor modifications. As the negative control of the experiments RT-negative tube without adding the RT enzyme was used. Quality of cDNAs was confirmed by the amplification of the housekeeping gene Phosphoglucomutase 1 (PGM).

Real-time quantitative polymerase chain reaction

To determine the transcript levels of the target genes, quantitative real-time PCR reaction was performed using SYBR GreenI Premix (Takara, Japan) and 200 ng/μl of each of the cDNAs, following the manufacturer's protocol with minor modifications. Amplifications were done in 40 cycles using Rotor-GeneTM 6000 machine (Corbette Life ScienceTM, Germany). To determine the maximum efficiency of each primer pairs, serial dilution of 10 pmol/μl of mixed primers was performed and 0.5μl for each primer was considered as the best efficiency in a final volume of 20 μl of reactions. PGM 1 and Hypoxanthine phosphoribosyltransferase (HPRT) were used as internal controls to normalize the target genes. The sequences of the related primers are listed in Table 1.

Statistical analysis

Table 1. Primers Sequences, F: Forward Primer, R: Reverse Primer bp: Base Pairs

Gene	Primer sequences	Amplicon (bp)
OIP5	F: CCCTTCCTAGTTGGCATTGA	
	R: GCACACCATTTTGTCACTGG	162
TAF7L	F: CTGGAACATGCTTGTACTGTCA	
	R: CAGCTTAGCAGCTAGTGGG	138
PGM1	F: AGCATTCCGTATTTCCAGCAG	
	R: GCCAGTTGGGGGTCTCATACAAA	120
HPRT	F: CCTGGCGTCGTGATTAGTGAT	
	R: AGACGTTCAGTCCTGTCCATAA	131

Table 2. Expression Analysis Results of the Cancer/Testis Genes, OIP5 and TAF7L in Breast Tumors and Cell Lines

	MDA-MB-231	MDA-MB-468	BT474	T47D	MCF7	SKBR3	Breast tumors
Breast cancer subtype	Claudin-low	Basal	Luminal B	Luminal A	Luminal A	HER2	NA
OIP5 expression	↑	*↑	↑	*↑	↑	*↑	*↑
TAF7L expression	*↑	↑	*↑	*↑	↓*	↑	↑

*Significant over expression: *↑, not significant over expression: ↑, significant down regulation: ↓*

Standard mode of REST 2009 software was used to analyze the relative gene expression data (Pfaff et al., 2002) using the amplification efficiencies and cycle thresholds from comparative quantification analysis in Rotor gene software. The final relative expression obtained relative to the normalizer genes and normal breast tissue. The pair wise fixed reallocation randomization test with 2000 iterations in the REST 2009 software was used to determine the significances.

Results

By employing the quantitative Real Time RT-PCR, six breast cancer cell lines, eight normal breast tissues, two normal testis tissues and 50 breast cancer tumors, were subjected for analyzing expression of the target genes. Expression of two cancer testis antigens; OIP5 and TAF7L was investigated in all tissue specimens. PGM1 and HPRT were used to normalize the gene expression as the internal controls. After normalizing the expressions levels, a combination of studied normal breast epithelial tissues showed very low levels of transcripts, whereas the highest levels of transcript was observed in normal testis with highly significant differences in gene expression fold changes in comparison with normal breast samples in both genes (p<0.00). MDA-MB-231 as the Claudin-low subtype of breast cancer showed over expression of both genes. The differences in expression ratio in comparison with normal breast was significant in the case of TAF7L (p<0.00) but not in OIP5 (p<0.169). MDA-MB-468 as the Basal subtype of breast cancer showed significant over expression of OIP5 in comparison with normal breast tissues (p<0.00), whereas over expression of TAF7L was not significantly different (p<0.169). In the cases of T47D and MCF7 as the Luminal A subtypes of breast cancer, significant over expressions in both transcripts was observe in T47D cell line (p<0.00), whereas MCF7 showed not significant but moderate over expression of OIP5 (p<0.49) and significant decrease in expression in the case of TAF7L(p<0.00) in comparison with normal breast tissues. BT474 as the Luminal B subtype of breast cancer cell line showed not significant, but moderate over expression of OIP5 (p<0.511) and significant fold changes in transcript level of TAF7L (p<0.00) in comparison with normal breast tissues respectively. SKBR3 as the HER2-enriched subtype of breast cancer cell lines, was significantly different in the case of OIP5 transcript levels
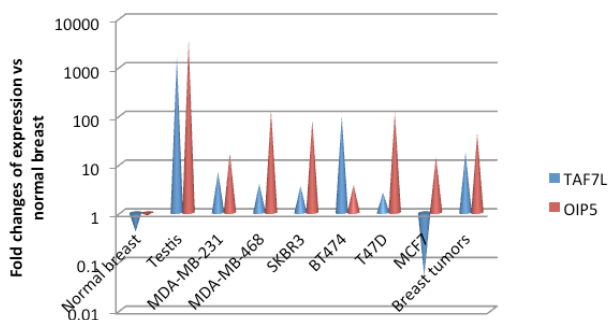

Figure 1. Comparative fold Expressions Changes of Oip5 and Taf7l In Comparison to Normal Breast Tissues in Breast Tumors and Cell Lines
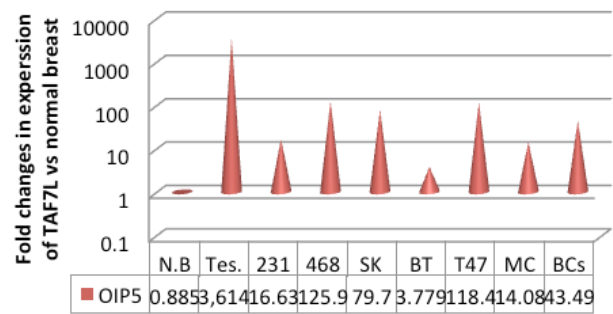

Figure 2. Fold Changes of OIP5 Expressions in Comparison to Normal Breast Tissues in Breast Tumors and Cell Lines. (The abbreviations are same as figure 1)
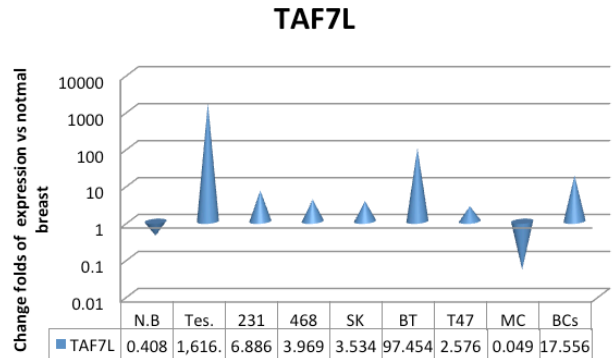

Figure 3. Fold Changes of TAF7L Expressions in Comparison to Normal Breast Tissues in Breast Tumors and Cell Lines. (The abbreviations are same as figure 1)

but not significant in TAF7L (p<0.331) in comparison with normal breast tissues (Figure 1).

A combination of studied breast tumors showed elevated levels of both gene transcripts. The differences in expressions fold changes in comparison with normal breast was significant in OIP5 transcripts (p<0.00) but not in the transcript levels of TAF7L (p<0.122). The results are summarized in Table 2.

Discussion

Breast cancer is a complex and heterogeneous disease (Deborah et al., 2011). Most part of breast cancer knowledge is based on in vivo and in vitro studies done on breast cancer cell lines. They provide an unlimited and easily handled source of self-replicating material, free of contamination with stromal cells. They are representative models of the tumors which they are derived from and powerful tools to test the efficiency of therapeutic drugs. Conversely, breast cancer cell lines have limitations. They represent only clonal isolates, separated from their stromal environment. Their sustained growth in culture might modify the phenotype and lead to an accumulation of genome alterations (Charafe-Jauffret et al., 2006). However our results of the expression pattern of studied genes showed significant or moderate over expression of both of the genes in studied cell lines, the MCF7 cell line seems to be different in TAF7L expression (table2 and figure), which needs further studies.

Testis is an immune privileged organ without expressing HLA molecules. CTAs are considered as

potential biomarkers and suitable antigens for targeting therapies. Unlike chemotherapy which kills healthy dividing cells in addition to tumor cells with profound side-effects, CTAs can be used for specific targeting of cancer cells using monoclonal antibodies or dendritic cell based immunotherapy (Saini et al., 2013). The T-cell responses to CT antigens are typically investigated by the screening of overlapping peptide panels with CD4+ orCD8+ T-cells from peripheral blood. Many HLA-restricted T-cell epitopes have been identified in this way, particularly for the MAGE-A, NY-ESO-1, and SSX genes, forming the basis for peptide-based CT cancer vaccine trials and for the monitoring the post-vaccination T-cell responses (Caballero et al., 2009). There are evidences for functional roles of CTAs including modulating the gene expression, contribution to tumor signaling pathways, tumor cell division and apoptosis (Whitehurst, 2014). There is a list of several known cancer/testis genes in breast cancer (Ghafouri-Fard et al., 2014). We previously reported over expression of the cancer/testis gene TSGA10 in breast tumors (Dianatpour et al., 2012) and a combination of different tumors which was correlated with some pathological features (Mobasheri et al., 2007).

The results of this study showed very low levels of OIP5 and TAF7L transcripts in the normal breast epithelial tissues whereas the highest levels of transcript was observed in normal testis with highly significant differences in gene expression ratio in comparison with normal breast samples in both genes which confirms the restricted expression pattern of the genes in normal testis. OIP5 was over expressed in a combination of studied breast tumors and all of the breast cancer cell lines but significant difference in the fold changes with normal breast was observed in breast tumors, MDA-MB-468, T47D and SKBR3 cell lines (Figure 2).

Opa Interacting Protein 5, also known as LINT-25 has restricted expression pattern in testis and in a lower levels in somatic cells, bone marrow, thymus and colon (Yazarlou et al., 2013). Sub cellular localization of OIP5 protein is mainly restricted to the nucleus and cytosol of the cells. OIP5 protein is essential for recruitment of centromere protein A (CENPA) to centromeres and normal chromosome segregation during mitosis.

Eukaryotic centromers are marked by CENPA as the unique form of histon H3 present in human chromosomes. Newly synthesized CENPA is deposited in nucleosomes at the centromers during late telophase/early G1 phase of the cell cycle. It makes nucleosomes stably associated with the centromere and divided equally to daughter centromers during S phase (Fujita et al., 2007). Considering its role in cell cycle control and chromatin organization, missregulation of OIP5 could be followed by serious consequences (Kimmins et al., 2004).

The transient expression of OIP5 in NIH3T3 cells resulted in a 2-fold increase in proliferation rate, highlights its oncogenic properties (Chun et al., 2010). Over expression of OIP5is reported in gastric and colorectal cancer (Nakamura et al., 2007; Chun et al., 2010). Over expression of OIP5 was associated with improved survival of the brain tumor glioblastoma (Freitas et al., 2013), and in a gender dependent expression pattern in female AML

patients as well (Yazarloo et al., 2013). Previous studies have demonstrated high expression of OIP5 mRNA in colorectal (Chun et al., 2010), gastric cancer (Nakamura et al., 2007) and clear cell renal carcinoma tumors and cell lines (Gong et al., 2013). In addition to the malignancies, OIP5 showed over expression in adipose tissues, lead to obesity (Inoue et al., 2014). The biological function and clinical significance of OIP5 in human breast cancer remains to be the subject of future researchers.

According to the results of this study, TAF7L is over expressed in a combination of breast tumors and all of the breast cancer cell lines except for MCF7. The fold changes of increased expression levels was significant in three cell lines, MDA-MB-231, BT474 and T47D but MCF7 showed significant decrease in expression levels in comparison with normal breast tissues (Figure 3).

The TAF7-like RNA polymerase II, TATA box binding protein (TBP)-associated factor, 50kDa (TAF7L) participates in a complex with TATA box Binding Proteins to form Transcription Factor IID. TAF7L may probably functions as a spermatogensis-specific component of the DNA-binding general transcription factor complex TFIID, a multimeric protein complex that plays a central role in mediating promoter responses to various activators and repressors. It predominantly expresses in testis and its expression is exchanged at the critical stages of germ cell development. In human, TAF7L may be essential for spermatogenesis maintenance (Akinloye et al., 2007). It likely cooperates directly with TBP-related factor 2 at promoters of a subset of post meiotic genes to regulate spermiogenesis (Zhou et al., 2013). Fascin homolog 1, actin-bundling protein which is involved in cytoplasmic protrusion and cell motility (Cheng et al., 2007) has been shown to be strongly down regulated in knockout Taf7l mice. Up regulation of FSCN1 has been reported in a number of human cancers and functions to promote cancer cell migration and invasion (Gan et al., 2008). Based on high throughput protein data, TAF7L has interaction with the protein kinase SRPK2 (serine/arginine-rich protein-specific kinase 2) which Promotes neuronal apoptosis by up-regulating cyclin-D1 expression. This is done by phosphorylation of SRSF2, leading to the suppression of the tumor suppressor p53/TP53 phosphorylation thereby relieving the repressive effect of p53/TP53 on cyclin-D1 expression (http://thebiogrid.org/119964). TAF7L plays an integral role in adipocyte gene expression as well, by targeting enhancers as a cofactor for PPARγ and promoters as a component of the core transcriptional machinery. The presence of the TAF7L subunit in TFIID, functions to promote long-range chromatin interactions during brown adipose tissue lineage specification (Zhou et al., 2014). Human papillomavirus (HPV) is associated with a subset of head and neck squamous cell carcinoma. TAF7L has been shown to be highly over expressed in HPV positive head and neck squamous cell carcinoma (Slebos et al., 2006). In colorectal cancers frameshift mutations inTAF7L gene is reported as well (Oh et al., 2014). In our previous study (Yazarloo et al., 2013) affected male AML compared to healthy males displayed down regulation of TAF7L in contrast to no significant difference in TAF7L expression in affected females compared to the healthy females.

Cancer/testis genes are divided into two main families. CT-X family which are located at X chromosome and non-CT-X which are distributed throughout the genome. TAF7L is located at the X chromosome and belongs to the CT-X family. About 50% of CT genes, including those which have been used in cancer immunotherapy, are CT-X antigens (Ghafouri-Fard, Modarressi, 2009). They are usually highly expressed in the spermatogonia, mitotically proliferating germ cells and frequently co-expressed in cancer cells. Non-X CT antigens are usually expressed in the spermatocytes and many have roles in meiosis. Their aberrant expression in cancer cells might cause abnormal chromosome segregation and aneuploidy (Simpson et al., 2005).

There are a little knowledge about the nature, causes, consequences and effects of cancer/testis antigens activation in different cancers and the effects of genetic (Miryounesi et al., 2014) and environmental factors (Azam et al., 20014) on their expression remains to be the subjects of further studies.

In final conclusion, because of the lack of validated biomarkers in breast cancer and importance of early detection of the disease to warranty the diagnosis and effective treatments in early stages, the potential candidates like the genes in this research needs more studies to explore the utilities as an early diagnostic biomarker and immunotherapeutic target in different cancers as well as breast cancer.

Acknowledgements

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Newly diagnosed without treatment