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In-Silico Target Prediction for hsa-mir-27a and Identification of Genes Involved in Breast Cancer

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ABSTRACT MicroRNAs (miRNAs) are key gene regulators in all living organisms. They play an important role in various diseases including breast cancer (BC). Mutations within *MIR27a* gene have been linked with various types of cancers including breast cancer. Several tools are being used to identify potential targets for crucial miRNA genes. In the present study, online target prediction tools were used to investigate target genes that may be regulated by the mature form of *MIR27a* (hsa-mir-27a-3p and has-mir-27a-5p). A total of 52 target genes were found for hsa-mir-27a while for hsa-mir-27a-5p determined 45 targets. These target genes were further explored for their potential role in BC. It was found that the target genes are involved in vital biological processes in cancer progression and development. In conclusion, *MIR27a* may regulate several genes that are vital for BC development and progression. *MIR27a* may act as a promising new biomarker for anti-breast cancer therapy.

INTRODUCTION

MicroRNAs (miRNAs) are small, highly conserved noncoding short (19-24 nt) RNA molecules that control gene expression post-transcriptionally, either by degradation of target mRNAs or by inhibition of protein translation. It is already known that miRNAs have a significant role in crucial biological pathways (Oliveto et al. 2017) More than fifty percent of all genes in the human genome are known to be regulated by miRNAs (Keller et al. 2014). Recently it is shown that abnormal expression of miRNAs is related to a broad range of human diseases (Chen et al. 2018; Li and Kowdley 2012). A large number of miRNAs have been discovered by cloning and size-fractionated RNA techniques. The researchers' previous studies have shown altered expression profile of selected miRNA in caloric metabolism and gastric cancer by using whole genome miRNA profiling studies using blood samples from human and related knockout mouse models (Shah et al. 2013; Shah et al. 2012; Shah et al. 2011). *MIR27a* is thought to be an oncomir promoting tumor growth and metastasis. It has been reported to be a marker for breast cancer progression and patient survival (Zhao et al. 2016). MiR-27a is involved in the modulation of growth, angiogenesis and metastasis of BC and gastric cancer cells (Ding et al. 2017; Zhou et al. 2016a; Tang et al. 2014). Targets genes of the majority of the miRNA are not known, but the predicted limit is from a single to hundreds of genes for a certain miRN As, which is based on target estimation by applying an array of bioinformatics methods. The biogenesis of miRNAs is a highly regulated process, and alteration in their regulation is linked with many diseases in human (Chen et al. 2018; Ding et al. 2017; Ha and Kim 2014). Expression of miRNAs is tissue-specific and also influenced by different developmental stages. Hence, miRNAs exhibiting abnormal expression levels in the situation of certain diseases are of great importance as therapeutic targets. As only a limited regulatory target is known, predicting and validating miRNA targets is one of the major hurdles in understanding miRNA biology. Due to diversity in miRNAs and difficulty in experimental identification of target genes, the specific function of most miRNAs is not known. It is necessary to find their target mRNAs to explore their post-transcriptional regulating properties and associations for ailments and diagnostics. Bioinformatics tools were used to predict target mRNAs for *MIR27a*.

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METHODOLOGY

Target Prediction

Mir-27a was selected based on its curial role in the modulation of breast cancer. Matured sequences of MIR27a are hsa-miR-27a-5p (MIMAT0004501)AGGGCUUAGCUGCUUGUGAGCA and hsa-miR-27a-3p (MIMAT0000084) UUCACAGUGGCUAAGUUCCGC. Twelve different target prediction tools were used for identification of target messenger RNAs for matured miRNAs of *MIR27a*. The prediction tools were selected on the basis of their availability on a single site (MirWalk 2.0) which reduced the amount of generated data by selection of filters. The target prediction tools used were Mirwalk, Microt4, Miranda, mirbridge, miRDB, miRMap, MiRNAMap, Pictar2, PITA, RNA22, RNAhybrid, and TargetScan. The predicted target genes were filtered based on the number of hits received and only those target genes were selected that were predicted by at least six target prediction tools.

Functions of the Predicted Target Genes

The filtered genes were enlisted, and previously published literature was searched for finding functions that may be associated with cancer, especially breast cancer. The data were searched using the keywords. Gene name and breast cancer/Gene name expression in breast cancer patients.

Grouping of Genes on the Basis of their Reported Activity in Cancer

The predicted genes were divided into groups as follows. Groups including all those predicted genes, which have been validated to be overexpressed or suppressed in different research studies in breast cancer with few from other human cancer such as hepatocellular carcinoma. Differentially expressed genes, genes involved in cancer progression, genes promoting tumorigenesis, genes involved in chromatin remodeling, cell migration inhibitors, tumor suppressors and cell proliferation promoters (Table 1).

Finding Gene Networks

The association of the predicted genes with one another was found by using gene MANIA (Multiple Association Network Integration Algorithm). Gene MANIA is quick and accurate and does not rely on a pre-specified association network (Mostafavi et al. 2008). The gene MANIA attributes were set as co-expression 20/235, co-localization 4/4, genetic interactions 10/10, pathway 6/6, physical interaction 244/244, predicted 42/42, and shared protein domains 2/2. Interactions were found among all the predicted genes, predicted targets of has-mir-27a-3p, hsa-mir-27-5p, overexpressed genes and down-regulated genes.

RESULTS

Predicted Target Identification for hsa-mir-27a-3p and hsa-mir-27a-5p and their Potential Role in Breast Cancer Progression

Target genes for the matured sequences of MIR27a (hsa-mir-27a-3p and hsa-mir-27a-5p) were investigated at the 3'-UTR of the mRNA of the target genes using bioinformatics tools. After filtering the results, 51 target genes were obtained for hsa-mir-27a-3p. Out of these genes, 40 are associated with cancer (Table 2) and 34 genes for hsa-mir-27a-5p were associated with

Table 1: Predicted target genes for has-mir-27a-(3p/ 5p) and their associated functions

Function	*Associated genes*
Over expressed genes	*TMSB10, APPBP2, ELAVL1, SLC23A2, BRPF3, PRX , DLL4, CKAP4, BTF3, LPCAT1, PRKCA, ANTXR1, NR1D2,* and *PTP4A2*
Low expressed genes	*CLTCL1, FBXW7, ELL2, PPP6C, HIVEP3, SMAD4, IQGAP2,* and *ZBTB4*
Cancer progression	*DVL2, MAGI3, ELAVL2, NR4A1, KATNBL1, LPCAT1, TEAD1, C6orf106,* and *PAX9*
Tumor promoters	*GSPT1, EYA1, CKAP4, LPAR6, LPCAT1, EN2, CPSF2,* and *PTP4A2*
Cell division effectors	*MKNK2, SATB2, PLK2, ORC5, CCSER1,* and *AF1*
Migration inhibition	*DVL2, SLC23A2, NAPA, KCNA1, PRKCA, SERPINB7, PPP1CC,* and *PCDH20*
Tumor suppressors	*UFL1, NGFR, SMARCA1, TMEM33, FBXW7, PPP6C, NOTCH2, PLK2, PSD2, TAOK1, ZBTB4, NR1D2, FGD6, PPP2R2A,* and *PCDH20*
Proliferation enhancers	*ELAVL1, EIF5, PRX, NAPA, CCR5, EIF1AX, PAX9,* and *SOWAHA*

Table 2: List of hsa-mir-27a-3p target genes that are associated with breast cancer

Target gene	*Associated function*
EDEM3	Accelerate degradation of misfolded glycoproteins in the ER. Alteration in glycan structures are early signs of malignancy. Variable expression in breast carcinomas (Potapenko et al. 2015)
PAX9	Potentially playing important roles in cancer progression (Robson et al. 2006); Loss of PAX2 expression enhances endometrial cancer malignancy (Monte et al. 2010) PAX5 expression in breast cancer cells enhances epithelial behavior (Vidal et al. 2010) promoting breast cancer cell proliferation and tumorigenesis (Zong et al. 2011)
PLK2	Regulates mitotic spindle orientation and mammary gland development. Loss of Plk2 leads to increased mammary epithelial cell proliferation and ductal hyper branching (Villegas et al. 2014)
KCNA1	Expression was reduced in human cancers, decrease correlated with increase in breast cancer aggressiveness (Lallet-Daher et al. 2013)
BRPF3	Significantly more amplified in basal-like *BRCA1* (Prat et al. 2014)
APPBP2	Highly expressed in breast cancer NCBI (Monni et al. 2001; Li et al. 2002)
PPP1CC	Interacts with *BRCA1* expressions (Winter et al. 2007), Inhibits cell migration (Xie et al. 2015)
TAOK1	TAOK inhibition or depletion increases the mitotic population, the percentages of mitotic cells (Koo et al. 2017)
SRL	Inhibits proliferation of human breast cancer cells (Savanur et al. 2014)
GXYLT1	Modifies O-glycosylate Notch EFG like repeats resulting in negative regulation of notch signaling (Crabtree et al. 2016)
HIVEP3	Down-regulated in breast cancer (Fujii et al. 2005)
STARD7	Down regulated in human breast adenocarcinoma MCF7 cells (Durand et al. 2004). Modulates ABCG2 multidrug transporter level, cell migration, proliferation, and biochemical and morphological differentiation marker expression in JEG3 cells (Flores-Martín et al. 2012)
NR1D2	Abundantly expressed in adipose tissue and functions in adipocyte differentiation, regulation of cell metabolism (Preitner et al. 2002), have a prosurvival function in HER2-positive breast cancer cells (Kourtidis et al. 2010)
KATNBL1	Contributes to tumor progression in various cancers including colorectal cancer (Oh et al. 2016)
VANGL1	VANGL1 protein complex modulates the migration, polarity and growth of breast cancer cells in association with NOS1AP and SCRIB genes (Anastas et al. 2012)
NGFR	Acts either as tumor suppressor or oncogene depending on cellular context (Tsang et al. 2012). Differentially overexpressed in basal and HER2-enriched tumor samples (Fernández-Nogueira et al. 2016)
FGD6	Depletion of FGD6 reduced colony formation in the MCF10A epithelial model (Hirsch et al. 2010). inhibition in BC coincided with an enhanced EGF-signaling (de Graauw et al. 2014)
ABCA1	ABCA1 protein mediates the transfer of cellular cholesterol across the plasma membrane to apolipoprotein, Facilitates cancer cell survival A-I (Smith and Land 2012). Increased levels of cholesterol are found in tumors as compared to normal tissue (Yoshioka et al. 2000)
SMARCA1	Chromatin remodeler, depletion promotes cancer cell growth (Takeshima et al. 2015)
MKNK2	Activates eukaryotic initiation factor 4E (elF4E), that increase proteins involved in cell cycle, apoptosis and angiogenesis regulation (Wang et al. 2016)
DVL2	Activation promoted migration of breast cancer cells involved in cell migration (Zhu et al. 2012)
EN2	Potential prostate cancer biomarkers (Pandh et al. 2012). Over-expression of EN is linked to tumor development in breast (Martin et al. 2005)
NR1D2	Onco-suppressors in breast-cancer (Garattini et al. 2016) adipocyte differentiation, regulation of cell metabolism, and thermogenic responses (Preitner et al. 2002)
TMSB10	Regulates tumorigenesis and metastasis; Upregulated in breast cancer cells and tissues (Zhang et al. 2017)
RELN	Loss of reeling protein expression is associated with decreased survival (Stein et al. 2010)
FBXW7	Increased mRNA levels significantly reduced in breast cancer compared to normal tissues (Wei et al. 2012). Tumor suppressor negatively regulates different oncoproteins (Welcker et al. 2004)
NKAIN1	Deregulated in the *PIK3CA*-mutated breast tumors (Cizkova et al. 2010)
ELL2	Down-regulated in prostate cancer (Zang et al. 2017)
DLL4	Critical regulator of tumor angiogenesis. cancer, Dll4 is up-regulated strongly in the tumor vasculature (Sainson and Harris 2007)
ORC5	Involved in the regulation of cell cycle and DNA replication (Li et al. 2014)
LPCAT1	Upregulation in breast carcinoma contributes to tumor progression (Abdelzaher and Mostafa 2015). Overexpression in colorectal adenocarcinomas (Mansilla et al. 2009)
CKAP4	Frequently upregulated in pancreatic and lung cancers (Kimura et al. 2016). CKAP4 expression was much higher in HCC tumor tissues compared with adjacent normal tissues and its expression was significantly correlated with tumor size (Li et al. 2014)
SOWAHA	Overexpression is associated with breast cancer cell proliferation (Imaoka et al. 2014)
MAGI3	Promotes the malignant transformation of human mammary epithelial cells (Ni and

Table 2: Contd...

Target gene	*Associated function*
EYA1	Overexpressed with cyclin D1 in luminal B breast cancer subtype EYA1 enhanced breast tumor growth in mice *in vivo* requiring the phosphatase domain (Wu et al. 2013)
SATB2	Responsible for chromatin remodeling and regulation of gene expression. Overexpression increased tumor cell migration and invasion (Aprelikova et al. 2010). Higher in malignant human breast cancer cells compared with normal breast tissue. Higher SATB1 expression is associated with shorter overall survival. Acts as a 'master genome organizer' in human breast carcinogenesis (Patani et al. 2009)
PRKCB	Mediate various physiological processes such as proliferation, differentiation, apoptosis, cellular motility, and angiogenesis (Griner and Kazanietz 2007). Up-regulated in breast tumor versus matched normal patient tissue (Ali et al. 2009)
WNK3	Promoting cell survival in a caspase-3-dependent pathway (Verissimo et al. 2006). Suppression of endogenous WNK3 by RNA interference accelerated the apoptotic response of HeLa cells (Moniz and Jordan 2010)
LPAR6	Overexpression of LPAR6 in HCC specimens associated with poor survival (hepatic cancer) supports the tumorigenicity of hepatocellular carcinoma (Mazzocca et al. 2015)
CDC42BPB	Vital to the survival of cancer cells (Fesik et al. 2003)

cancer (Table 3). Data mining for cancer-related role of the predicted genes was performed and it was found that fourteen genes were found to have overexpression in breast cancer in various studies, these include, *TMSB10, APPBP2, ELAVL1, SLC23A2, BRPF3, PRX, DLL4, CKAP4, BTF3, LPCAT1, PRKCA, ANTXR1, NR1D2,* and *PTP4A2*. Information is shown in Table 1 and Table 2. Eight genes had been reported to have overexpression in breast cancer cells and tissues as compared to normal cells and tissues. The down-regulated genes are *CLTCL1, FBXW7, ELL2, PPP6C, HIVEP3, SMAD4, IQGAP2,* and *ZBTB4*.

Of these genes, nine are involved in cancer progression while eight genes act as tumor promoters. Six genes affect spindle fibers orientation and chromatin remodeling. Eight of them inhibit the migration of cancer cells. Fifteen are reportedly involved in the inhibition of tumor growth and development. Furthermore, eight genes were found to positively affect the proliferation of cancerous cells. Co-expression, pathway, co-localization, similar domain, and genetic interactions were investigated.

Deregulated Genes Interactions

For the overexpressed genes, there was 63.65 percent co-expression, 34.09 percent involvement in pathways, 1.14 percent co-localization and 1.14 percent genetic interactions. The overexpressed genes were found to have high similarity index of expression across different conditions. For down-regulated genes no significant association was found, co-expression 73.23 percent, predicted 15.84 percent, pathway 10.34 percent, genetic interactions 0.37 percent, and co-localization 0.22 percent. When all the deregulated genes were compared it was observed that they are related by having a 73.23 percent of similar expression (Table 4).

hsa-mir27a-3p Target Predicted Genes Network

Interaction among the predicted genes was found with the help of gene MANIA online tools. Search conditions were set according to the following default arrangement. Co-expression 20/235, co-localization 4/4, genetic interactions 10/10, pathway 6/6, physical interaction 244/244, predicted 42/42 and shared protein domains 2/2. Co-expression shows that the genes are expressed together across different experimental conditions; Co-localization refers to the fact that the genes are expressed inside the same tissue or location; Pathways interaction means the protein of the genes are involved in the same reaction in a common pathway; Predicted are functions of the orthologous genes interaction obtained from a different organism; Genetic interactions means altering expression of one gene affects the expression of the other; Physical interaction means that product interact in a protein interaction study; Shared protein domain means that proteins produced by the gene are part of the same protein, enzyme or complex.

Table 3: Important target genes for hsa-mir-27a-5p and their associated functions

hsa-mir-27a-5p	*Associated functions*
PSD2	Tumor suppressor; early loss of expression of EFA6B contributes to the malignant progression of breast cancer (Zangari et al. 2014)
WDFY3	Autophagy (Simonsen et al. 2004) (Cullinane et al. 2013)
PRKCA	Over expression reduces proliferation and migration of breast cancer cells (Lønne et al. 2010); Higher expression in TNBC cells (Hsu et al. 2014)
EIF5	Promotes cell proliferation (Homma et al. 2005)
CCSER1	Contribute to the chromosomal instability of cancer; deficiency creates defects in cell division (Patel et al. 2013)
ELAVL1	Increased at all stages of breast cancer; promotes breast cancer cells proliferation (Zhang et al. 2017); Increased invasiveness (Gubin et al. 2010)
IQGAP2	Reduced expression in breast cancer tissues; low expression reduces patients survival; Reduced expression in higher stages of breast cancer (Kumar et al. 2017)
C60rf106	Promotes the malignant progression of breast cancer (Jiang et al. 2015)
TMEM33	Overexpression induces apoptosis in breast cancer cells (Clarke et al. 2017)
TSHZ3	Required for the survival of breast cancer cells harboring 19q12 amplification (Natrajan et al. 2012); Downregulated in breast cancer cells (Yamamoto et al. 2011)
PPAT	Increased expression in lung cancer (Goswami et al. 2015)
CPSF2	Overexpressed in human breast cancer and functions as a tumor promoter (Binothman et al. 2017)
SLC23A2	Enhances cancer cell inhibition in association with Vit-C; Overexpressed in BC cells, helps in autophagy of BC cells (Hong et al. 2013)
PCDH20	Significantly suppressed cell proliferation, clonogenicity, migration and tumor formation in hepatocellular cancer cells (Lv et al. 2015)
NOTCH2	Tumor suppressor in colorectal cancer (Chu et al. 2011); Differential expression in subgroups of breast tumors (Fu et al. 2010)
SERPINB7	Suppressed the invasiveness and motility of malignant cancer cells (Chou et al. 2012)
SMAD4	Decreased in ductal breast carcinoma (Liu et al. 2015):[illegible](Stuelten et al. 2006); Induced apoptosis in breast cancer cells (Li et al. 2005)
ZBTB4	Downregulated in breast cancer patients; Tumor-suppressor in breast cancer cells (Kim et al. 2012)
ANTXR1	Up-regulated in tumor vasculature (Andersen et al. 2016)
PTP4A2	Promotes breast tumor formation in the mouse model (Hardy et al. 2010). Low expression is associated with shorter survival in breast cancer patients (Zhao et al. 2015). Increased expression increases the patient's survival (Andres et al. 2013): Overexpression in breast cancer tissues (Ottenhoff-Kalff et al. 1995); (Andres et al. 2013): Overexpression in BC cells (Hardy et al. 2010)
GSPT1	Proto-onco gene (Malta-Vacas et al. 2009)
PPP6C	Have a protective role during breast cancer; lower expression in breast tumors (Zhong et al. 2011)
EIF1AX	Overexpression results in cell proliferation (Yu et al. 2014)
PAF1	Cell-cycle regulation (Jaehning 2010) Tumorigenesis; Candidate oncogene (Chaudhary et al. 2007)
BTF3	Increased expression in epithelial carcinoma (Symes et al. 2013)
PPP2R2A	Tumor suppressor (Beca et al. 2015) (Kalev et al. 2012)
NAPA	Inhibits cell invasiveness (Vasse et al. 2001); Stimulate cell aggregation, inhibits proliferation (Thibout et al. 1999)
NR4A1	Promotes breast cancer invasion and metastasis (Zhou et al. 2014)
UFL1	Tumor suppressor (Wu et al. 2010); Overexpression promotes the proliferation of lung cancer (Kim et al. 2013)
CCR5	Increase metabolic activity of cancer cells, Enhance the proliferation of breast cancer cells (Gao et al. 2016); Increased expression in breast cancer cells (Velasco-Velázquez et al. 2014)
TEAD1	Promotes breast cancer (Wang et al. 2015), the initiation and progression of cancer (Zhou et al. 2016)
CLTCL1	Down-regulated in breast cancer early invasiveness (Sens-Abuazar et al. 2012)
PRX	Overexpression in breast cancer tissues compared to other tissues; Higher expression in breast cancer as compared to other tissues cancer (Cha et al. 2009); Silencing inhibits tumor proliferation in breast cancer (Chua et al. 2010)
MYOD1	Inhibits the proliferation of breast cancer cells (Cai et al. 2016)

The genetic influence on each other is less and also has very less existence in cells and tissues. Collectively all predicted target genes for has-mir-27a-3p had co-expression 49.74 percent, predicted 25.20 percent, pathway 18.36 percent, physical interactions 5.02 percent, co-localization 1.40 percent, and shared protein domains 0.28 percent. The 27a-3p predicted target genes showed a 49.7 percent similar expression, while they tend to have a lower tendency of becoming part of a protein domain. However, they are involved in the regulation of different pathways sharing 25.2 percent of reactions involved in various pathways. These genes are less affected by perturbation from one another (Table 4).

hsa-mir-27a-5p Target Predicted Genes Network

Co-expression 39.46 percent, pathway 32.06 percent, physical interactions 16.08 percent, predicted 7.83 percent, co-localization 4.16 percent, genetic interactions 0.41 percent. For the 27a-5p targets the co-expression rate is 39.46 percent, next comes involvement in pathways and there is very less genetic interaction predicted for them (Table 4).

hsa-mir-27a-3p and 27a-5p Combined Targets Interactions

Predicted 45.01 percent, co-expression 31.48 percent, pathway 16.30 percent, physical interactions 4.56 percent, genetic interactions 1.88 percent, shared protein domains 0.46 percent and co-localization 0.31 percent. When all the predicted target genes are co-analyzed it is observed that the co-expression of these genes is reduced to 31.48 percent. They do not express in the same tissues or cells and share protein domains in a very small percentage (Table 4).

DISCUSSION

Only those predicted genes were enlisted which were predicted by at least 6 target prediction tools. For hsa-mir-27a-3p a total of 51 target genes were found while for has-mir-27a-5p; 45 targets were determined. The genes were explored for their role in breast cancer using the online available literature. It was found that the target genes are involved in vital processes that regulate the formation and development of tumors. Nine genes, that are predicted targets of hsa-mir-27a, have been reported to be down-regulated in breast cancer patients such as *NKAIN1* and *PIK3CA* mutated breast tumors and *STARD7* in human breast adenocarcinoma *MCF* cells and *HIVEP3*, *ZBTB4*, and *KCNA1* in breast and other human cancer. Besides breast cancer tumor aggression in gastric cancer is increased by mutations *PIK3CA* (Kim et al. 2017). *FBXW7* has been reported to have reduced mRNA level in breast cancer cells as compared to normal tissues, similarly, FBXW7 disruption increased cancer progression by suppressing apoptosis and promoting cell proliferation in breast cancer cells (Xia et al. 2017). In another study it is been stated that inhibition of *FBXW7* by mir-367a promoted tumor growth in breast cancer patients (Xiao et al. 2017) while *IQGAP2* has lower expression in breast tissues and higher stages of breast cancer and is associated with survival of breast cancer patients (Kumar et al. 2017; Wei et al. 2012).

The expression levels of *CLTCL1* are reduced during breast cancer early invasiveness and

Table 4: Illustrated interaction among the predicted genes using gene mania online tools

Category	*Co-expression (%)*	*Pathways (%)*	*Predicted (%)*	*Co-ocalization l (%)*	*Genetic interactions (%)*	*Physical interactions (%)*	*Shared protein domains (%)*
All predicted target genes	31.48	16.30	45.01	0.31	1.88	4.56	0.46
Hsa-mir-27a-5p all predicted target genes	39.46	32.06	7.83	4.16	0.41	16.08	-
Hsa-mir-27a-3p all predicted target genes	49.74	18.36	25.20	1.40	-	5.02	0.28
All over expressed genes	63.65	34.09		1.14	1.13	-	-
All deregulated genes	73.23	10.34	15.84	0.22	0.37	-	-

SMAD4 has been reported to be down-regulated in ductal breast carcinomas. This gene has been identified as a driver mutation of tumorigenesis in dendritic cell carcinoma (Davila et al. 2017). In contrast to down-regulated genes; certain genes were documented as up-regulated in various breast tissues and cancer types such as *NGFR* is over-expressed in basal and HER2-enriched tumor samples (Fernández-Nogueira et al. 2016).

Overexpression of *TMSB10*, *CPSF2*, *AP-PBP2*, *PTP4A* and *CCR5* is reported in breast cancer cells and tissues (Binothman et al. 2017; Zhang et al. 2017; Velasco-Velázquez et al. 2014; Andres et al. 2013; Li et al. 2002; Monni et al. 2001; Ottenhoff-Kalff et al. 1995). Similarly, variable expression for *EDEM3* has been reported in breast carcinomas (Potapenko et al. 2015). Significantly high regulation of *BRF3* in basal-like *BRCA1*, *NRID2* in adipose tissue and (*DLL4* and *ANTXR1*) in tumor vasculature have been known in previous research work (Andersen et al. 2016; Prat et al. 2014; Sainson and Harris 2007; Preitne et al. 2002). *ANTXR1* has been used as a member in targeted therapy against triple negative breast cancer treatment in recent studies (Byrd et al. 2017).

Increased expression of *PPAT* in lung cancer and *BTF3* in other epithelial carcinomas was found in two separate studies (Goswami et al. 2015; Symes et al. 2013). Two independent studies have reported overexpression of *PRX* in breast cancer tissues as compared to other tissue cells and cancers. In addition, *PRKCB* and *DLL4* are involved in angiogenesis. *PRKCB* has been reported to be a good predictor for non-small cell lung cancer (*NSCLC*) in recent research (Liu et al. 2017).

Previous investigations have shown that *WNK3* suppression accelerated the apoptotic response in HELA cells. Griner and Kazanietz (2007) have reported the involvement of *PRKCB* in apoptosis while *TMEM33* induced apoptosis in breast cancer when overexpressed (Clarke et al. 2017). *PLK2* and *SMARCA1* regulated mitotic spindle orientation and remodeling while *STAB2* is responsible regulation of gene expression besides chromatin remodeling. *PLK2* down-regulation has been reported in other cancer like B-cell neoplasm (Syed et al. 2006). Cell survival is hallmark of cancerous cells. The predicted target gene *WNK3*, *ABACA1*, and *CDC42BPB* are reported to promote cell survival. In different cellular contexts, *NGFR* acts either as a tumor suppressor or an oncogene. *FGD6* depletion reduced the process of colony formation in MCF10A cells, *SRL*, *FBXW7*, and *MYOD1* inhibits proliferation of breast cancer cells and negatively regulates different oncoproteins (Cai et al. 2016; Savanur et al. 2014; Welcker et al. 2004). *NRID2* is oncosuppressor in breast cancer and *NOTCH2* in colorectal cancer (Garattini et al. 2016; Chu et al. 2011). *ZBTB4*, *PSD2*, *UFL1*, and *PPP2R2* are a tumor suppressor in breast cancer cells and early loss of *PSD2* contributes to the progression of breast cancer. *SLC23A* helps in autophagy of breast cancer cells.

PAX9 potentially play important role in tumorigenesis and proliferation. *PLK2* loss leads to increased mammary epithelial cell proliferation and ductal hyper-branching, inhibition or depletion of *TAOK1* increases the mitotic population of cells (Koo et al. 2017). *EIF5*, *CCR5*, and *SOWAHA* overexpression are associated with an increase in metabolic activity and breast cancer cell proliferation (Gao et al. 2016; Imaoka et al. 2014; Homma et al. 2005). *ELAVL1* increased at all stages of breast cancers and promotes breast cancer cell proliferation and invasion (Li et al. 2017; Gubin et al. 2010). *EIF1AX* over-expression results in cell proliferation, *PAF1* regulates cell cycle and *PRKCA* overexpression reduces proliferation and migration of breast cancer cells.

Overexpression of *LPCAT1* and down-regulation of *PAX9* contributes to breast cancer progression and malignancy. *CKAP4* is significantly correlated with tumor size. *KATNBL1*, *C6orf106*, *TEAD1*, and *EN2* contribute to tumor progression in breast, hepatocellular and colorectal cancer (Oh et al. 2016; Zhou et al. 2016b; Jiang et al. 2015; Martin et al. 2005). *EYA1* enhanced tumor growth in mice in vivo requiring the phosphatase domain. *MAG13* promotes malignant transformation of human mammary epithelial cells (Ni and Kuperwasser 2016). *TMSB10* regulates tumorigenesis and metastasis (Zhang et al. 2017). *PCDH20* and *PRKCA* significantly suppress migration and tumor formation in hepatocellular and breast cancer cells. *NR4A1* promotes breast cancer invasion and metastasis. *SERPINB7* and *NAPA* suppress the

invasion and motility of malignant cancer cell. *CPSF2* and *PAF1* promote tumor (Binothman et al. 2017; Chaudhary et al. 2007). *PTP4A2* promotes breast tumor formation in the mouse model. *ORC5* is involved in the regulation of cell cycle and DNA replication. *NR1D2* regulates cell metabolism and thermogenic responses, depletion *SMARCA1* promotes cancer cell growth while *PPP1CC* interacts with *BRCA1* gene.

CONCLUSION

Matured miRNAs (hsa-mir-27a-3p and hsa-mir-27a-5p) codded by *MIR27a* gene regulates several genes in the human genome that are associated with breast cancer. Deregulation of *MIR27a* gene due to mutation may affect the functions of these predicted target genes. Experimental validation of interaction between the target genes and hsa-mir-27a-3p/5p can help in better understanding the initiation, proliferation, tumorigenesis, and metastasis in breast cancer.

RECOMMENDATIONS

Further *in-vitro* study is required to investigate the interaction of miR-27a gene with predicted cancer-associated genes reported in the current study.

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