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Gene Expression Profiling of Triple Negative Breast Cancer Cells Treated with Afatinib

Qudus Ibrahim[1], Ruchi Patel[1], Elvis Boamah[1], Dolapo Ajayi[1] and Michael Danquah[1*]

[1]*Department of Pharmaceutical Sciences, Chicago State University, 9501 South King Drive, Chicago, IL, 60628, USA.*

Authors' contributions

This work was carried out in collaboration between all authors. Author QI conducted literature search, performed experiments and analyses and wrote first draft of the manuscript. Authors RP, EB and DA performed experiments and literature search. Author MD conceptualized, supervised the study and revised the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Purpose: To investigate gene expression profile changes in triple negative breast cancer cells (MDA-MB-231) treated with afatinib.
Methods: Differential expression of 84 genes commonly involved in breast cancer carcinogenesis was examined in MDA-MB-231 cells treated with afatinib (5 μM) and compared to untreated cells. Total RNA was extracted using RNeasy mini kit and subsequently assessed by real-time PCR using the Human Breast Cancer RT^2 Profiler PCR Array. Relative gene expression was computed using the $\Delta\Delta C_t$ approach and a fold change equal to or greater than 2 was considered significant.
Results: Treatment of MDA-MB-231 cells with afatinib (5 μM) for 24 h resulted in significant differential expression of several genes commonly involved in breast cancer carcinogenesis. Specifically, 33 of the 84 genes examined exhibited greater than two-fold differential expression when MDA-MB-231 cells were exposed to afatinib. Three genes (*CTSD*, *ESR2* and *ID1*) were upregulated while thirty genes were downregulated in afatinib treated cells compared to control. Core analysis of differentially expressed genes using the Ingenuity Pathway Analysis (IPA) software identified five regulatory networks pertinent to cell cycle, cancer, cellular growth and

*Corresponding author: E-mail: mdanquah@csu.edu;

proliferation. This led to phosphoinositide-3-kinase (PI3K)/Akt and mitogen-activated protein kinase (MAPK)/ERK being identified as pathways impacted by afatinib.
Conclusions: Our findings elucidate molecular targets with altered expression in MDA-MB-231 cells exposed to afatinib. Based on RT^2-PCR array analysis, afatinib increased expression of key tumor suppressor genes and down-regulated expression of pivotal oncogenes. This knowledge could contribute to the design and development of effective afatinib based combination therapies for treating TNBC.

Keywords: Afatinib; triple negative breast cancer; breast cancer; gene expression profiling; RT^2 profiler PCR array; EGFR inhibitors.

1. INTRODUCTION

Breast cancer is the most prevalent form of cancer and the foremost cause of cancer related death affecting women in the United States [1]. Approximately 246,660 new cases of breast cancer are predicted to occur in 2016 with 40,450 mortalities projected regardless of therapeutic advances [1]. Of this number, approximately 10 to 17% of breast cancer patients have triple negative breast cancer (TNBC) [2-5]. Clinically, TNBC are an aggressive molecular subtype of breast tumors that lack expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER 2) [6-8]. Collectively, the prognosis for TNBC patients is poor with median survival being less than one year for women with metastatic TNBC. A couple of reasons contribute to this bleak outcome. First, endocrine therapy (e.g., tamoxifen or aromatase inhibitors) or HER2 targeted therapies (e.g., lapatinib or Herceptin) routinely used in treating breast cancer lack therapeutic basis and are ineffective in TNBC. Second, TNBC exhibits a high rate of early-occurring metastasis as well as a propensity for recurrence. The absence of effective targeted therapies for treating TNBC had led to reliance on chemotherapy. For example, anthracycline- and taxane-based chemotherapy is now being explored for treating TNBC with modest benefit [9-11]. Nonetheless, low therapeutic to toxicity ratios associated with anticancer agents employed in TNBC chemotherapy has mitigated their use and currently there is no preferred standard form of chemotherapy for TNBC. Hence, there is an urgent need for targeted therapies against TNBC and several studies are investigating signature molecules that can be used as target sites in TNBC treatment.

Overexpression of the epidermal growth factor receptor (EGFR/HER1) has been demonstrated to occur in more than 60% of TNBC cases [12-14]. Its expression is associated with reduced apoptosis, increased proliferation and survival of cancer cells and resultant inferior treatment outcome [15,16]. A number of therapeutic strategies have been developed to inhibit EGFR. One approach utilizes monoclonal antibodies targeting the extracellular domain of EGFR. Examples include cetuximab and panitumumab which have been used in combination with anticancer agents in clinical trials with mixed results [17-20]. A second approach exploits small molecule tyrosine kinase inhibitors (TKIs) which bind directly to the tyrosine kinase domain of the EGFR. Examples of widely-used first generation EGFR-targeting TKIs include erlotinib, gefitinb and lapatinib. Recently, erlotinib and gefitinib have been used in treating non-small cell lung cancers (NSCLC) harboring an EGFR-mutation. Lapatinib has also shown promising results for metastatic breast cancer treatment in combination with capecitabine and has been approved by the Food and Drug Administration (FDA) [21-24]. Despite the wide application of TKIs for treating solid tumors, it is only recently that a TKI (erlotinib) in combination with carboplatin and docetaxel is being evaluated for treating metastatic TNBC (NCT00491816). One potential reason limiting the use of TKIs for treating TNBC could be that first generation EGFR-targeting TKIs (erlotinib, gefitinb and lapatinib) are reversible EGFR inhibitors and prolonged administration results in many patients becoming resistant to the drug. In contrast, afatinib (BIBW2992) is an anilinoquinazoline ATP-competitive inhibitor which covalently binds to and irreversibly inhibits EGFR (HER1), HER2 and HER 4 with high selectivity [25]. It is extremely effective against wild-type and mutant EGFR including the L858R/T790M double mutation of EGFR known to be resistant to erlotinib, gefitinib and lapatinib [25-27]. We recently showed afatinib alone or in combination with cyclopamine to potently inhibit cell proliferation and induce apoptosis in MDA-MB-231 breast cancer cells (metastatic basal-like

TNBC model) and MCF-7 cells which is HER2 negative [28]. In that study, we explored the potential of afatinib as an EGFR-targeted therapy and speculated afatinib efficacy to be dependent on EGFR expression. However, our findings revealed a need for better understanding of the effects of afatinib on MDA-MB-231 cell line at the genomic level if it is to be used to design and develop therapeutic approaches against TNBC.

The purpose of this study is to survey the gene expression profile of a focused panel of 84 breast cancer related genes following exposure of MDA-MB-231 cells to afatinib. Better understanding of key breast cancer genes impacted by exposure of MDA-MB-231 cells to afatinib is urgently needed since this knowledge could potentially facilitate rationale development of new effective afatinib-based combination therapy strategies for treating TNBC.

2. MATERIALS AND METHODS

2.1 Materials

Human breast cancer cell lines MDA-MB-231 were purchased from American Type Culture Collection (ATCC, Manassas, VA) and stored in liquid nitrogen. Dulbecco's Modified Eagle Medium (DMEM), TrypLE Express and antibiotic-antimycotic were obtained from Life Technologies (Carlsbad, CA). Fetal Bovine Serum (FBS) was obtained from Atlanta Biologicals (Flowery Branch, GA). Afatinib was purchased from LC-Laboratories (Woburn, MA). All other reagents were obtained from Sigma-Aldrich (St. Louis, MO) and used as received unless otherwise stated.

2.2 Cell Culture and Drug Treatment

MDA-MB-231 breast cancer cell lines were recovered from liquid nitrogen and maintained in DMEM supplemented with 10% Fetal Bovine Serum (FBS) and 1% Antibiotic-Antimycotic. Cells were incubated with complete medium in a humidified incubator of 5% CO_2 at 37°C. Cells were sub-cultured every 3–4 days to maintain exponential growth. For experiments, cells were seeded in 6-well plates at a density 4 x 10^5 of viable cells per well following counting using a Countess automated cell counter (Life Technologies, Carlsbad, CA) and allowed to grow for 24 h. Subsequently, cells were treated with afatinib (5 µM) for 24 h.

2.3 Human Breast Cancer RT^2 Profiler PCR Array

Human breast cancer RT2 Profiler PCR Array was performed as previously described [29]. Total RNA was first extracted per the manufacturer's protocol using RNeasy mini kit from Qiagen (Valencia, CA) and RNA concentration and quality measured using an Eppendorf BioPhotometer Plus (Hauppauge, NY). Subsequently, cDNA synthesis was performed using RT^2 First Stand Kit (Qiagen, Valencia, CA) following the manufacturer's protocol and stored at -20°C until used. Samples for the Human Breast Cancer RT^2 Profiler PCR Array (Qiagen, Valencia, CA) were prepared by mixing 1350 µL of 2x RT^2 SYBR Green mastermix, 102 µL cDNA reaction mixture 1248 µL of RNase-free water and pipetted into 96-well pcr array plates to assess expression of pertinent breast cancer genes. RT^2 Profiler PCR Arrays were then run on Eppendorf Mastercycler ep realplex model 4 (Hauppauge, NY) using 95°C for 10 min, 45 cycles of 95°C for 15s and 60°C for 1 min as PCR cycling condition. A web-based PCR Array Data Analysis Software (www.SABiosciences.com/pcrarraydataanalysis.php) was used to analyze cycle thresholds obtained from the real-time PCR. $\Delta\Delta C_t$ determined for each gene following afatinib-treatment was compared to the control array to compute relative gene expression. A fold change equal to or greater than 2 was considered significant.

2.4 Ingenuity Pathway Analysis

Core analysis on the dataset gene files generated using RT^2 Profiler PCR Array was performed using the Ingenuity Pathway Analysis (IPA) program (https://analysis.ingenuity.com/) to explore regulatory networks. Analysis was done using the gene ID and fold change greater than 2. The flexible format was used for analyzing raw data and gene identified by RefSeq accession numbers.

3. RESULTS

3.1 Identification of Differentially Expressed Genes in Afatinib Treated MDA-MB-231 Cells

Breast cancer associated gene expression changes following treatment of MDA-MB-231 cells with afatinib (5 µM) for 24 h were identified

by performing a Human Breast Cancer RT^2 profiler PCR array. Analyses RT^2 profiler PCR array data revealed expression changes in 33 of the 84 genes studied (Table 1 and Figs. 1 – 3). The remaining 51 genes did not display a significant change compared to control (untreated MDA-MB-231 cells). The differentially expressed genes were categorized based on the following nine functional roles: (1) Signal transduction, (2) Epithelial to Mesenchymal Transition (EMT), (3) Angiogenesis, (4) Cell Adhesion Molecules, (5) Proteolysis, (6) Apoptosis, (7) Cell Cycle, (8) DNA Damage and (9) transcription factors. A total of 3 genes exhibited increased expression while 30 genes demonstrated decreased expression. Among the 3 most upregulated genes, one coded for signal transduction proteins and transcription factors (Estrogen receptor 2 [*ESR2*]) (Fig. 1A and C), one for angiogenesis (Inhibitor of DNA binding 1 [*ID1*]) (Fig. 3A) and one for proteolysis (Cathepsin D [*CTSD*]) (Fig. 1C).

Regarding the 30 most downregulated genes, eight coded for signal transduction proteins (Catenin (cadherin-associated protein), beta 1 [*CTNBB1*], Retinoblastoma 1 [*RB1*], Adenomatous polyposis coli [*APC*], Secreted frizzled-related protein 1 [*SFRP1*], Phosphatase and tensin homolog [*PTEN*], Baculoviral IAP repeat containing 5 [*BIRCA5*], Notch 1 [*NOTCH1*] and Tumor protein p73 [*TP73*]) (Fig. 1A); two for epithelial-to-mesenchymal transition (*EMT*) proteins (Catenin (cadherin-associated protein), beta 1 [*CTNBB1*] and Notch 1 [*NOTCH1*]) (Fig. 1B) and seven for transcription factors (Catenin (cadherin-associated protein), beta 1 [*CTNBB1*], GATA binding protein 3 [*GATA3*], Hypermethylated in cancer 1 [*HIC1*], Notch 1 [*NOTCH1*].

Retinoic acid receptor, beta [RARB], Retinoblastoma 1 [*RB1*] and Tumor protein p73 [*TP73*]) (Fig. 1C). Additionally, five downregulated genes coded for apoptosis proteins (Adenomatous polyposis coli [*APC*], Cadherin 1, type 1, E-cadherin [*CDH1*], Interleukin 6 [*IL6*], Retinoic acid receptor, beta [*RARB*] and Stratifin [*SFN*] (Fig. 2A); eight for cell cycle proteins (Adenomatous polyposis coli [*APC*], Cyclin A1 [*CCNA1*], Cyclin-dependent kinase 2 [*CDK2*], monoclonal

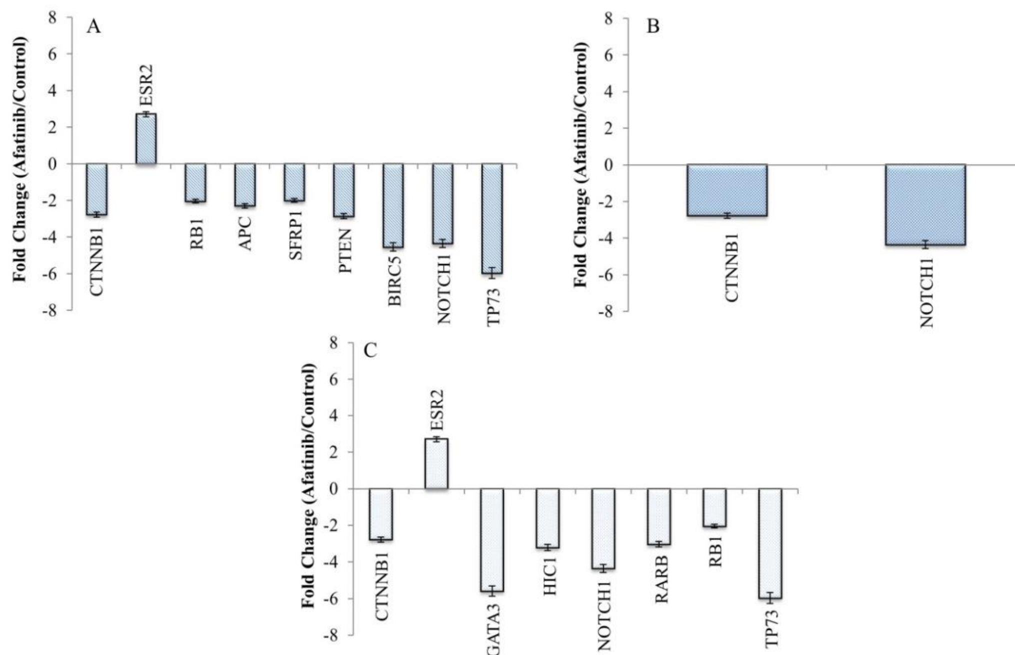


Fig. 1 Gene expression in MDA-MB-231 breast cancer cell line following treatment with afatinib (5 µM) for 24 h using a differential cutoff of two-fold. (A) Signal Transduction; (B) Epithelial-to-Mesenchymal Transition (EMT) and (C) Transcription Factors.

antibody Ki-67 [*MKI67*], Phosphatase and tensin homolog [*PTEN*], Ras association (RalGDS/AF-6) domain family member 1 [*RASSF1*], Retinoblastoma 1 [*RB1*] and Stratifin [*SFN*] (Fig. 2B) and five genes for DNA Damage proteins (Adenomatous polyposis coli [*APC*], Breast cancer 2, early onset [*BRCA2*], MutL homolog 1 [*MLH1*], Stratifin [*SFN*] and Tumor protein p73 [*TP73*]) (Fig. 2C). Also, ten downregulated genes coded for angiogenesis proteins (Cadherin 13, H-cadherin [*CDH13*], Catenin (cadherin-associated protein), beta 1 [*CTNBB1*], Epidermal growth factor [*EGF*], Interleukin 6 [*IL6*], Notch 1 [*NOTCH1*], Plasminogen activator, urokinase [*PLAU*], Phosphatase and tensin homolog [*PTEN*], Serpin peptidase inhibitor [*SERPINE1*], Slit homolog 2 [*SLIT2*] and Thrombospondin 1 [*THBS1*]) (Fig. 3A); seven for cell adhesion molecules (ADAM metallopeptidase domain 23 [*ADAM23*], Adenomatous polyposis coli [*APC*], Cadherin 1, type 1, E-cadherin [*CDH1*], Catenin (cadherin-associated protein), beta 1 [*CTNBB1*], Phosphatase and tensin homolog [*PTEN*] and Thrombospondin 1 [*THBS1*]) (Fig. 3B) and three for proteolysis (ADAM metallopeptidase domain 23 [*ADAM23*], Cystatin E/M [*CST6*] and Plasminogen activator, urokinase [*PLAU*] (Fig. 3C).

Table 1. Functional grouping of genes differentially expressed following afatinib treatment for 24 h. Gene names in bold are upregulated, while gene names in standard type are downregulated

Gene	Description	Gene category	Upregulated/ Downregulated
ADAM23	ADAM metallopeptidase domain 23	Cell Adhesion Molecules/ Proteases	Downregulated
APC	Adenomatous polyposis coli	Signal Transduction/ Cell Adhesion Molecules/Apoptosis/Cell Cycle/DNA Damage and Repair	Downregulated
BIRC5	Baculoviral IAP repeat containing 5	Signal Transduction	Downregulated
BRCA2	Breast cancer 2, early onset	DNA Damage and Repair	Downregulated
CCNA1	Cyclin A1	Cell Cycle	
CDH1	Cadherin 1, type 1, E-cadherin (epithelial)	Cell Adhesion Molecules/Apoptosis	Downregulated Downregulated
CDH13	Cadherin 13, H-cadherin (heart)	Angiogenesis/ Cell Adhesion Molecules	Downregulated
CDK2	Cyclin-dependent kinase 2	Cell Cycle	Downregulated
CST6	Cystatin E/M	Proteases	Downregulated
CTNNB1	Catenin (cadherin-associated protein), beta 1, 88kDa	Signal Transduction / Epithelial-to-Mesenchymal Transition/ Angiogenesis/Cell Adhesion Molecules/ Transcription Factors	Downregulated
CTSD	Cathepsin D	Proteases	Upregulated
EGF	Epidermal growth factor	Angiogenesis	Downregulated
ESR2	Estrogen receptor 2 (ER beta)	Signal Transduction/Transcription Factors	Upregulated
GATA3	GATA binding protein 3	Transcription Factors	Downregulated
HIC1	Hypermethylated in cancer 1	Transcription Factors	Downregulated
ID1	Inhibitor of DNA binding 1, dominant negative helix-loop-	Angiogenesis	Upregulated

Gene	Description	Gene category	Upregulated/ Downregulated
	helix protein		
IL6	Interleukin 6 (interferon, beta 2)	Angiogenesis/Apoptosis	Downregulated
MKI67	Antigen identified by monoclonal antibody Ki-67	Cell Cycle	Downregulated
MLH1	MutL homolog 1, colon cancer, nonpolyposis type 2 (E. coli)	DNA Damage and Repair	Downregulated
NOTCH1	Notch 1	Signal Transduction / Epithelial-to-Mesenchymal/Transcription Factors	Downregulated
PLAU	Plasminogen activator, urokinase	Proteases	Downregulated
PTEN	Phosphatase and tensin homolog	Signal Transduction / Cell Adhesion Molecules/ Cell Cycle	Downregulated
RARB	Retinoic acid receptor, beta	Apoptosis/Transcription Factors	Downregulated
RASSF1	Ras association (RalGDS/AF-6) domain family member 1	Cell Cycle	Downregulated
RB1	Retinoblastoma 1	Signal Transduction / Cell Cycle/ Transcription Factors	Downregulated
SERPINE1	Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1	Angiogenesis	Downregulated
SFN	Stratifin	Apoptosis/Cell Cycle/DNA Damage and Repair	Downregulated
SFRP1	Secreted frizzled-related protein 1	Apoptosis	Downregulated
SLIT2	Slit homolog 2 (Drosophila)	Angiogenesis	Downregulated
THBS1	Thrombospondin 1	Angiogenesis/ Cell Adhesion Molecules	Downregulated
TP73	Tumor protein p73	Signal Transduction /Apoptosis/ DNA Damage and Repair/ Transcription Factors	Downregulated

3.2 Gene Networks by IPA

To reveal pathways implicated in response to afatinib treatment, relationships between highly differentially expressed genes in MDA-MB-231 cells were determined using Ingenuity Pathway Analysis (IPA). The IPA program constructed 5 significantly interconnected gene networks. The first network (Fig. 4A) consisted of 13 focus molecules pertaining to Cellular Movement, Cancer, Organismal Injury and Abnormalities. Among these, only *CTSD* was upregulated. In contrast, the following genes were downregulated: *BRCA2, CDH1, CDH13, CST6, GATA3, HIC1, PLAU, SERPINE1, SFRP1, SLIT2, TFF3* and *THBS1*. The second network (Fig. 4B) comprised 9 focus molecules concerned with Cell Cycle, Cancer and Cellular Development. Only *ID1* was upregulated while downregulated genes included *APC, BIRC5, CCNA1, CDK2, RARB, RASSF1, RB1* and *SFN*. No upregulated genes were present in the third network (Fig. 4C) which contained 5 focus molecules associated with Cancer, Gastrointestinal Disease and Hepatic System Disease. However, downregulated genes included *ADAM23, CTNNB1, IL6, SLIT2* and *TP73*. The fourth network consisted of 4 focus molecules related to Cellular Movement, Cellular Development, Cellular Growth and Proliferation. Downregulated genes include *EGF, KRT8, NOTCH1* and *PTEN*. No upregulated genes were present. Finally, the fifth network contained 3 focus molecules associated with Organ Morphology, Reproductive System Development and Function and Cell Cycle. In this network,

ESR2 was upregulated while *MKI67* and *MLH1* were downregulated.

4. DISCUSSION

A number of studies have examined gene expression profiles of several solid tumors treated with EGFR inhibitors including afatinib [30-33]. However, most of these studies conducted in breast cancer have focused on HER2-positive breast cancer and little attention has been given to the effect of EGFR inhibitors on gene expression in TNBC. In this work, we studied the impact of afatinib on the expression of 84 key breast cancer genes in the TNBC cell line MDA-MB-231. Our aim was to furnish findings that could potentially inform the rationale development of combination therapies for treating TNBC based on afatinib.

We have previously shown afatinib to potently inhibit breast cancer cell proliferation alone or in combination with cyclopamine. In particular, we found the IC_{50} value of afatinib in MDA-MB-231 cells after treatment for 24 h to be approximately 5 μM [28]. Higher concentrations kill the vast majority of cells which make it impossible to assess gene expression changes. Hence, we chose to treat MDA-MB-231 cells with 5 μM afatinib in the current study. Of the 84 genes examined, 3 were highly upregulated and 30 were down-regulated following treatment with afatinib for 24 h. To delineate the functional role of the differentially expressed genes, we organized them in the following nine groupings for analyses: Angiogenesis, Apoptosis, Cell Adhesion Molecules, Cell Cycle, DNA Damage, Epithelial to Mesenchymal Transition (EMT), Proteolysis, transcription factors and Signal transduction. Since signal transduction comprises numerous signaling pathways, the signal transduction group was further sub-categorized and examined under the following groups: Steroid Receptor-Mediated, Hedgehog, Glucocorticoid, Classical WNT, PI3K/AKT, NOTCH and MAPK. In our study, no expression changes were observed for glucocorticoid and hedgehog signaling genes. However, three Steroid Receptor-Mediated sub-category genes (*CTNNB1, RB1 and ESR2*) were found to be significantly differentially expressed. *CTNNB1 and RB1 were downregulated while ESR2 was*

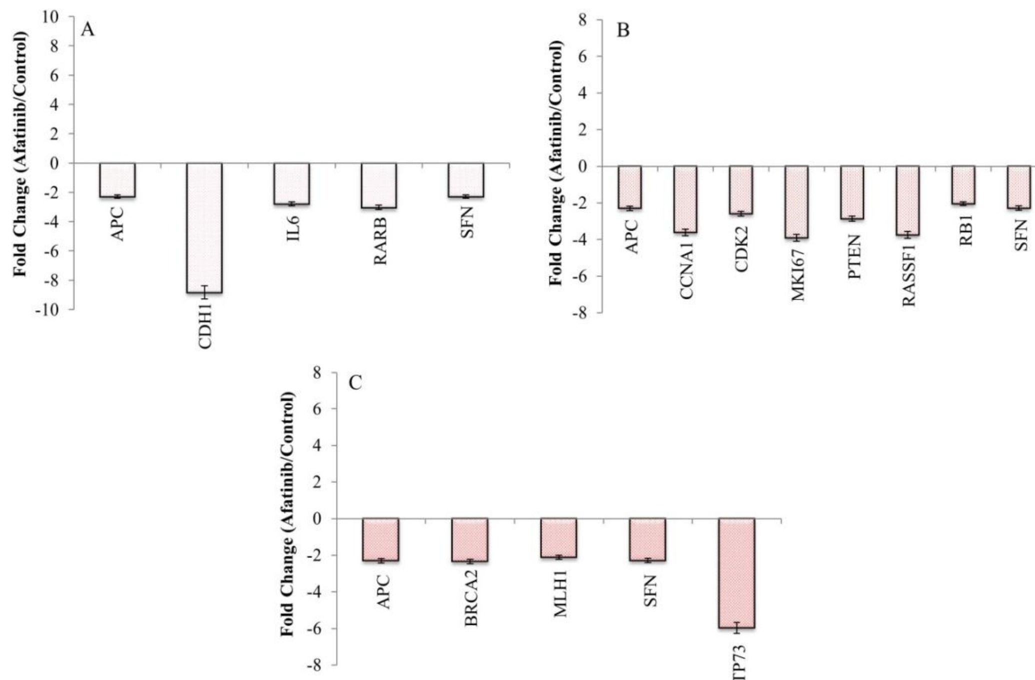


Fig. 2 Gene expression in MDA-MB-231 breast cancer cell line following treatment with afatinib (5 μM) for 24 h using a differential cutoff of two-fold. (A) Apoptosis; (B) Cell Cycle and (C) DNA Damage and Repair.

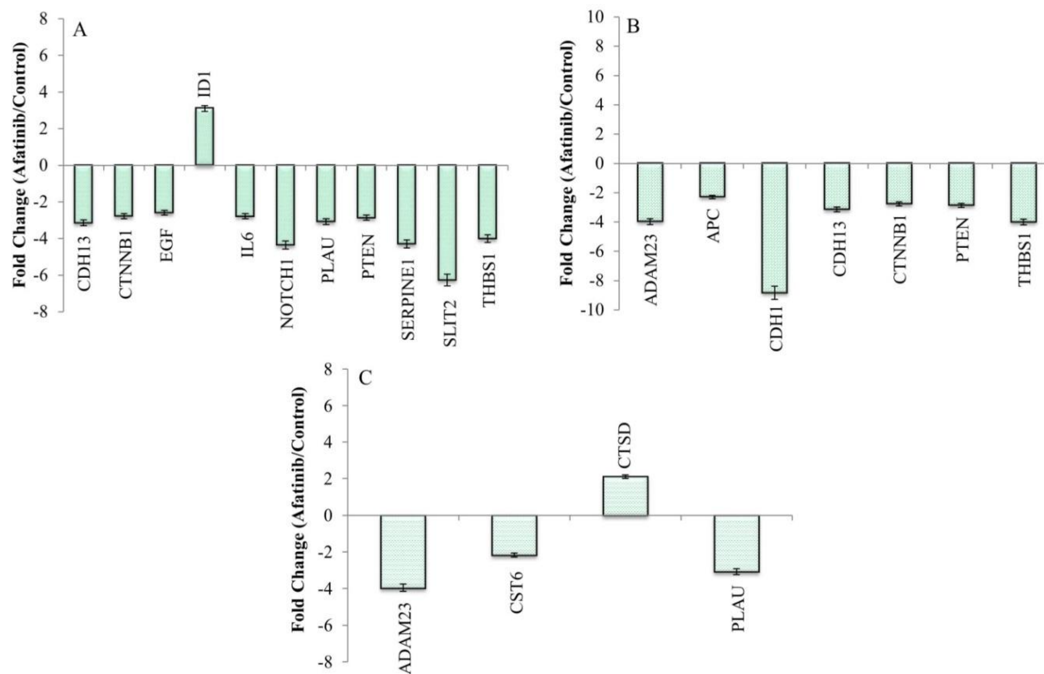


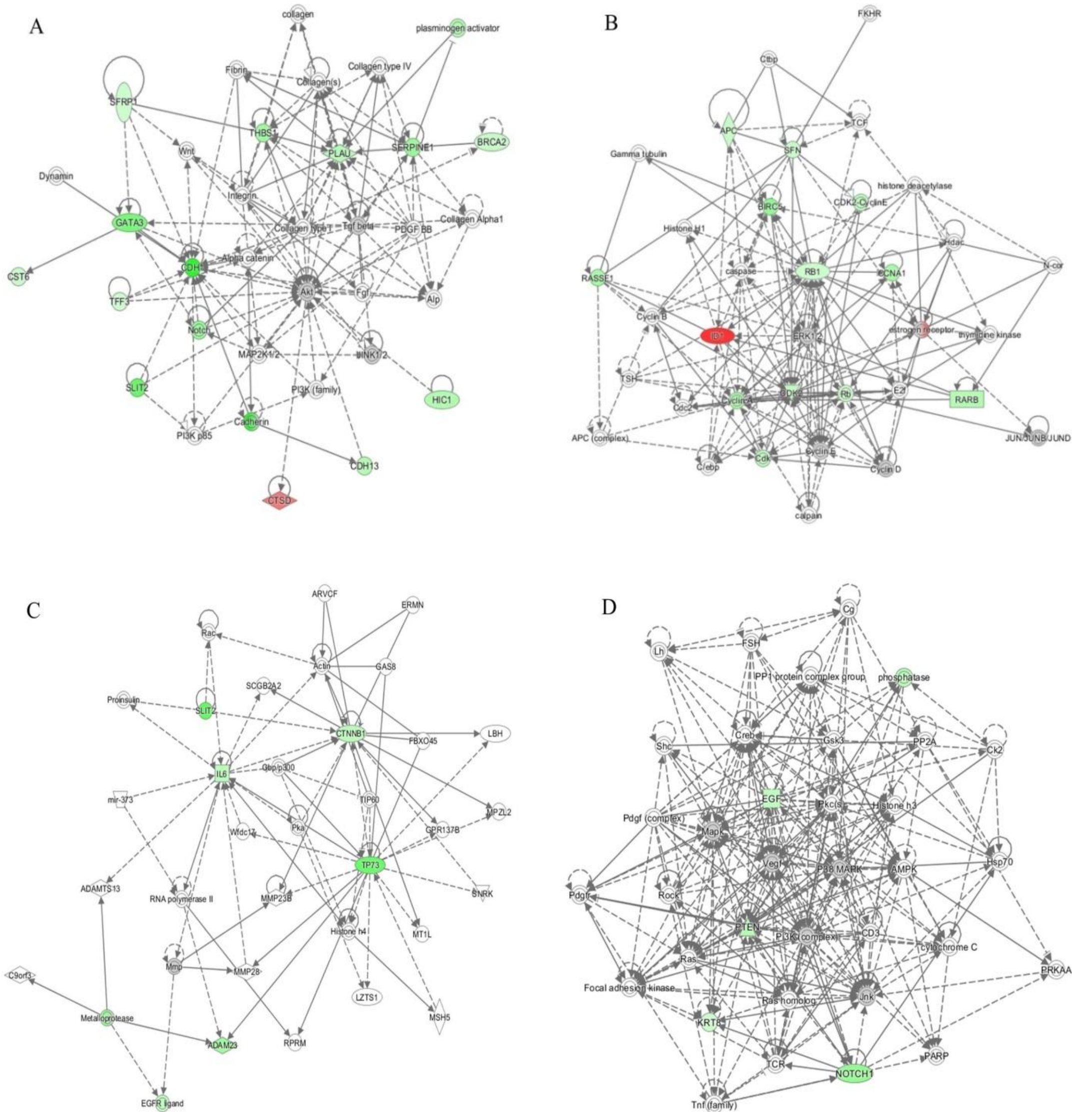
Fig. 3 Gene expression in MDA-MB-231 breast cancer cell line following treatment with afatinib (5 μM) for 24 h using a differential cutoff of two-fold. (A) Angiogenesis; (B) Cell Adhesion Molecules and (C) Proteolysis.

upregulated. *ESR2* (Estrogen receptor 2) is an important transcription factor involved in breast cancer carcinogenesis which plays a pivotal role in signal transduction as a steroid receptor-mediated molecule. It encodes estrogen receptor beta (ER-β). Unlike estrogen receptor alpha (ER-α) associated with tumor formation, ER-β has been shown to be a potent tumor suppressor with anti-proliferative abilities which may oppose ER-α effects in reproductive tissues [34,35].

EMT plays a crucial role in invasion and metastasis of breast cancer and its importance is well documented in the literature [36,37]. In our study, we examined changes in the expression levels of five EMT regulators (*CTNNB1, NOTCH1, SRC, TGFβ1* and *TWIST1*) in MDA-MB-231 cells treated with afatinib. Our findings reveal no changes in the expression of *SRC, TGFβ1* and *TWIST1*. In contrast, expression of *CTNNB1* and *NOTCH1* was downregulated. Notch activity can initiate a series of cascading molecular events resulting in slug-induced EMT and its accompanying coding of proto-oncogene Beta-Catenin by CTNNB1. Hence, it may be expected that both *CTNNB1*and *NOTCH1* are downregulated [38].

Importantly, only two of the differentially expressed genes (*ID1* and *CTSD*) pertaining to apoptosis, cell cycle, DNA damage and repair, angiogenesis, cell adhesion and proteolysis were significantly upregulated in our study. *ID1* (Inhibitor of DNA binding 1) is a part of the ID protein family and its overexpression has been highly correlated with tumor angiogenesis in ER-negative and node-positive subtypes of invasive breast cancer [39,40]. On the other hand, CTSD (Cathepsin D) is a lysosomal protease with high expression levels in several solid tumors including breast cancer [41]. CTSD is implicated in the pathogenesis of breast cancer since it stimulates cell growth, migration and angiogenesis and plays a role in apoptosis [42-44]. In this regard, our results of increased ID1 and CTSD expression in afatinib treated MDA-MB-231 cells are rather unexpected and warrant further investigation. In our study, the majority of downregulated genes in the above-mentioned functional groupings were oncogenes. Interestingly, the most downregulated gene was CDH1 which is a known tumor suppressor gene. It is unclear the molecular mechanism governing this outcome and further investigations are necessary to elucidate this result. Generally, it can be speculated based on the findings of this

study that afatinib increases expression of key tumor suppressor genes and downregulates expression of pivotal oncogenes in the TNBC MDA-MB-231. Nonetheless, there some unexpected results and additional studies are needed to confirm them. The interaction of gene expression data generated with the human breast cancer RT^2 profiler PCR array following treatment of MDA-MB-231 cells with afatinib was examined using IPA analysis. Regulatory networks with the two highest number of focus molecules revealed phosphoinositide-3-kinase (PI3K)/Akt and mitogen-activated protein kinase (MAPK)/ERK to be the pathways most impacted by afatinib. This result is in agreement with the literature [45].



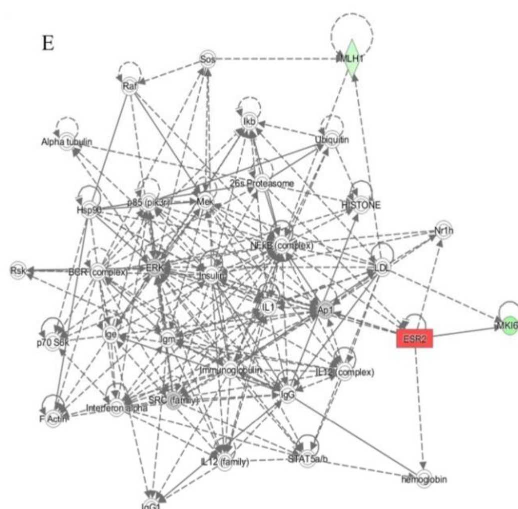


Fig. 4 IPA generated networks related to: (A) Cellular Movement, Cancer, Organismal Injury and Abnormalities; (B) Cell Cycle, Cancer, Cellular Development; (C) Cancer, Gastrointestinal Disease, Hepatic System Disease, (D) Cellular Movement, Cellular Development, Cellular Growth and Proliferation and (E) Organ Morphology, Reproductive System Development and Function, Cell Cycle. Networks comprise nodes and edges. Genes/gene products are represented by nodes while the different shapes capture unique function of the various classes of nodes. Edges reflect relationship between nodes. Upregulated and down-regulated molecules are colored red and green, respectively. Molecules included from the ingenuity pathway knowledge database are colored white while molecules colored gray did not satisfy the specified cutoff of 2.

5. CONCLUSION

In conclusion, the present study showed that thirty-three key breast cancer genes involved in carcinogenesis were differentially expressed in MDA-MB-231 cells treated with afatinib. Five regulatory networks confirming the importance of PI3K/Akt and MAPK/ERK signaling in afatinib therapy were identified using IPA database. Together, our findings provide a preliminary identification of possible molecular targets in MDA-MB-231 cells affected by afatinib. This information can facilitate the rationale design of afatinib-based combination therapy for treating TNBC based on afatinib. However, additional concentrations of afatinib (above the IC50), as well as additional cell lines, would need to be examined to confirm validity of the results.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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