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Down-regulation of miRNA 451a promotes many oncogenic signaling pathways in breast cancer patients

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The present study was designed to investigate the expression status of the micro RNA 451a (miR-451a) in BC patients through multi-layered bioinformatics analysis. The miR-451a was down-regulated in BC patients and through the KEGG pathway analysis we were able to find 8 genes (*CDKN2B, CDKN2D, ATF2, EIF2A3K, RAB5A, CAV1, UCK1,* and *PMM2*) encoded molecules of which can potentially participate in different pathways like cell cycle, FOXO signaling pathway, influenza A, Viral carcinogenesis, Endocytosis, HTLV-I infection, Metabolic pathways, and ubiquitination pathways. While *PSMB8* acts as the major target of miR-451a and is involved in different pathways of cancer proliferation, metastasis, and growth. The *PSMB8* protein is a component of the immuno-proteasome and participates in the ubiquitination-based degradation of the proteins. It's concluded that miR-451a acts as a tumor suppressor molecule and its down-regulation turns on many oncogenic pathways and thus promotes cancer.

Keywords: *CDKN2B*, *CDKN2D*, FOXO signaling, microRNA451a, *PSMB8*, *RAB5A*, *UCK1*

Breast cancer (BC) is the second leading cause of cancer-related mortalities worldwide. It is the most commonly diagnosed cancer among women[1]. Despite improvements in early detection and treatment, BC remains the leading cause of carcinoma-associated mortalities in women, generally due to the development of recurrent, chemoresistance and/or metastasis disease[1-4]. During the first diagnosis, about 5% of patients suffer from distant metastases as well as up to 10–15% of patients develop distant metastases within the first 3 years[5].

The miRNAs (miRs) are short, single-stranded RNAs that serve as key regulators of gene expression. The miRs are small (19–22 bases in length) non-coding RNAs, which negatively regulate the expression of protein-coding genes by either promoting mRNA degradation or inhibiting translation[6]. In BC, various miRs are known to be differentially expressed as compared to normal controls. Several earlier studies have demonstrated that many miRs target specifically various cancer-related genes to induce cancer initiation, progression, metastasis, and drug resistance[7]. In BC, some miRs upregulate the expression of various oncogenes while others stimulate tumor suppressor genes[8-13]. Moreover, it has been noticed that the miRs' expression profiles of each intrinsic subtype of BC differs from other types[14-16]. Several miRs' expression is either upregulated or down-regulated in BC. The miRs also regulate metabolic pathways *e.g*. reduce the uptake of glucose and glutamine and prevent lactate build-up in the imatinib resistant cells[17] and involved in epigenetic[18].

The miR-451a is encoded by the gene present on human chromosomal region 17qll.2. The miR-451a takes part in various physiological and pathological processes, containing hematopoietic system differentiation[19], embryonic development, epithelial cell polarity[20] and nervous system development[21]. It is widely dysregulated in human malignancies, including papillary thyroid carcinoma, pancreatic, and ductal adenocarcinoma, colorectal cancer, gastric cancer, breast cancer, glioma, and cutaneous basal

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Abbreviations: Activating Transcription Factor 2, *ATF2*; Breast cancer, BC; caveolin 1, *CAV1*; Cyclin-dependent kinase 4 inhibitor B, *CDKN2B*; Cyclin-dependent kinase 4 inhibitor D, *CDKN2D*; Cytidine to cytidine monophosphate, CMP; epithelial-mesenchymal transition, EMT; Eukaryotic translation initiation factor 2 alpha kinase 3, *EIF2AK3*; Major histocompatibility complex class I, MHCclass I;Member RAS Oncogene Family, *RAB5A* ; miRs, miRNAs; Non-small cell lung cancer, NSCLC; Phosphomannomutase 2, *PMM2*; Proteasome 20S subunit beta 8, *PSMB8*; The Cancer Genome Atlas Program, (TCGA); TPM, transcripts per million; Uridine monophosphate, UMP; Uridine monophosphate, UMP; Uridine-Cytidine Kinase 1, *UCK1*

cell carcinoma. Serum exosomal miR-451a has diagnostic utility in diffuse large B-cell lymphoma and hepatocellular carcinoma. Furthermore, miR-451a is down-regulated in non-small cell lung cancer (NSCLC) tissues and lung cancer cells. The miR-451a is significantly down-regulated during the treatment of BC. Its higher level has been associated with disease-free survival[22]. It is involved in several cancer-related biological processes, including proliferation, apoptosis, angiogenesis, epithelial-mesenchymal transition (EMT), drug resistance, and metastasis. It sometimes acts as a tumor suppressor gene in cancers and modulates multiple pathways by targeting different downstream mRNAs[23]. The miR-451a has a key role in the RAS-RAF-ERK and associated signaling pathways[24].

However, a careful literature review revealed that no report has been found on clinicopathological-specific features related to the expression of miR-451a. Similarly, identification of the miR-451a targeted genes and their pathway enrichment, expression correlation with miR-451a expression, promoter methylation level, genetic alterations, and copy number variations is yet to be uncovered. Therefore, in the present study, we comprehensively examined the potential oncogenic role/tumor suppressor role of miR-451a in BC through a multi-layered comprehensive bioinformatics approach. Our results suggested that miR-451a is a potential oncogenic biomarker of BC and its dysregulation results in the altered expression of various genes which ultimately results in the activation of key oncogenic pathways.

Material and Methods

The data related to miR-451a and its target genes‘s expression, promoter methylation, and mutational status in BC cell lines and patients’ tissues was taken from the publically available databases including TCGA (available at: www.ualcan.path.uab.edu), miRDB (available at: www.mirdb.org)., and many others discussed in the corresponding sections. These databases contain experimental data of standard molecular biology laboratories worldwide. This manuscript involved data mining and analysis of data using various *in silico* tools to investigate the role of miR-451a in BC progression.The methodology of analysis has been summarized in (Fig. 1).

Exploration of miR-451a differential expression

The miR-451a’s expression analysis in normal and cancerous breast tissues and its association with several other parameters *i.e*. stage, race, gender, was carried out using the information of the publically available online database UALCAN (www.ualcan.path.uab.edu). The UALCAN[25] is a platform containing TCGA microRNA-seq level 3 data of approximately more than 31 malignancies. During our analysis first, we pressed the "Analysis" option that showed the "search-box" for micro RNA querying in the Breast Invasive Carcinoma dataset. The database has an "Explore" option that displayed graphic or whisker box plot representation of miRs differential expression results. The database uses quartile ranges from minimum to maximum values that indicate differential expression *i.e*., ranging from lower to higher, respectively. The expression of transcripts was measured as the number of transcripts per million (TPM).

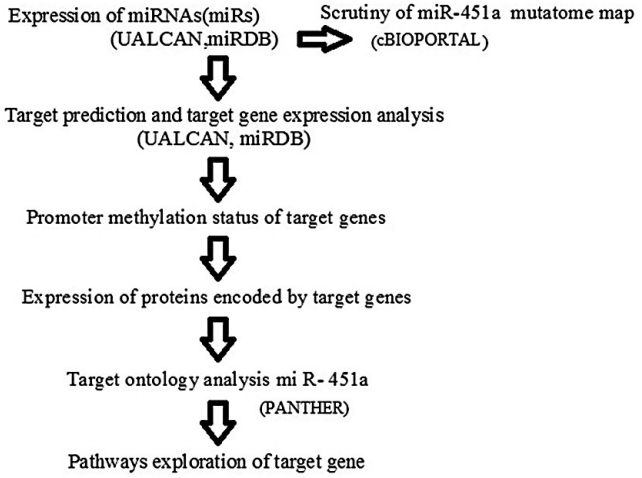

Fig. 1 — Experimental strategies used for analysis miRNA’s target prediction and expressional analysis data was retrieved through UALCAN and miRDB databases 2. Mutation analysis was accomplished through cBIPORTAL, 3. Predicted Target’s gene expression analysis was carried through UALCAN and miRDB, 4.Promoter’s methylation status of target genes was investigated through UALCAN, 5. Target ontology analysis of miRNA was carried out using Panther, 6. All the retrieved information was used to design and explore target genes’ related signalling pathways

Scrutiny of miR-451a mutatome map

The miR-451a mutatome map retrieval was carried out using the cBioPortal cancer genome server (www.cbioportal.org) which is a huge repository of cancer genome data. We selected the "Breast Invasive Carcinoma TCGA, Cell 2015" dataset by querying miRs in the gene "search box" and finally pressed the "submit" option that displayed a map of mutations from the "mutation" option. The map covers mutation types, positions, and frequencies in the corresponding miRs gene reported in the cancer dataset.

Prediction of miR-451a target genes and expression analysis of target genes

miRs target prediction helps in identifying miR-targeted genes. The miRDB (available at: www.mirdb.org) is an online database for miRs target genes prediction and functional annotations of the targeted genes[26,27]. The potential targets are found using the target score value. This data set not only provides information about the target genes of miRs but also about the seed location of miRs in the respective gene (data provided in the Suppl. Table 7).

For expression analysis of miR-451a targeted genes, all target gene expression status was checked through UALCAN (www.ualcan.path.uab.edu) and documented in terms of TPM. The expression information of miR-451a target genes was further searched through miRDB (available at: www.mirdb.org). For this purpose in the query box we wrote miR-451a and clicked go, after that we selected BT-20 invasive ductal carcinoma cell line from the available list of total cell lines[26]. This database revealed the miR-451a' targeted gene expression levels in terms of the RPKM method (Reads per Kilobase of transcript per Million, mapped reads). The RPKM value of 20+ was considered as an up-regulated, RPKM value (5-20) was referred to as a moderate expression while an RPKM value (1-5) was mentioned as a low expression. Targeted genes with high or moderate expression are more likely to be breast cancer-relevant genes in BT-20 cell lines of invasive ductal carcinoma. The expressed genes were further investigated using UALCAN (www.ualcan.path.uab.edu) to document the effect of their differential expression on patients' survival and promoter's methylation and total protein expression status as available in CPTAC dataset.

Ontology analysis of miR- 451a target genes

For target ontology analysis, predicted targets for amiRs are evaluated using Gene Oncology (GO) terms. Further categories like biological process and molecular function are identified based on statistics. During the process, miRDB first retrieves predicted miRs targets and then submits them to the PANTHER server http://pantherdb.org/webservices/go/overrep.jsp for statistical analysis. The final GO enrichment results are presented on the PANTHER website. The cutoff value is determined based on the highest fold enrichment value and p-value < 0.05.

Results

The results validated that the down regulated expression of miR-451a in BC patients turns on expression of many oncogenes and oncogenic signaling pathways. The information can be exploited for designing new drugs, planning appropriate treatment strategy to control BC, and improving patients' survival.

Differential expression of miR-451a

The miR-451a's expression (3236.144 TPM) was found to be decreased as compared to normal in primary tumor breast cancer patients (447.605 TPM). The expression of miR 451a was down-regulated in all cancer stages *i.e*. stage 1 (526.377 TPM), stage 2 (413.899 TPM), stage 3 (443.18 TPM), and stage 4 (260.453 TPM). Patients of various populations exhibited differential expression of miR451a; Caucasian (421.851 TPM), African-American (402.977 TPM), and Asian (485.536 TPM). While higher expression was observed in the Asian population than in the other two populations. The miR451a's expression was greater in females (449.586 TPM) than in males (260.453 TPM) indicating a higher impact on the endocrine system in females than in both normal and male patients (Fig. 2).

Mutatome map of miR-451a

Breast Invasive Carcinoma TCGA Cell 2015 dataset results revealed no genetic alteration in miR-541a genes of breast cancer samples (Fig. 3).

Target genes prediction and expression analysis of target genes of miR-451a

There were 40 predicted targets for miR-451a in miRDB with no gene exhibiting a 100 target score (Suppl. Table 1). However, There were 27 predicted targets for miR-451a within expression levels >=1 and 2 in cell line BT-20 which further decreased in number as expression level increased (Suppl. Table 2). The scored values of all predicted target genes are provided in (Suppl. Table 3).

Among the target genes *CDKN2D, ATF2, EIF2AK3, UCK1, RAB5A, PSMB8, PMM2* showed up regulation in BC patients. While *CDKN2B* and *CAV1* were down-regulated BC patients (Suppl. Table 4).

The *CDKN2D, ATF2, EIF2AK3, UCK1, RAB5A, PSMB8*, and *PMM2* mRNA expression was increased as compared to normal (15.477, 45.107, 28.523, 37.906, 76.563, 155.609, 37.212 TPM, respectively), in primary tumor breast cancer patient (43.058, 53.048, 43.492, 46.416, 93.283, 347.888, 83.926 TPM, respectively). But the expression of *CDKN2B* was decreased as compared to normal (59.704 TPM) in primary tumor breast cancer patients (28.041 TPM).

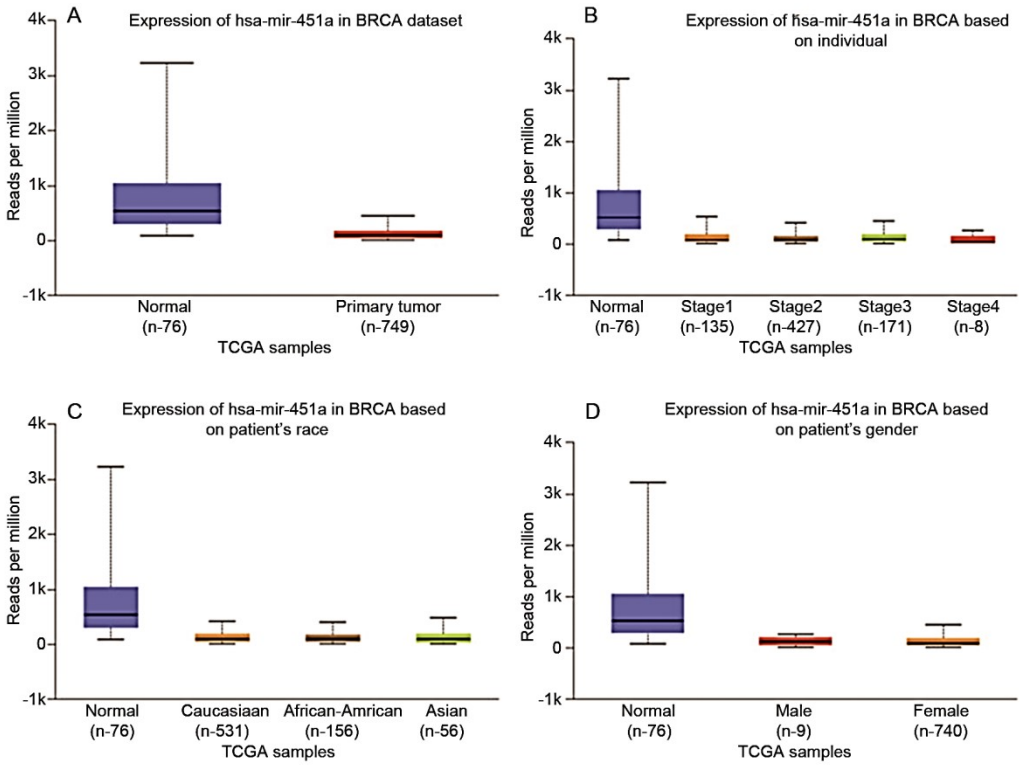

Fig. 2 — miRNA 451a expression in (A) breast invasive carcinoma data sets; (B) individual cancer stage; (C) patient's Race; and (D) patient's gender. The p-value of 0.05 was used as a significant cut off criterion

CAV1 expression decreased as compared to normal (1457.13 TPM) in primary tumor breast cancer patients (137.637 TPM). Data are summarized in (Suppl. Table 4).

Promoter methylation status of target genes

The promoter regions of all target genes *i.e.*, *CDKN2D*, *ATF2, EIF2AK3, UCK1, RAB5A*, *PSMB8, PMM2, CDKN2B,* and *CAV1* were found to be hyper-methylated in primary tumors (0.181,0.036, 0.08, 0.197, 0.351, 0.311, 0.26, 0.08 and 0.231 β- value, respectively), when compared with normal methylation status (0.207,0.039, 0.087, 0.41, 0.465, 0.342, 0.288, 0.097 and 0.288 β-value, respectively). Data of all other minor genes methylation status data is provided in (Suppl. Table 5).

Expression of proteins encoded by target genes

The upregulated expression of *CDKN2D, ATF2, EIF2AK3, RAB5A, PSMB8, PMM2*, and *CAV1* proteins was observed as compared to normal (0.0, 0.313, 0.0,1.134, 0.471, –0.204 and 2.31 Z- value, respectively), in primary tumor breast cancer patient (1.783, 1.875, 1.936, 2.489, 1.859,0.43, and 2.616 Z-value, respectively). Detailed information has been summarized in (Suppl. Table 6).

Target ontology analysis miR- 451a

During the target ontology analysis of miR-451a, no information regarding the biological and molecular function of miR-451a was found in panther-related datasets.

Pathways exploration of target gene

The *PSMB8* is the component of the immunoproteasome. It takes part in the ubiquitin (Ub)-proteasome pathway (UPP) of protein degradation. Ubiquitin is conjugated to the target proteins through ATP dependent process that involves three enzymes E1, E2, and E3, and forms a ubiquitin chain. A chain of five Ub molecules attached to the protein substrate is sufficient for the complex to be recognized by the proteasome (immune-proteasome). In addition to ATP-dependent reactions, Ub is removed and the protein is linearized and injected into the central core of the immunoproteasome, where it is digested into peptides. The peptides are degraded to amino acids by peptidases present in the cytoplasm or used in antigen presentation. The amino acids released after protein degradation are used for cell reprogramming, DNA repair, and synthesis. While for antigen presentation MHC I detect antigen and elicits a response[28,29]. *PSMB8* plays a role in the detection of intercellular

proteins (Fig. 11) which are destined to be degraded through the ubiquitin pathway[30]. We speculate that in cancer defective *PSMB8* protein will lead to defective antigen presentation due to a decrease in the size of MHC1 after successive rounds of antigen presentation[31].

UCK1 gene encodes a uridine-cytidine kinase which phosphorylates uridine to uridine monophosphate (UMP), cytidine to cytidine monophosphate(CMP), and also their analogs using ATP and GTP as phosphate donors (Fig. 9). It is unable to phosphorylate deoxyribonucleosides and purine nucleosides[32]. The increased expression of *UCK1* observed in breast cancer patients as compared to normal persons in our study is comparable with previous findings where no significant association of *UCK1* methylation status has been reported in HCC[33,34]. While the role of its analog UCK2 has been known in breast cancer[35,36]. Similarly, the promoter hypermethylation status of *UCK1* in breast cancer patients has also been previously reported[33]. The reduced expression of *CAV1* observed in breast cancer patients of our study is to the findings of various previous studies[37,38].

CDKN2B is either deleted or mutated in various tumors and hence considered a tumor suppressor gene. It is a cyclin-dependent kinase inhibitor. It forms a complex with *CDK4/CDK6* (Fig. 4). This complex inhibits the activation of CDK kinases which play a role in the control of cell cycle G1 progression. Its expression is induced by TGFβ which is involved in growth inhibition[32].

CDKN2D is a cyclin-dependent kinase inhibitor that encodes a protein belonging to the family of *INK4*. The encoded protein forms a stable complex with *CDK4/CDK6* (Fig. 4). Its highest expression is observed in the S phase while the low is detected in the G1 phase[32].

The transcription factor *ATF2* belongs to the leucine zipper family of DNA-binding proteins and is encoded by the *ATF2* gene (Fig. 5). The encoded protein plays a role in various functions *e.g.*, stimulation of CRE-dependent transcription, histone acetyltransferase, and DDR (cell DNA damage response) complex[32].

Various extracellular factors (such as growth factors, cytokines, drugs, and stress) activate upstream kinases like *ATM*, *ERK*, *p38*, *VRK1*, and *PKC* which phosphorylate *ATF2* at its corresponding phosphorylation sites; ZF domain(amino acids, position 25-49), HAT(amino acids, position 289-314) and bZIP (amino acids, position352-415). Followed by the phosphorylation activated *ATF2* is translocated

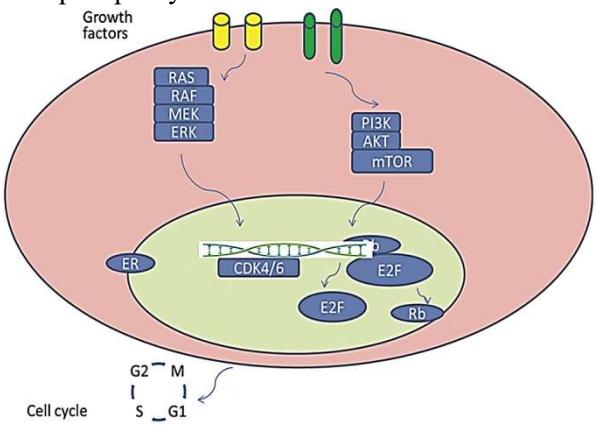

Fig. 4 — Cross talk of CDK family kinases (especially CDK4/CD6)and various oncogenic pathways. During the cell cycle DNA synthesis is initiated due to the elevated level of cyclin D1 which in turn increases the CDK4/6 levels through upstream signaling of mitogens. The CDK4/6 complex further phosphorylates Rb present in the form of the Rb-E2F complex. The dephosphorylated Rb dissociates from the complex and is thus activated. The Rb protein limits the expression of E2F target genes which are involved in cell progression, DNA replication, and mitotic expression. CDK4/6 inhibitor inhibits CDK4/6 mediated phosphorylation of Rb and thus causes cell cycle arrest in Go/G1 phase[39,40]

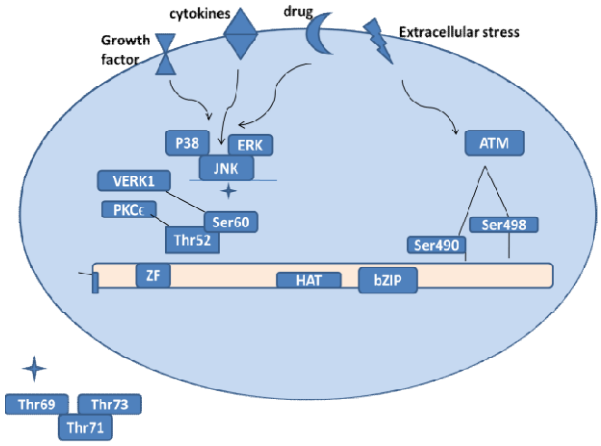

Fig. 5 — ATF2-related signaling pathways

Fig. 3 — An overview of genetic alterations contributing towards microRNA 451a copy number regulation (reported in TCGA Cell 2015 BRIC dataset)

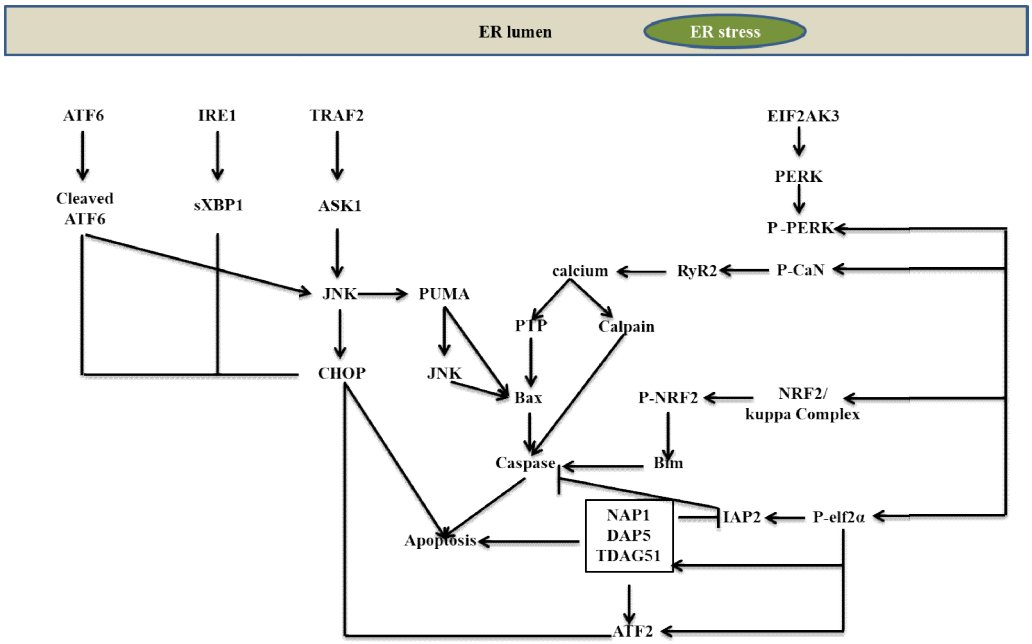

Fig. 6 — The EIF2AK3 signaling pathway. It works by initiating the PERK signaling pathway which activates many downstream effectors. The components of the PERK signaling pathway include PERK/eIf2α/ATF4, PERK/CAN, PERK/eIf2α/TDAG51, PERK/eIf2α/IAP2 and PERK/NRF2. All pathways associated with the PERK pathway induce apoptosis due to ER stress. EIF2AK3 is also known as PERK, PEK, and WRS[42]

to the nucleus where it binds c-JUN and mediates gene expression of genes harboring AP-1 or CRE-like binding sequence[41].

Sometimes due to tumor formation rate of protein synthesis enhances and as a result the folding capacity of the endoplasmic reticulum (ER) increases which subsequently elevates nascent protein levels within ER lumen leading to ER stress. It initiates PERK (protein kinase RNA-like endoplasmic reticulum kinase) signaling pathway thus phosphorylating eIF2α at position Ser51 (Fig. 6). It promotes selective protein translation which is directly or indirectly linked to the expression of proteins such as *ATF4* involved in pro-survival and pro-death[32] .

RAB5A is a protein-coding gene belonging to the RAS family. It encodes a small GTPase which becomes active in GTP- bound form while inactive in GDP-bound form (Fig. 6). It plays a role as an effector protein that mediates various cellular responses like intracellular membrane trafficking and formation of transport vesicles *etc*.[32].

RAB5A exhibits various biological functions and is regulated by GDP/GTP exchange. In *RAB5A-GTP* bound form activated promotes tumorigenesis[44]. RabGTPases can transform between the GTP-bound activated form and GDP-bound inactivated form[45]. The GTP-Rab is located on the plasma membrane,

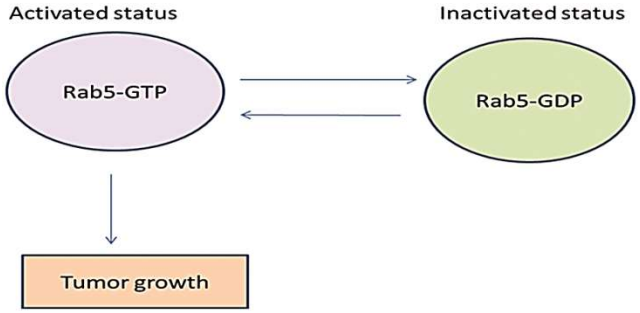

Fig. 7 — RAB5A-associated cellular signaling pathway

and GDP-Rab is located in the cytoplasm. The transformation between the activated and inactivated forms requires three crucial regulators: GDP dissociation inhibitor (GDI), guanine nucleotide exchange factor (GEF), and GTPase activating protein (GAP). As shown in (Fig. 7), GDI is a circulating factor that regulates the binding and unloading of RabGTPases on the plasma membrane. After being released by GDI, Rab is activated by GEF, which catalyzes the conversion of GDP to GTP. Then, Rab-GTP may perform its roles by recruiting the downstream effectors. The inactivation of Rab-GTPases involves the following steps: GAP inactivates RabGTPases by catalyzing the hydrolysis of GTP. GDI binds with inactivated Rab-GDP to form a complex, impeding the interaction between Rab

proteins and their effectors. Then, inactivated Rab proteins are transferred from the plasma membrane into the cytoplasm to start a new cycle[44].

The *CAV1* is a tumor suppressor gene that encodes protein present in the plasma membrane of caveolae. Th *CAV1* protein takes part in the Ras-ERK pathway by activating cytosolic tyrosine kinase (Fig. 8). It promotes cell cycle progression and negatively regulates Ras-p42/44 mitogen-activated pathway[32].

The pyrimidine synthesis occurs through two processes one is the de novo synthesis while the other is the salvage process. It is initiated by the formation of carbamyl from glutamine. It further reacts with N-carbamyl aspartate to produce dihydroorotate through enzyme activity of trifunctional enzyme CAD including carbamyl phosphate synthetase, aspartate transcarbamylase, and dihydroortatase. Dihydroortatase converts dihydroorotate into orotate accompanied by chain transmission. The phosphorylated orotate produces UMP through the reaction catalyzed by the bifunctional enzyme UMPS (uridine monophosphate synthase). Similarly, for the salvage process, the cell obtains uridine and cytidine from the extracellular environment mainly from two nucleoside transport family genes SLC28A along with SLC29A subsequently uridine and cytidine convert to UMP and CTP, respectively[46]. This pathway generates pyrimidine molecules for DNA replication,

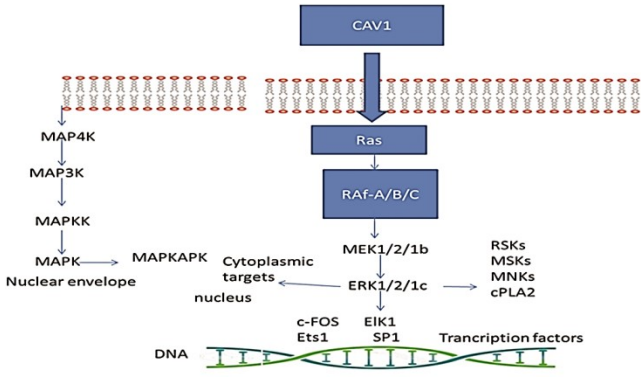

Fig. 8 — MAPKs (Mitogen-activated protein kinases) pathway activated by mitogen and growth factor. CAV1 acts as a mitogen and activates various kinases of the MAPKssignaling pathway which are present in the cytoplasm and followed by phosphorylation translocated to the nucleus. MAPKsfurther activates downstream effectors such as MAP4K, and MAP3K and mitogen-activated protein kinase activated protein kinases such as Ras are involved in cell cycle progression ERK expression is important for development. Its hyperactivation leads to cancer development and progression. The Ras/Raf/MAPK (MEK)/ERK pathway is the most important signaling cascade among all MAPK signal transduction pathways. It plays a key role in the survival as well as the development of tumor cells[43]

and RNA synthesis, as well as for cellular bioenergetics. Increased pyrimidine synthesis supports the uncontrolled growth of tumors and is a characteristic of cancer[47] (Fig. 9).

The protein encoded by the *PMM2* gene catalyzes the isomerization of mannose 6-phosphate to mannose 1-phosphate, which is a precursor to GDP-mannose and is necessary for the synthesis of dolichol-P-oligosaccharides (Fig. 10). Mutations in this gene have been shown to cause defects in glycoprotein biosynthesis, which is manifested as carbohydrate-deficient glycoprotein syndrome type I[32].

The process of glycan synthesis involves mannose related pathway. The deficiency in *PMM2* leads to decreased levels of Man-1-P which reduce their flux through the pathway and reduced N-glycosylation. This deficiency can be treated by direct provision of Man-1-P[48,49]. Interestingly, a previous report showed that miR-451a expression is controlled by glucose levels and thus regulates cancer aggressiveness through the AMP-activated protein kinase pathway and mTOR activation in glioblastoma[50]. Thus, miR-451a-regulated pathways may be involved in glucose-related metabolic pathways and thus promote cancer aggressiveness[51].

The ubiquitin in the presence of ATP and three enzymes E1, E2, and E3 is added to the target proteins mainly on their lysine amino acid. The polyubiquitinated protein is recognized by 26S proteosome mainly by their important component immunoproteasome where it deubiquitinates by deubiquitinases, unfolded and its proteolysis occurs. The peptides produced are further broken down to amino acids or detected by MHCI through the process of antigen presentation[52].

Regulation of immunoproteasome gene *PSMB8* occurs by both cell-intrinsic and –extrinsic factors

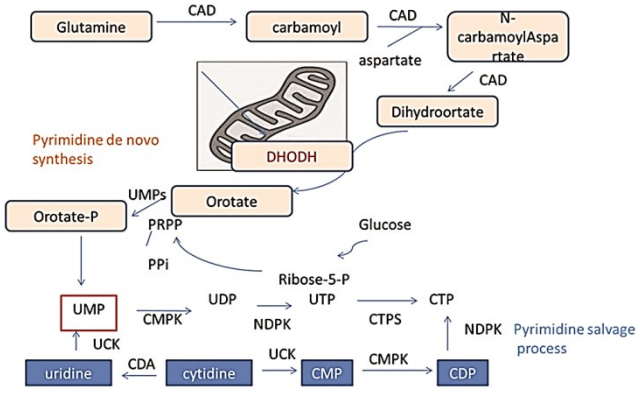

Fig. 9 — UCK1-related cellular signaling pathway of pyrimidine metabolism

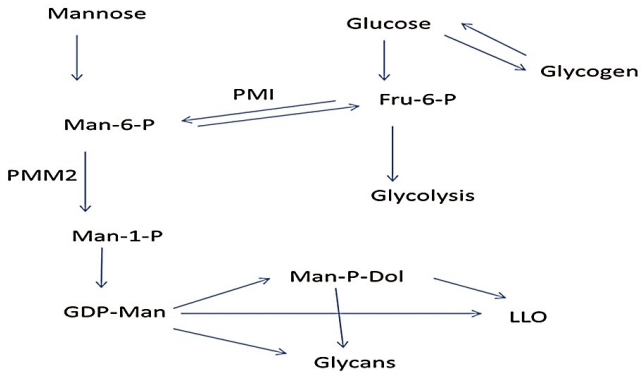

Fig. 10 — The PMM2-related cellular signaling pathway

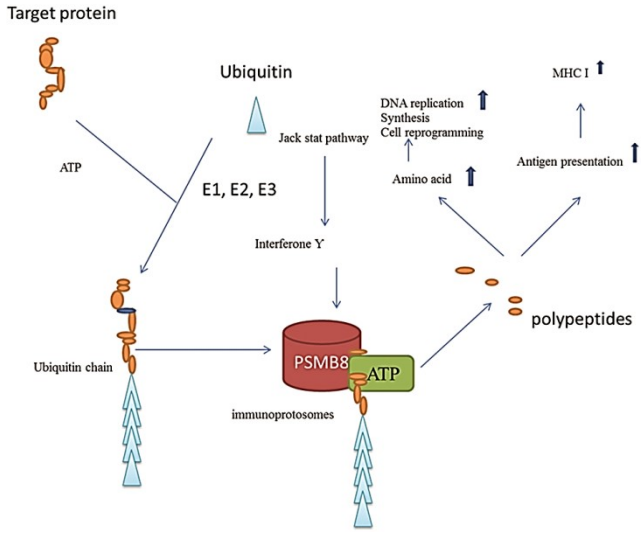

Fig. 11 — Role of PSMB8 (immunoproteasome) in ubiquitination-mediated decay of proteins

in various carcinomas (Fig. 11). In breast cancer, it is a cancer cell-extrinsic process correlating with the presence of IFN-γ-secreting tumor-infiltrating lymphocytes. Previous studies have reported that lymphocyte infiltrates and IFN-γ secretion in solid tumors are favorable prognostic markers. It is further known that high expression of *PSMB8* is correlated with improved survival in patients with breast cancer[53].

Discussion

The miR-451a is dysregulated in many human cancers and thus promotes carcinogenesis[54]. Various studies have reported that miR-451a is necessary for the development and maintenance of normal tissues and it may be down-regulated during the transition to cancer[55,56]. Our Results are comparable with previous findings where down-regulation and tumor suppressor role of miR-451a have been reported[57,58].

The miR-451a regulates BC cell growth, invasion, and apoptosis *via* targeting different genes such as *CDKN2D, CDKN2B, ATF2, EIF2AK3, RAB5A, CAV1, UCK1, PMM2*, and *PSMB8* which play role in different biological pathways. Our findings of *CDKN2D*, *ATF2*[41,59], *EIF2A3K*[60], *RAB5A*[61,62], and *PSMB8's*[53] up regulated expression and their promoter's hyper methylation status in cancer patients have been confirmed by previous studies.

The up regulation of *CDKN2D* was observed which was comparable with previous finding where up regulation of *CDK* inhibitors acts as cell growth regulator and halts cell cycle's G1 progression[63]. Therefore *CDKN2D* acts as an oncogene while contrasting results are also available where down regulation of *CDKN2D* is linked with proliferation of cervical cancer[64]. The down regulation of *CDKN2B* confirmed its role as tumor suppressor which was in contrast with previous finding where higher expression has been found to promote tumor growthvia activation of TGF-β1/Smad2/3 signalling and thus acts as an oncogene gene[65].

The up regulation of *ATF2* observed in our study contradicts the findings of a previous study which reported that *ATF2* acts as a tumor suppressor by inhibiting the cancer promoting protein TROP2. Therapeutic *TROP2* targeting might prevent particularly the first steps in metastasis, *i.e*., the de-adhesion and invasion of colon cancer cells[66]. Therefore decrease in ATF2 expression is associated with cancer invasiveness.

EIF2A3K's up regulation was observed in present study. This protein is involved in the phosphorylation of eukaryotic translation-initiation factor 2 (EIF2), leading to its inactivation, and thus decreases the global protein synthesis and enhances oxidative stress in cell[67].The up regulation of *EIF2A3K* detected in the present study may thus globally reduce the cellular proteins' expression including many oncogenic proteins and tumor suppressor proteins.

The *RAB5A*'sup regulation examined in the current study is comparable with previous finding. The *RAB5A's* regulates vesicular membranes transport and control the delivery many proteins to different membrane bound organelles which ultimately results in altered signaling pathway. The role of *RAB5A* is known in theinvasion, migration, metabolism, autophagy, exosomes' secretion and drug resistance in cancer[68].

Similarly *PSMB8* up regulation has been with the relapse free survival in Triple negative BC patients[69]. Proteosomal degradation is involved in the normal

turn-over of the proteins. However, the pro-inflammatory cytokines and oxidative stress activate immune-proteosome and regulate ubiquitin mediated proteins degradation. The oxidative stress and pro-inflammatory cytokines are known to promote carcinogenesis[70].

The miR-451a acts as tumor suppressor molecule which negatively regulates the expression of its target genes[24]. Therefore it seems to be a potential suitable candidate which can be targeted during cancer therapies. The results of present study have shown that the miR-451a suppresses tumour development and therefore carcinogenesis is promoted by its down-regulation. We hypothesize that cancer cells which have lost expression of miR-451a, if regain the lost expression, the oncogenes targets of miR-451a can be down-regulated. The down-regulation of oncogenes will help to control cancer initiation, progression and metastasis. The proposed hypothesis is based upon a previously published article reporting that the induction of two miRs (miR-145-5p and miR-203a-5p) in imatinib resistant chronic myeloid leukemia (CML) cells helps to overcome the drug resistance [17].

The role of miR-451a in carcinogenesis is further strengthened by an earlier study which reported that the intracellular Notch1 (ICN1) promotes degradation of transcriptional activator of miR-451a (*i.e.*, E2a tumor suppressor) and as a result the expression of miR-451a is repressed[71]. In the absence of miR-451a expression of MYC an oncogene that is known to act as regulator of apoptosis and cell cycle[72]. The major target of miR-451a identified during present study, PSMB8, is known to enhance the rate of cytokine-induced apoptosis in normal human beta-cells[70]. However, less is known regarding role of PSMB8 and other components of immune-proteosome in BC[69]. It is therefore valuable to investigate this aspect of carcinogenesis.

Conclusion

The miR-451a acts as a tumor suppressor molecule which plays a vital role in normal cell growth and differentiation[23]. The down-regulation of miR-451a leads to BC progression by facilitating the up-regulation of various oncogenes (*i.e.*, *CDKN2D, ATF2, EIF2AK3, RAB5A,UCKI, PMM2,* and *PSMB8*). The up-regulated oncogenic proteins play oncogenic role by turning on many oncogenic pathways like activation of cyclin-dependent kinases, activating transcription factor 2, PERKs signaling pathway, MAPKs pathway, up-regulated degradation of proteins by ubiquitination pathway, glycan synthesis pathway, pyrimidines synthesis pathways and is thus involved in cell reprogramming and dysregulated process of DNA replication, and the antigen presentation. Therefore, the present study concluded that miR-451a is a potential biomarker of BC which may serve as a new drug target during treatment and thus can facilitate designing an effective anticancer treatment strategy.

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Conflict of interest

Both the authors declare no conflict of interest.

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