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========== **BIOINFORMATICS** ==========

In-silico Elucidation of the Role of ABC-Transporter Genes Expression Regulation by OncomiRs (miR-21, miR-15, and miR-let-7) in Drug Efflux and Chemoresistance in Breast Cancer

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Abstract. Breast cancer is the most common and aggressive malignancy in females with a high prevalence rate of 77.9 million worldwide. Chemotherapy and tyrosine kinase inhibitors have been used to treat invasive and malignant tumors; however, invasive tumors have showed resistance to conventional therapies. ABC transporters play a crucial role in breast cancer due to their chemo-resistance and drug efflux abilities. Additionally, chemo-resistant roles of ABC transporters have been reported in several cancers such as cervical cancer, colon cancer, esophageal squamous cell carcinoma, glioma and HCC. The goal of this study was to identify the tumor suppressor role of miR-21, miR-15 and miR-let-7 to chemo-resistant genes majorly ABCA1, ABCB1 and ABCC1 in breast cancer. TargetScan, miRWalk, and miRDB were employed to predict microRNA-mRNA interactions. MC-Sym and RNAComposer were utilized for the tertiary structure prediction of shortlisted miRNAs and mRNAs. For molecular docking and visualization, HDOCK and PyMOL were employed. The present study identified 10, 7 and 13 interactions between microRNAs (miR-21, miR-15, and miR-let-7) and oncogenes (ABCA1, ABCB1, ABCC1) through miRWalk, miRDB and TargetScan respectively. RNA22 predicted the binding sites of microRNAs such as 22 miR-21, 11 miR-15 and 58 miR-let-7 on ABCA1, ABCB1 and ABCC1, respectively. Out of multiple docked complexes, the top three were shortlisted for visualization based on maximum confidence score and least binding affinity. The present study identifies the interactions of two novel (miR-15a-5p and let-7c-5p) microRNAs with ABCA1, ABCB1 and ABCC1 regions due to their maximum interactions. The findings of this research may help in developing miRNA drugs that could target ABC transporters specifically ABCA1, ABCB1 and ABCC1 to inhibit increased drug efflux and chemoresistance in breast cancer.

Key words: *ABC-transporters, miRNA, oncomiR, miR-21, miR-15, miR-let-7, breast cancer.*

INTRODUCTION

Cellular changes lead to uncontrolled growth and division of abnormal cells in breast tissue producing a mass of tumor called breast cancer (BC) [1]. It is marked as the major cause of cancer- related deaths and the most prevalent malignancy worldwide. According to GLOBOCAN, the estimated prevalence (5-year) rate of BC is 77.9 million globally with the highest prevalence rate of 32.1 million cases in Asia and 21.3 million cases in Europe [2].

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The use of hormones for targeted therapy has been reported to be unhelpful in treating BC that lack hormone receptors [3]. Therefore, chemotherapy is the most common adopted treatment option. Therefore, drugs such as taxane and anthracycline are used as the chemotherapeutics for BC treatment that interfere with cell division, motility and damage DNA in cancer cells [4]. Individuals that lack pathological complete response (pCR) i.e. disappearance of all invasive tumor cells in breast after the chemotherapy, are more likely to have a metastatic disease and an early recurrence [5]. Resistance to chemotherapy is a significant hindrance in the treatment of breast cancer as alterations in multiple signaling pathways govern changes such as drug efflux, bulk tumor cells, cancer stem cells (CSC) and tumor microenvironment (TME) [6]. Transporter mediated drug efflux is one of the most validated chemoresistance. Numerous ABC transporters are actively involved in chemoresistance of numerous solid tumors, including BC [7].

ATP-binding cassette (ABC) transporter gene family consists of 48 genes that are involved in transporting molecules such as peptides, cholesterol, sterol, bile acid and iron transport via ATP hydrolysis across cellular lipid membranes [8]. These transporters are divided into 7 sub-families based on their gene structure, amino acid sequence, domain organization, and phylogenetic analysis [9]. ABCA1, a member of the ABC1 sub-family, consists of 2261 amino acids (aa) and is involved in the transportation of cholesterol and phospholipids in the cellular lipid removal pathway [10]. It has been reported that increased ABCA1 is a specific biomarker of TNBC and its overexpression is related to lymph node metastasis [11]. ABCB1 comprises 1280 aa and is a member of MDR/TAP sub-family. It is an ATP-dependent drug efflux pump, which is responsible for decreased drug accumulation and resistance to anticancer drugs [12]. ABCC1 is a member of the MRP sub-family and consists of 1531 aa. It is involved in multidrug resistance and functions as an organic anion transporter [13]. Furthermore, upregulated ABC transporters such as ABCC1, confer resistance to drugs (anthracyclines, taxanes, vinca alkaloids, tyrosine kinase inhibitors and methotrexate) due to activation of the hedgehog pathway, which is involved in carcinogenesis, invasiveness, recurrence and CSC maintenance [14]. It has been reported that multiple tyrosine kinase inhibitors (TKI) such as lapatinib, sorafenib, erlotinib, apatinib and nilotinib, have been shown to inhibit ABC transporters (ABCG2, ABCB1, ABCC1, ABCC10, ABCC11). Moreover, TKIs are also transported out of the cells by ABC transporters, thus conferring an altered pharmacokinetics of TKIs in cancer individuals [15]. A recent research reported the increased expression of certain ABC transporters in BC e.g., ABCA1, ABCG2 and ABCB1 were responsible for primary chemoresistance in BC while ABCB1 and ABCC1 were identified to be the key players in the prognosis of BC [7]. Moreover, ABC transporters (ABCA1, ABCB1 and ABCC1) showed resistance against chemotherapy and TKIs [16]. Therefore, there is an ultimate need to develop miRNA-based anticancer therapy as tumor suppressor miRNAs would directly target the oncogenes and suppress the tumor development.

MicroRNA (miRNA) regulates gene expression by binding to specific targets and suppressing their function. They are small (22 nucleotides), endogenous and non-coding RNAs that regulate gene expression at posttranscriptional level [17]. MiRNAs that regulate oncogenes by suppressing their expression, which leads to prevention of cancer progression, are termed as anti-oncogenes. Recent studies have reported the upregulation of a tumor suppressor (TS) miRNAs, namely let-7 family miRNAs, which suppress oncogenic mRNAs. [18]. Upregulation of these TS miRNAs leads to decreased proliferation of cancer [19]. Transfecting of cancer cell lines with certain TS miRNAs targeting key oncogenes that can lead to tumor repression. It has been reported that introduction of let-7 into lung adenocarcinoma cell lines could reduce the colony formation by suppressing the cell proliferation [20]. A similar research reported the suppression of an oncogene, ERBB2/3, that is responsible for breast tumor cell proliferation. Similarly, introduction of miR-125a/b into breast tumor cells, promote inhibition of ERBB2/3 transcription and translation, which leads to tumor growth suppression [21]. It has been reported that miR-15a acts as TS miRNA in chronic lymphocytic lymphoma (CLL) by downregulating Bcl2 and Mcl1, which in turn regulate the intrinsic apoptotic pathway [22]. Only a single

published research reported the TS role of miR-21-5p in colorectal cancer in which pyroptosis is induced via TGFBI regulation [23].

Consequently, the analysis of miRNA-mRNA interaction should be performed in order to have a detailed understanding of the mechanism of action of TS miRNAs [24]. Thus, the study of siRNA-mRNA interaction will lead to the development of miRNA preparations that can be used as therapeutic drugs against breast cancer caused by dysregulation of ABC transporters, especially ABCA1, ABCB1 and ABCC1.

The interaction study provides insight into gene binding sites (3'-UTR, 5'-UTR, and CDS) that TS miRNAs interact with, as well as the binding conformations and free energy values that assure the miRNA's regulatory role in gene degradation and silencing.

This study focuses to identify the interaction regions, preferred binding orientation, strength and affinity of miR-21, miR-15 and miR-let-7 with drug resistant ABC family members namely ABCA1, ABCB1 and ABCC1. Identification of binding interactions between TS miRNAs and ATP-binding cassette transporters will help develop the foundations of targeted therapy based on the use of new and effective antitumor oncomiRs against drug-resistant oncogenes in breast cancer.

METHODOLOGY

Overview

The interactions of TS miRNAs (miR-21, miR-15, and miR-let-7) with drug resistant oncogenes (ABCA1, ABCB1, ABCC1) were analyzed in order to identify the binding potential of miRNA-mRNA complexes. The overall workflow is illustrated in Figure 1.

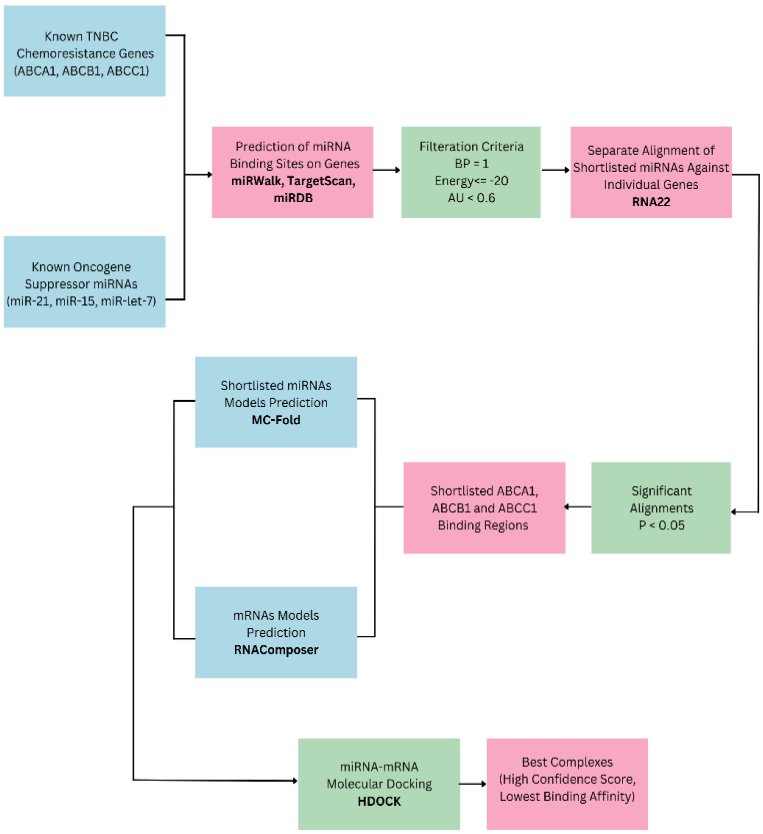

Fig. 1. General strategy followed for the prediction of miRNA-mRNA interactions.

Prediction of miRNA interactions with ABC family

To predict the interaction of the miRNAs (miR-21, miR-15 and miR-let-7) with target genes (ABCA1, ABCB1 and ABCC1), miRWalk (v3) (http://mirwalk.umm.uni-heidelberg.de/),

miRDB (v6.0) (https://mirdb.org/) and TargetScan (v8.0) (https://www.targetscan.org/vert_80/) were used. MiRWalk contains predicted data obtained through a machine-learning algorithm and includes miRNA-target interactions that are experimentally verified [25]. MiRDB employs a high throughput sequencing approach to identify miRNA-target interactions [26]. Similarly, TargetScan uses the complementarity approach to predict the target genes of miRNAs by searching the conserved sites that match the seed region (6 nucleotides (nt) long) of miRNAs [27]. In order to find common miRNAs in three databases that form strong and stable interactions with ABCA1, ABCB1, and ABCC1, the filter function of the dplyr (v1.0.10) package in R (v4.2.2) was used. Binding probability 1, AU-content < 0.6 (refers to the section of transcript that is rich with AU and is laying 30 nucleotides up- and downstream of predicted site) with binding energy $E \leq -20$ (denotes increased binding strength) was applied as filtration criteria [28]. The resulting miRNA-mRNA interactions (for each miRNA and each gene) that were common among all databases were selected for alignment analysis.

Alignment between shortlisted miRNAs and ABC genes

RNA22 is an alignment tool that aligns miRNAs with target genes through the combination of G:U wobbles and bulges in the seed region of a heteroduplex. It predicts non-canonical targets as well as the ones that are beyond 3'-UTR [28]. The alignment of the shortlisted miRNAs with ABCA1, ABCB1 and ABCC1, RNA22 was performed with default parameters, i.e. minimum number of paired-up bases in heteroduplex was kept at 12 while the maximum folding energy was set at −12 kcal/mol. Moreover, sensitivity and specificity values of 63 % and 61 % respectively were retained, and seed size of 7 was selected with one unpaired base allowed without limiting the maximum number of G:U wobbles in the seed region [29]. The sequences of shortlisted miRNAs were retrieved from miRBase database (v22) (https://www.mirbase.org/) [30]. The NCBI database was used to retrieve the nucleotide sequences of ABCA1, ABCB1 and ABCC1 using accessions: NM_005502.4, NM_000927.5 and NM_004996.4 respectively. The alignment results were filtered in order to get the significant alignments. The filtration was performed using the filter function of dplyr applying *p*-value criteria of < 0.05 to alignment results.

3D structure prediction of ABC mRNAs

To overcome the constraints imposed by multiple miRNA 3D structure prediction tools, a decision was made to focus on 500 nt regions that demonstrated the highest frequency of miRNA-mRNA interactions, as determined by the least energy criterion, for subsequent analysis. To extract the nucleotide regions of ABC family genes that showed maximum interactions with miRNAs, scatter plots for each gene were generated using the scatter method of Python (v3.9.12) library seaborn (v0.11.2). The binding sites and folding energy of the predicted miRNA-mRNA interactions were plotted which showed ABCA1, ABCB1 and ABCC1 regions with maximum and stable miRNA interactions. The nucleotide sequences of these regions were extracted for their model prediction through RNAComposer (v1.0) (https://rnacomposer.cs.put.poznan.pl/) [31]. RNAComposer is an automated manually curated database of RNA 3D structure elements, and it is known to predict 3D structure using the machine translation system of RNA FRABASE database [32]. It is restricted to take only 500 nt as input, and CONTRAfold was selected as a secondary structure prediction method.

Shortlisted miRNAs 3D structure prediction

For the prediction of 3D structures of miRNAs that showed maximum interaction with all of the genes, MC Fold (https://www.major.iric.ca/MC-Fold/) and MC Sym (https://www.major.iric.ca/MC-Sym/) were employed. MC Fold is a secondary structure prediction tool, which deploys the generation of loops through decomposition of RNA structure

[33]. MiRNA sequences were given as input to MC Fold and among the multiple predicted secondary structures, the one with minimum energy was selected and then submitted to MC Sym, to predict the stable tertiary structures. MC Sym generates structures by examining the conformational search space of RNA [34].

Molecular docking and visualization

To perform the miRNA-mRNA molecular docking of shortlisted miRNAs and genes, HDOCK (v2021) (http://hdock.phys.hust.edu.cn/) was utilized. It is an *ab initio* docking web-server, which employs template based modeling to identify the complexes with lowest binding affinity and stable conformation [35]. The structures of the shortlisted miRNAs and the gene regions were provided as input with default parameters. A total of 10 complexes were generated for each miRNA-mRNA docked model, and the one with high confidence and least binding affinity score was selected. Hence, from all the predicted complexes, only three were shortlisted for visualizations, based on minimum binding affinity score and maximum confidence score. For visualization of these shortlisted complexes, PyMOL (v2.5.4) (https://pymol.org/2/) was employed. It identifies the nucleotides of miRNA and mRNA that interact with each other in a stable docked complex. PyMOL is a molecular graphics tool that visualizes small molecules such as nucleic acids, proteins, trajectories and electron densities [36].

RESULTS

Predicted miRNA-ABC genes interactions

MiRWalk, TargetScan and miRDB predicted a total of 924, 322 and 341 miRNA-mRNA interactions, respectively. However only stable and strong interactions of microRNAs particularly miR-21, miR-15 and miR-let-7 against ABC family genes were shortlisted. ABC family genes notably ABCA1, ABCB1 and ABCC1 showed a total of 18, 13 and 28 stable interactions with aforementioned microRNAs. Hence, only 30 predicted miRNA interactions that were common among the three databases were shortlisted. It was found that miR-21, miR-15 and miR-let-7 showed 10 interactions with ABCA1. Moreover, seven interactions were found for miR-21, miR-15 and miR-let-7 with ABCB1 while miR-21, miR-15 and miR-let-7 showed 13 interactions with ABCC1. The miRNA-mRNA interactions for each gene are represented individually in Table 1, Table 2 and Table 3.

Table 1. Predicted miR-21, miR-15 and miR-let-7 interactions with ABCA1 through miRWalk, TargetScan and miRDB

miRNA	miRNA sequence
miR-21-5p	UAGCUUAUCAGACUGAUGUUGA
miR-21-3p	CAACACCAGUCGAUGGGCUGU
miR-15a-5p	UAGCAGCACAUAAUGGUUUGUG
miR-15a-3p	CAGGCCAUAUUGUGCUGCCUCA
let-7i-3p	CUGCGCAAGCUACUGCCUUGCU
let-7f-1-3p	CUAUACAAUCUAUUGCCUUCCC
let-7e-3p	CUAUACGGCCUCCUAGCUUUCC
let-7d-3p	CUAUACGACCUGCUGCCUUUCU
let-7b-3p	CUAUACAACCUACUGCCUUCCC
let-7a-3p	CUAUACAAUCUACUGUCUUUC

Table 2. Predicted miR-21, miR-15 and miR-let-7 interactions with ABCB1 through miRWalk, TargetScan and miRDB

miRNA	miRNA sequence
miR-15a-3p	CAGGCCAUAUUGUGCUGCCUCA
let-7i-3p	CUGCGCAAGCUACUGCCUUGCU
let-7g-3p	CUGUACAGGCCACUGCCUUGC
let-7f-1-3p	CUAUACAAUCUAUUGCCUUCCC
let-7c-5p	UGAGGUAGUAGGUUGUAUGGUU
let-7b-5p	UGAGGUAGUAGGUUGUGUGGUU
let-7a-3p	CUAUACAAUCUACUGUCUUUC

Table 3. Predicted miR-21, miR-15 and miR-let-7 interactions with ABCC1 through miRWalk, TargetScan and miRDB

miRNA	miRNA sequence
miR-21-3p	CAACACCAGUCGAUGGGCUGU
miR-15a-3p	CAGGCCAUAUUGUGCUGCCUCA
let-7g-3p	CUGUACAGGCCACUGCCUUGC
let-7f-1-3p	CUAUACAAUCUAUUGCCUUCCC
let-7e-3p	CUAUACGGCCUCCUAGCUUUCC
let-7d-5p	AGAGGUAGUAGGUUGCAUAGUU
let-7d-3p	CUAUACGACCUGCUGCCUUUCU
let-7c-5p	UGAGGUAGUAGGUUGUAUGGUU
let-7b-5p	UGAGGUAGUAGGUUGUGUGGUU
let-7b-3p	CUAUACAACCUACUGCCUUCCC
let-7a-5p	UGAGGUAGUAGGUUGUAUAGUU
let-7a-3p	CUAUACAAUCUACUGUCUUUC
let-7a-2-3p	CUGUACAGCCUCCUAGCUUUCC

Alignments between miRNAs-mRNAs

RNA22 predicted the alignments of shortlisted miRNAs with each gene. The results revealed 22 alignments between miRNAs-ABCA1, 11 between miRNAs-ABCB1 and 58 between miRNAs-ABCC1. These alignments denoted miRNA binding sites on mRNAs and exhibited certain binding hotspots with low binding energy, which showed the strength and stability of miRNA-mRNA resulting complex.

Shortlisting of ABCA1, ABCB1 and ABCC1 regions

To shortlist the ABCA1, ABCB1 and ABCC1 regions, which showed maximum and stable interactions with miRNAs, three scatter plots, were generated (Fig. 2, Fig. 3 and Fig. 4). Three, two and eight regions of ABCA1, ABCB1 and ABCC1 respectively were recognized that showed maximum miRNA-mRNA interactions (Table 4, Table 6, Table 8). Out of 22 miRNA-ABCA1 interactions, the nine miRNAs were shortlisted which showed maximum binding with ABCA1 regions Table 5. Among the nine shortlisted miRNAs, three showed interactions with region 1, 3 with region 2 and 3 with region 3. Moreover, 5 out of 11 miRNAs were shortlisted which showed increased binding with ABCB1 regions Table 7. It was observed that two miRNAs showed interaction with region 1 while three miRNAs with region 2. Furthermore, among the 58 miRNA-ABCC1 interactions, 35 were shortlisted based on their highest binding affinity with ABCC1 regions Table 9. Out of 35 shortlisted miRNAs, four showed interactions with region 1, 7 with region 2, 4 with region 3, 3 with region 4, 3 with region 5, 6 with region 6, 4 with region 7 and 4 with region 8.

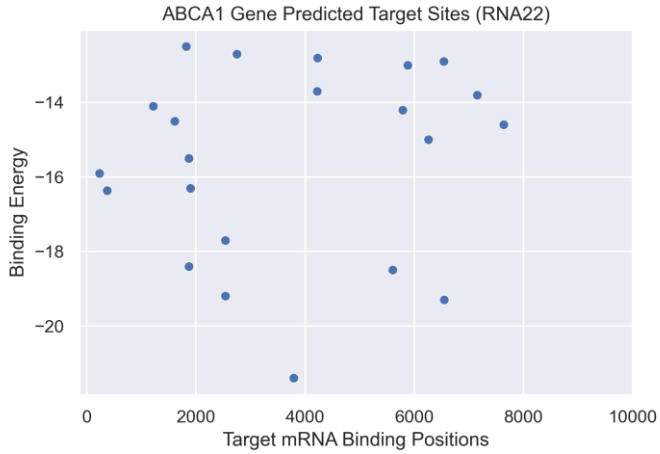

Fig. 2. Visualization of hotspot miRNA-mRNA interaction regions at various ABCA1 regions that show maximum miRNA binding with low energy. The *x*-axis represents the mRNA binding positions while the *y*-axis represents the binding energy of miRNA-mRNA interaction. Due to high number of interactions between 1600-2100, 2400-2900 and 6100-6600, the miRNAs interacting in these regions were shortlisted.

Table 4. Shortlisted regions of ABCA1 for prediction of mRNA 3D structures

Region name	Nucleotide length
Region 1	1600–2100
Region 2	2400–2900
Region 3	6100–6600

Table 5. Shortlisted ABCA1 regions, the shortlisted miRNAs present within those regions and their interactions along with folding energy predicted through RNA22

Gene region	miRNA	Predicted target site	Folding energy	*p*-value
Region 1	miR_21_5p	1821	-12.5	1.67E-01
	miR_15a_5p	1874	-18.4	1.67E-01
	miR_21_3p	1903	-16.3	1.67E-01
Region 2	let_7i_3p	2543	-19.2	3.03E-02
	let_7d_3p	2543	-17.7	3.03E-02
	miR_15a_3p	2752	-12.7	1.04E-01
Region 3	miR_21_5p	6262	-15	9.48E-02
	let_7d_3p	6543	-12.9	1.20E-01
	let_7i_3p	6547	-19.3	1.20E-01

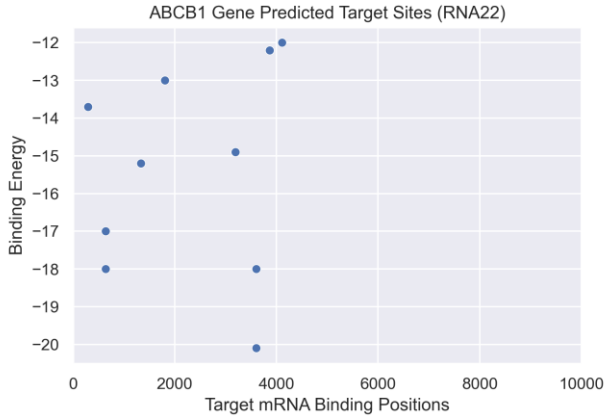

Fig. 3. Visualization of hotspot miRNA-mRNA interaction regions at various ABCB1 regions that show maximum miRNA binding with low energy. The *x*-axis represents the mRNA binding positions while the *y*-axis represents the binding energy of miRNA-mRNA interaction. Due to high number of interactions between 200-700 and 3150-3650, the miRNAs interacting in these regions were shortlisted.

Table 6. Shortlisted regions of ABCB1 for prediction of mRNA 3D structures

Region name	Nucleotide length
Region 1	200–700
Region 2	3150–3650

Table 7. Shortlisted ABCB1 regions and corresponding miRNAs, their interaction probabilities, and the folding energy predicted by RNA22

Gene region	miRNA	Predicted target site	Folding energy	*p*-value
Region 1	let_7b_5p	292	-13.7	2.73E-01
	let_7c_5p	643	-18	3.61E-01
Region 2	let_7g_3p	3198	-14.9	1.12E-01
	let_7c_5p	3605	-18	6.28E-02
	let_7b_5p	3605	-20.1	6.28E-02

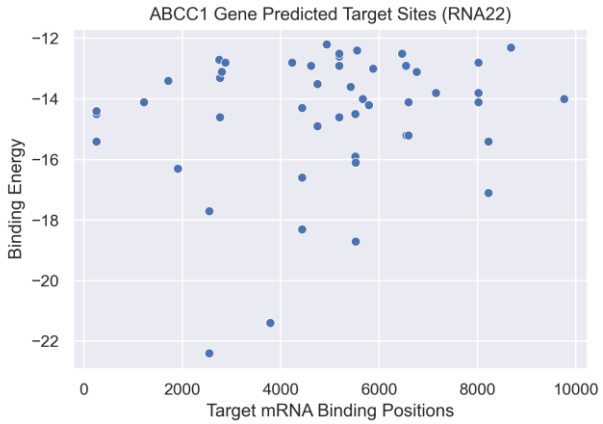

Fig. 4. Visualization of hotspot miRNA-mRNA interaction regions at various ABCC1 regions that show maximum miRNA binding with low energy. The *x*-axis represents the mRNA binding positions while the *y*-axis represents the binding energy of miRNA-mRNA interaction. Due to the large number of interactions in fragments with coordinates 1–500, 2400–2900, 4200–4700, 4900–5300, 5400–5800, 6500–7000, 8000–8500 and 9500–10000, the miRNAs interacting in these regions were shortlisted.

Table 8. Shortlisted regions of ABCC1 for prediction of mRNA 3D structures

Region name	Nucleotide length
Region 1	1–500
Region 2	2400–2900
Region 3	4200–4700
Region 4	9500–10000
Region 5	8000–8500
Region 6	5400–5800
Region 7	4900–5300
Region 8	6500–7000

Table 9. Shortlisted ABCC1 regions and corresponding miRNAs, their interaction likelihoods and folding energy predicted by RNA22

Gene region	miRNA	Predicted target site	Folding energy	p-value
R1	let_7d_5p	252	-15.4	1.61E-02
	let_7c_5p	252	-14.4	1.61E-02
	let_7b_5p	252	-14.5	1.61E-02
	let_7a_5p	252	-14.4	1.61E-02
R2	let_7d_3p	2543	-17.7	3.03E-02
	let_7g_3p	2544	-22.4	3.03E-02
	miR_15a_3p	2752	-12.7	1.04E-01
	let_7b_5p	2762	-14.6	1.04E-01
	let_7c_5p	2765	-13.3	1.04E-01
	let_7a_5p	2765	-13.3	1.04E-01
	let_7d_5p	2796	-13.1	1.04E-01
R3	let_7b_3p	4230	-12.8	2.49E-02
	let_7a_5p	4431	-14.3	2.39E-01
	let_7c_5p	4433	-16.6	2.39E-01
	let_7b_5p	4433	-18.3	2.39E-01
R4	let_7c_5p	9763	-14	7.21E-03
	let_7b_5p	9763	-14	7.21E-03
	let_7a_5p	9763	-14	7.21E-03
R5	let_7b_5p	8015	-14.1	2.78E-01
	let_7a_5p	8019	-12.8	2.78E-01
	let_7c_5p	8021	-13.8	2.78E-01
R6	let_7a_2_3p	5419	-13.6	9.05E-02
	let_7c_5p	5517	-15.9	9.47E-03
	let_7b_5p	5517	-14.5	9.47E-03
	let_7d_5p	5518	-18.7	9.47E-03
	let_7a_5p	5518	-16.1	9.47E-03
	miR_15a_3p	5788	-14.2	1.04E-01
R7	let_7b_5p	5186	-12.6	2.58E-01
	let_7d_5p	5189	-14.6	2.58E-01
	let_7c_5p	5189	-12.5	2.58E-01
	let_7a_5p	5189	-12.9	2.58E-01
R8	let_7d_3p	6543	-12.9	1.20E-01
	let_7g_3p	6548	-15.2	1.20E-01
	let_7c_5p	6597	-15.2	1.20E-01
	let_7b_5p	6597	-14.1	1.20E-01

Molecular docking between miRNAs-mRNAs

HDOCK was employed to perform the molecular docking of the 49 shortlisted miRNAs with gene regions. For each shortlisted miRNA interaction with the corresponding mRNA region, 10 complexes were generated. A top complex with lowest binding affinity score was selected for each miRNA-mRNA interaction. Molecular docking analysis yielded nine miRNA–ABCA1, five miRNA–ABCB1 and 35 miRNA–ABCC1 docking complexes, and among each group, the single best complex with the highest confidence score and lowest binding affinity was shortlisted in Table 10. The binding affinity and confidence score represents the association strength of miRNA–mRNA complex which denotes that the complex is in preferred orientation and is stable. Let-7c-5p interacts with both ABCB1 and ABCC1 but with different binding affinity. The let-7c-5p–ABCC1 complex was shown to have least binding affinity (-629.76) as compared to let-7c-5p–ABCB1 complex which showed a binding affinity of –572.68.

Table 10. Shortlisted miRNA–mRNA docked complexes based on lowest binding affinity score and highest confidence score predicted through HDOCK

Gene name	Complex	Binding affinity score	Confidence score
ABCA1	miR-15a-5p-R1	-543.88	0.9996
ABCB1	let-7c-5p-R2	-572.68	0.9998
ABCC1	let-7c-5p-R7	-629.76	0.9999

Shortlisted complexes visualization

The visualization of shortlisted complexes was performed through PyMOL in order to identify the nucleotides that interact between miRNA-mRNA interactions. It was observed that miR-15a-5p interacts with ABCA1–R1 at 16 different sites with a binding affinity of -543.88 (Fig. 5). Furthermore, let-7c-5p interacts with ABCB1(R2) with a binding affinity of -572.68 at 20 sites (Fig. 6). The let-7c-5p showed 14 interactions with ABCC1(R7) with a -629.76 binding affinity score (Fig. 7). The results revealed that let-7c-5p–ABCB1(R2) showed maximum interactions while let-7c-5p–ABCC1(R7) showed least number of interactions.

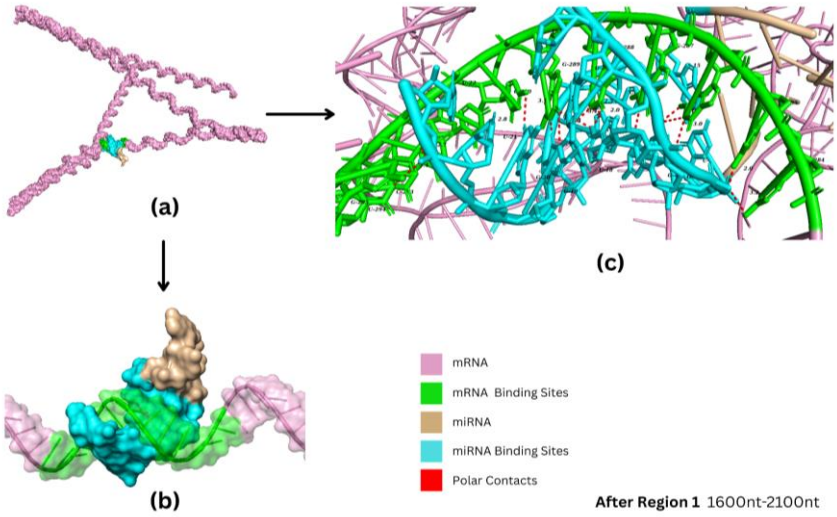

Fig. 5. MiR-15a-5p–ABCA1(R1) interaction: a) MiRNA–mRNA complex interaction is demonstrated as surface representation. b) The ligand (miRNA) is denoted by wheat color while its binding site is represented by cyan color. Similarly, the pink color represents the receptor (gene region) while the green color indicates the receptor binding site. c) The polar interactions between the receptor and the ligand are represented by red dotted lines.

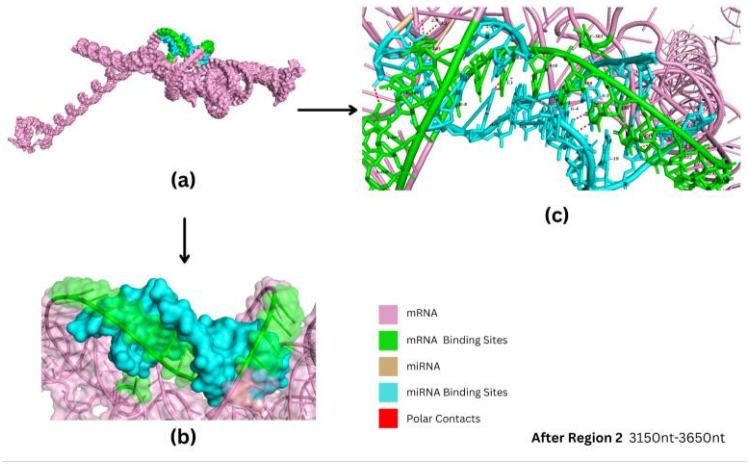

Fig. 6. Let-7c-5p–ABCB1(R2) interaction: a) miRNA–mRNA complex interaction is demonstrated as surface representation. b) The ligand (miRNA) is denoted by wheat color while its binding site is represented by cyan color. Similarly, the pink color represents the receptor (gene region) while the green color indicates the receptor binding site. c) The polar interactions between the receptor and the ligand are represented by red dotted lines.

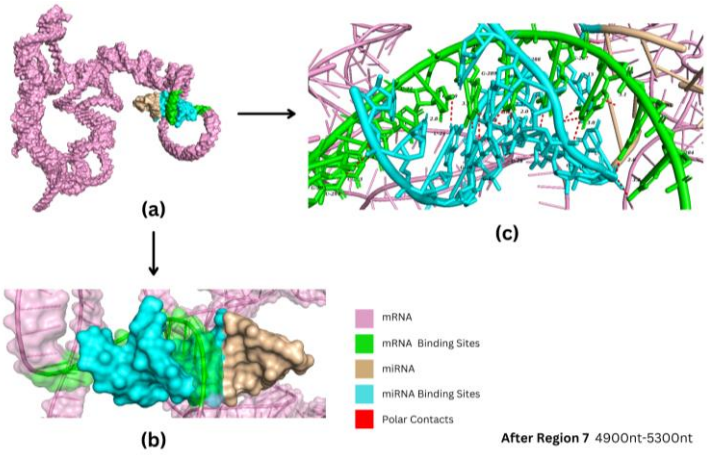

Fig. 7. Let-7c-5p–ABCC1(R7) interaction: a) MiRNA–mRNA complex interaction is demonstrated as surface representation. b) The ligand (miRNA) is denoted by wheat color while its binding site is represented by cyan color. Similarly, the pink color represents the receptor (gene region) while the green color indicates the receptor binding site. c) The polar interactions between the receptor and the ligand are represented by red dotted lines.

DISCUSSION

The resistance to chemotherapy is promoted by ABC transporters which are responsible for decreased drug accumulation and increased efflux from tumor cells. ABC family consists of membrane transporters that are involved in the transportation of molecules across cells that require ATP hydrolysis [37]. The ABC transporters are involved in key physiological processes and their dysregulation causes several pathologies including cancer [38]. Based on gene structure, domain organization and amino acid sequence, the ABC transporters are classified into multiple subfamilies. ABCA1 is the largest member of the ABC family and is responsible for transporting cholesterol and phospholipids to apolipoproteins. This transport enables the process of reverse cholesterol transport by helping cells to shed excess cholesterol to make high density lipoproteins [39]. Besides cholesterol transport, ABCA1 also functions in immune system cells as an engulfment receptor on macrophages [40]. ABCB1 encodes P-glycoprotein (P-gp) which is a multidrug resistant protein [41]. It acts as an efflux carrier and transports multiple substrates

out of the cell such as chemotherapeutic drugs, antibiotics and anti-epilepsy drugs, thus interfering with therapeutic drug delivery [42]. ABCC1 is an organic anion transporter which plays a crucial role in multidrug resistance of tumor cells and enables them to proliferate aberrantly. Its increased expression is involved in the progression of multiple cancers such as breast cancer, lung cancer and prostate cancer due to enhanced chemoresistance [43].

Several studies have established the role of ABC transporters in the transport of tumor enhancing molecules as well as the interaction of multiple proteins that influence the tumor progression and aggressiveness [43]. Moreover, ABC transporters are also involved in reduced therapeutic drug accumulation in tumor cells that effectively hinders chemotherapy [44]. It has been reported that miRNA mediated regulation of the ABC transporter family suppresses tumor progression [45].

For the identification of miRNAs that inhibit ABCA1, ABCB1 and ABCC1, miRWalk, TargetScan and miRDB were employed which predicted a total of 30 common interactions for three shortlisted miRNAs (miR-21, miR-15 and miR-let-7) and three genes. Among these interactions, 10 were found for miRNA-ABCA1, 7 for miRNA-ABCB1 and 13 for miRNA-ABCC1. These interactions may help in identifying the binding potential of the shortlisted miRNAs with these genes that ultimately affect the process of protein synthesis [46]. To identify the binding sites of shortlisted miRNAs on target genes, RNA22 was employed. The employment of standard RNA22 parameters by similar research predicted the possible binding sites of 242 miRNAs against the genome of influenza C virus. In the present study, the possible binding sites of 22 miRNAs against ABCA1, 11 miRNAs against ABCB1 and 58 miRNAs against ABCC1 were predicted. The aligned miRNAs such as miR-21-5p, let-7b-5p and let-7d-5p have been identified to be associated with cancer development. It has been reported that overexpression of miR-21-5p downregulates transforming growth factor beta-induced (TGFB1) which inturn induces pyroptosis (inflammatory cell death) in colorectal cancer cells [23]. The upregulation of let-7b-5p in human mucosal melanoma shows antitumor effects by suppressing MTDH and CALU. Therefore the downregulation of these genes disrupts signaling pathways (MAPK, Wnt/β-catenin, PI3K/AkT) and protein folding in endoplasmic reticulum (ER) which suppresses tumor progression [47]. Furthermore, suppression of HMGA1 by overexpression of let-7d-5p enhances p53 signaling pathway, which in turn inhibits the tumor proliferation and facilitates apoptosis and chemotherapeutic sensitivity in ovarian cancer [48].

RNAComposer and MC Sym were utilized for the tertiary structure prediction of shortlisted miRNAs and mRNAs. The shortlisting of miRNA-mRNA interactions depends upon the maximum binding affinity and target miRNA and mRNA abundance. For ABCA1, ABCB1 and ABCC1 3, 2 and 8 mRNA regions were shortlisted respectively. ABCA1 showed maximum binding with nine miRNAs, while ABCB1 with five miRNAs and ABCC1 with 35 miRNAs. HDOCK was employed for molecular docking in order to identify the stable miRNA-mRNA complex with least binding affinity. Out of 49 docked complexes, nine were generated for miRNAs-ABCA1, 5 for miRNAs-ABCB1 and 35 for miRNAs-ABCC1 however among them only the top complex that showed maximum confidence score and least binding affinity was shortlisted. Out of the 3 shortlisted complexes, 2 (miRNA-ABCA1, miRNA-ABCB1) showed interactions with mRNA at CDS while one (miRNA-ABCC1) showed interaction with 5’-UTR. The canonical binding site of miRNA in target gene for its regulation through mRNA degradation is 3’-UTR [49]. On the contrary, miRNA binding with the CDS region or 5’-UTR of the target gene leads to gene silencing through translational inhibition [50]. Therefore, it is critical to identify miRNA targets and their corresponding biological processes for the functional characterization of miRNAs. Several studies reported the TS role of miR-15a-5p. It was found that SNHG12 knockdown by miR-15a-5p upregulation promotes SALL4 inhibition, which in turn suppresses proliferation, migration, and invasion of BC cells [51]. Moreover miR-15a-5p targets CCND1 and PD1 in CD8+ T-cells in colon cancer and HCC respectively, thus suppressing the tumor growth by cellular proliferation and migration repression [52]. The TS

role of let-7c-5p in esophageal squamous cell carcinoma, erythroleukemia and HCC has been reported by multiple researchers [53]. Overexpression of let-7c-5p in MCF-7 BC cells inhibits ERCC6 gene expression which results in proliferation and apoptosis repression [54]. Furthermore, let-7c-5p inhibits glioma progression by targeting RGS16, which hinders PI3k/AkT pathway [55]. Nevertheless, a study demonstrated the role of let-7c-5p as an anticancer drug in cervical cancer, which obstructs tumor proliferation and migration by targeting transcription factors and signal transduction pathways [56].

The present study identified the binding affinities of shortlisted miRNAs with ABCA1, ABCB1 and ABCC1. It was observed that let-7c-5p binds with ABCB1 and ABCC1 however it showed least binding affinity with ABCC1 (-629.76) interacting at 14 different sites. On the contrary, let-7c-5p showed a maximum number of interactions with ABCB1 i.e. 20 with a binding affinity of -572.68. Thus, these strong interactions demonstrate a stable complex of more specificity and potential efficacy. Among the three shortlisted complexes, 2 showed strong miRNA interactions with CDS region of target genes indicating a hotspot region on the gene structure where TS miRNAs bind to suppress the gene expression.

In the research, highly significant regions of ABCA1, ABCB1 and ABCC1 were identified that could be targeted by novel TS miRNAs (miR-15a-5p, let-7c-5p) in order to suppress the oncogenes in breast cancer. These miRNAs have been reported to act as TS in multiple carcinomas such as BC, HCC, colon cancer, cervical cancer, esophageal squamous cell carcinoma and glioma [57]. The binding of TS miRNAs on oncogenic mRNAs may lead to decreased drug efflux and increased accumulation of anticancer drugs in cells [57]. Furthermore, regulation of ABC family genes leads to appropriate transportation of molecules across the cells in the tumor microenvironment [58]. Therefore, the identification of mRNA regions of ABCA1, ABCB1 and ABCC1 indicating strong interactions with TS miRNAs, suggests the use of miRNAs in cancer therapy. Hence, predicted TS miRNAs could target ABC family oncogenes and suppress their functions making the tumor cells more susceptible to chemotherapy.

CONCLUSION

Breast cancer is the most common type of cancer among women across the globe [59]. The overexpression of ABC transporters such as ABCA1, ABCB1 and ABCC1 leads to increased drug efflux and chemoresistance breast cancer. Therefore, this research identified 22, 11 and 58 interactions between miRNAs (miR-21, miR-15, and miR-let-7) and ABCA1, ABCB1 and ABCC1 respectively. Out of these miRNA-mRNA interactions, molecular docking analysis revealed three complexes with maximum confidence score and least binding affinity. It was observed that miR-15a-5p interacts with ABCA1 at 16 binding sites while let-7c-5p interacts with ABCB1 and ABCC1 at 20 and 14 sites respectively. Additionally, miR-15a-5p and let-7c-5p showed interactions with ABCA1 and ABCB1 mRNAs at CDS regions, whereas let-7c-5p showed interaction with ABCC1 at 5’-UTR. In this study the TS role of miRNAs have been identified that may target the oncogenes (ABCA1, ABCB1 and ABCC1) and suppress their function which results in decreased drug efflux and chemoresistance. The present study identified interactions and significant binding regions of two novel miRNAs (miR-15a-5p and let-7c-5p) that can suppress ABCA1, ABCB1 and ABCC1 expression to inhibit chemoresistance in TNBC. Moreover, TS role of these miRNAs have been reported previously to inhibit multiple carcinomas including HCC, cervical cancer, glioma, colon cancer and esophageal squamous cell carcinoma. Thus, aforementioned miRNAs have been proposed as anticancer drugs to provide new aspects in breast cancer treatment.

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