Journal of Applied Biology & Biotechnology Vol. 13(6), pp. 80-87, November-December, 2025
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2025.v13.i6.9

In silico analyses to identify the potential genes and microRNA targets in pathways mediating radiation-induced adaptive response in breast cancer

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ARTICLE INFO

Article history:
Received on: 07/04/2025
Accepted on: 24/06/2025
Available online: 16/09/2025

Key words:
Radiation-induced adaptive response,
Radiotherapy,
Breast cancer,
Gene interactions,
miRNA targets.

ABSTRACT

Radiation therapy is implemented as palliative treatment using X-ray either via conventional/fractionated regime to treat breast cancer patients where radio-resistance remains a challenge. Low followed by higher doses of radiation results in non-targeted effect of radiation known as (RIAR), which is considered a causal factor for the occurrence of radio-resistance. Genes were collected from NCBI database, literature, and enriched using Enrichr database. Understanding *RIAR* gene-gene interactions is essential for unravelling complex biological mechanisms in *RIAR*; hence, interactions among genes were performed using Cytoscape (v 3.10.2) and Search Tool for the Retrieval of Interacting Genes databases. The microRNAs (miRNAs) corresponding to these genes and those having roles in breast cancer were retrieved from miRNA databases, namely, miTarBase, miRDB, miRNet, and enriched using enrichMiR. Our study analysed 69 genes associated with RIAR pathways. About 19 miRNAs among RIAR and breast cancer were found to be enriched with several gene overlaps. These genes and miRNAs have profound roles in processes such as DNA repair, cell survival, cell proliferation, oxidative stress, cell cycle regulation, inflammation, cell migration, and cytoskeletal interactions. Our results suggest these miRNAs may serve as potential targets in altering RIAR. Targeting these miRNAs further can alter genes involved in RIAR-associated mechanisms, thereby improving the radio-therapeutic efficacy.

1. INTRODUCTION

Cancers pose a serious burden to global public health, with the number of cases and associated deaths rising annually [1]. Radiotherapy (RT) has been shown to be an effective palliative strategy and treatment as it increases longevity with disease-free survival in the affected. During RT, the ionizing radiation (IR) such as X-ray for multiple doses is delivered in fractions either via the conventional or fractionated regime. Radio-resistance refers to the ability of cancer cells to resist the cell-killing effects of radiation, thereby leading to reduced/compromised efficacy. Thus, the effectiveness of RT remains a challenge due to the development of resistance followed by recurrence and relapse even after the completion of therapy among a considerable number of breast cancer patients [2]. While therapeutic effects are mediated through direct DNA damage and cell killing, the resistance has been postulated to be mediated by non-targeted effects. One such phenomenon of non-targeted effect of IR is the adaptive response, specifically known as the radiation-induced adaptive response (RIAR) implicated in the development of radio-resistance. The low dose followed by high doses of IR serves to confer a radio-protective effect in normal cells whereas it reduces the therapeutic efficacy in cancer cells [3].

In vitro laboratory experiments with cell lines have demonstrated the existence of RIAR phenomenon followed by exposure to radiochemicals and radiation [4,5]. Various types of end-points (cytogenetic, genetic, proteomic, metabolic, or epigenetic) were employed to demonstrate the radio-resistance in those systems [6]. Scrutiny of literature has shown that many genes and gene products are involved in mediating the response; identifying the ideal candidate

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and inhibiting the molecule/pathway are proposed as new targets for cancer RT [7,8]. There are a lot of challenges in the existing therapeutic targets because of their specificity, sensitivity, and lack of standardization. Hence, an advancement in the categorization of genetic and epigenetic biomarkers via the *in silico* approaches may be helpful in identifying the pathways and molecules in mediating the response and confers resistance. The molecular mechanisms of RIAR have not been completely understood till date as this phenomenon remains challenging. Thus, understanding the gene-gene interactions is essential for unraveling the complex biological mechanisms in RIAR.

Evaluating the microRNAs (miRNAs) can serve as potential therapeutic targets to predict the RT response as they are profound theragnostic biomarkers [9]. Many miRNAs regulate the expression of radiation and cancer-related pathways; hence, targeting specific miRNAs of the target genes involved particularly in cellular pathways/mechanisms that mediate RIAR, which alter gene expression patterns during RT thereby paving the way for new possibilities in altering RIAR. Performing *in silico* studies can identify suitable targets that can pave the way for opting genes and miRNAs for future *in vitro* studies. Thereby, more appropriate studies can be performed to target miRNAs and alter gene expression during cancer RT. Therefore, we hypothesize that *in silico* analyses could reveal detecting and targeting the possible miRNAs associated with genes that might be potentially involved/mediated in the pathways of RIAR-associated mechanisms. The reason for the choice of breast tissue selected as the primary basis in this study for exploring RIAR is due to the possibilities of higher radio-resistance occurring in these types of cancers and an effective radiation therapy for cancer patients [10]. The objective of this *in silico* study is to find out the miRNAs that target the genes playing a crucial role in pathways associated with RIAR, using literature databases, integrating the gene set enrichment analysis (GSEA), and gene-gene interactions to predict the most possibly occurring miRNAs in RIAR and breast cancer. miRNAs function as post-transcriptional regulators by binding to messenger RNA and either inhibit the translation or stimulate degradation. Enriching miRNAs between the RIAR and breast cancer might help in finding the most appropriate miRNAs that will target genes altering the RIAR phenomenon during breast cancer RT. Though the studies have shown the role of miRNAs in mediating cell-cell communication and intercellular signal transduction for the occurrence of RIAR in fibroblasts as a protective mechanism, however, the knowledge of miRNAs in RIAR during clinical RT remains limited [7]. Therefore, this study remains novel in miRNA target prediction for the occurrence of RIAR phenomenon during breast cancer RT. The final miRNAs listed in this study can be further explored for *in vitro* experimental models as it can be candidate targets for future validation, it may be beneficial for altering the RT regimes (conventional or hyper-fractionated RT) in the clinic for enhanced therapeutic efficacy in breast cancer patients. Here, we provide detailed information on the rationale of gene interactions and highly enriched miRNA in breast cancer-associated genes that play a vital role in RIAR.

2. MATERIALS AND METHODS

2.1. Study Design

This study consists of various steps, namely (i) gene data collection (ii) GSEA, (iii) gene–gene interactions followed by (iv) miRNA data retrieval, and (v) miRNA enrichment analysis to predict highly interacting miRNA. All the steps were used to identify the list of highly possible miRNAs that may play a major role in altering the RIAR-associated pathways during RT.

2.2. Gene Data Retrieval

Genes from databases namely, National Centre for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/) and relevant publications having functions in RIAR-associated pathways were retrieved using keywords, namely, "*Radiation-Induced Adaptive Response/radio-adaptive response*," "*radio-resistance*," "*gene expression and radiotherapy*," "*breast cancer*." About 69 driver genes were associated with the pathways involved in RIAR and have been found to be either upregulated or downregulated during exposure to low followed by high doses of IR.

2.3. GSEA

GSEA provides a functional interpretation of gene expression datasets by relating genes to their specific biological pathways and mechanisms. The genes that were retrieved from Gene databases were subjected to investigate the biological role using the Enrichr database (https://maayanlab.cloud/Enrichr/) with HTML5 list. "WikiPathways 2024 Human" pathway analysis was chosen as the basis for retrieving the pathways in which the genes are involved. We have analysed the highly interactive genes involved in RIAR that when explored can lead to effective radio-therapeutic strategies.

2.4. Gene-gene Interactions

The gene interactions were explored using the Cytoscape (v 3.10.2) (https://cytoscape.org/index.html) and Search Tool for the Retrieval of Interacting Genes (STRING) (http://string-db.org/) tools based on the information of genes available from the NCBI database and BioGRID version 4.4 (https://thebiogrid.org/).

2.5. miRNA Data Retrieval

The target miRNAs for RIAR-associated genes were retrieved from miTarBase (https://mirtarbase.cuhk.edu.cn/~miRTarBase/miRTarBase_2019/php/index.php), miRDB (https://mirdb.org/), and miRNet (https://www.mirnet.ca/miRNet/home.xhtml) databases with a criterion of these genes that have been already reported to be validated by quantitative polymerase chain reaction were considered. The structure (5'-3') of miRNAs were retrieved from RNA central database version 24 (https://rnacentral.org/). The miRNAs pertaining to breast carcinoma were retrieved from miRNet database.

2.6. miRNA Enrichment Analysis

The retrieved miRNAs were collectively fed into the enrichMiR database (https://ethz-ins.org/enrichMiR/) for miRNA enrichment analysis. The overall genes in common with the associated miRNAs were predicted. The highly enriched genes with the number of gene overlaps were determined.

2.7. Statistics

The enriched gene data provided various software generated statistical scores resulting from three tests namely, (i) Fisher's exact test employed in a wide range of gene list enrichment analysis programs; (ii) odd's ratio; and (iii) a combined score that is calculated by multiplying the log of *P*-value from the Fisher's exact test by the z-score using the test correction. The combined score was considered for correlating the highly enriched genes involved in RIAR along-with many diseases. The gene interactions were given by an interaction score; a score of 0.999 was considered to be highly interacting and had a strong correlation in specific pathways. STRING scores are indicators of confidence ranking from 0 to 1, for which a score >0.5 is considered acceptable for even

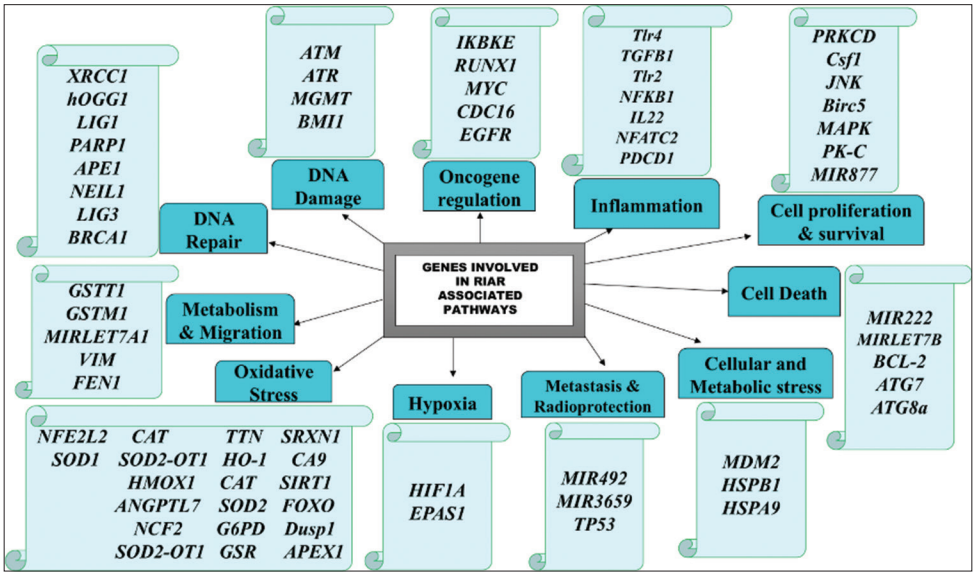

Figure 1: Analysis of genes playing a role in radiation-induced adaptive response-associated pathways.

	Base Excision Repair (6)	Immune Regulation (5)	miRNA TF regulation (8)	DNA Repair (10)	TP53 Network (5)	ATM signaling (5)	Cellular response by ATM (5)	Cellular response by ATR (4)	DNA Damage response (6)
Oxidative Stress (6)	0	1	2	0	0	1	0	0	0
Base Excision Repair (6)		0	0	6	2	0	1	1	2
Immune Regulation (5)			1	0	0	1	0	0	0
miRNA TF regulation (8)				2	2	2	2	1	2
DNA Repair (10)					5	2	4	3	5
TP53 Network (5)						2	4	3	4
ATM signaling (5)							2	2	2
Cellular response by ATM (5)								4	5
Cellular response by ATR (4)									4

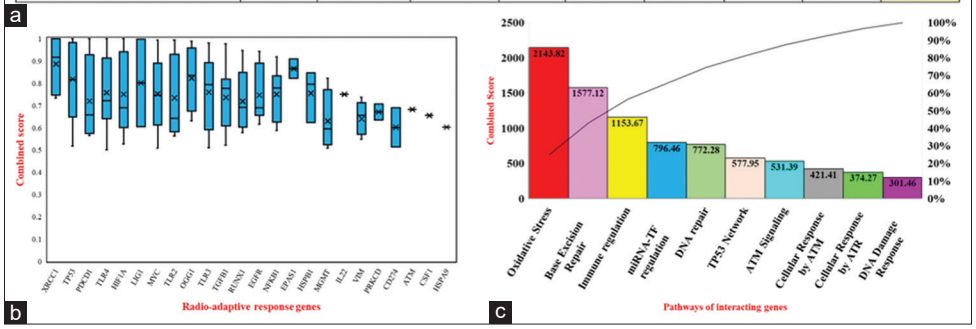

Figure 2: Gene Enrichment Analysis of genes involved in inducing radiation-induced adaptive response (RIAR) phenomenon. (a) Common genes intersecting among the RIAR-related pathways. (b) Order of highly enriched genes involved in the major pathways associated with RIAR. (c) Pathways of enriched genes based on "WikiPathways 2024 Human" presented with their combined scores.

the second set of interactors. Since many genes had higher interaction scores, a score of 0.9 was considered a stronger interaction [11]. The average local clustering coefficient was determined to be 0.517 upon the highest confidence interval of 0.9. Since many genes had the highest interaction scores that were statistically significant, the genes with a minimum of 0.98 and a maximum of 0.99 were considered. Predicted miRNA-target interactions were obtained using the TargetScan database (8.0). Interactions were filtered based on the context++ score percentile [12], and only those with a percentile above 80 (i.e., top 20% most confident predictions) were considered for

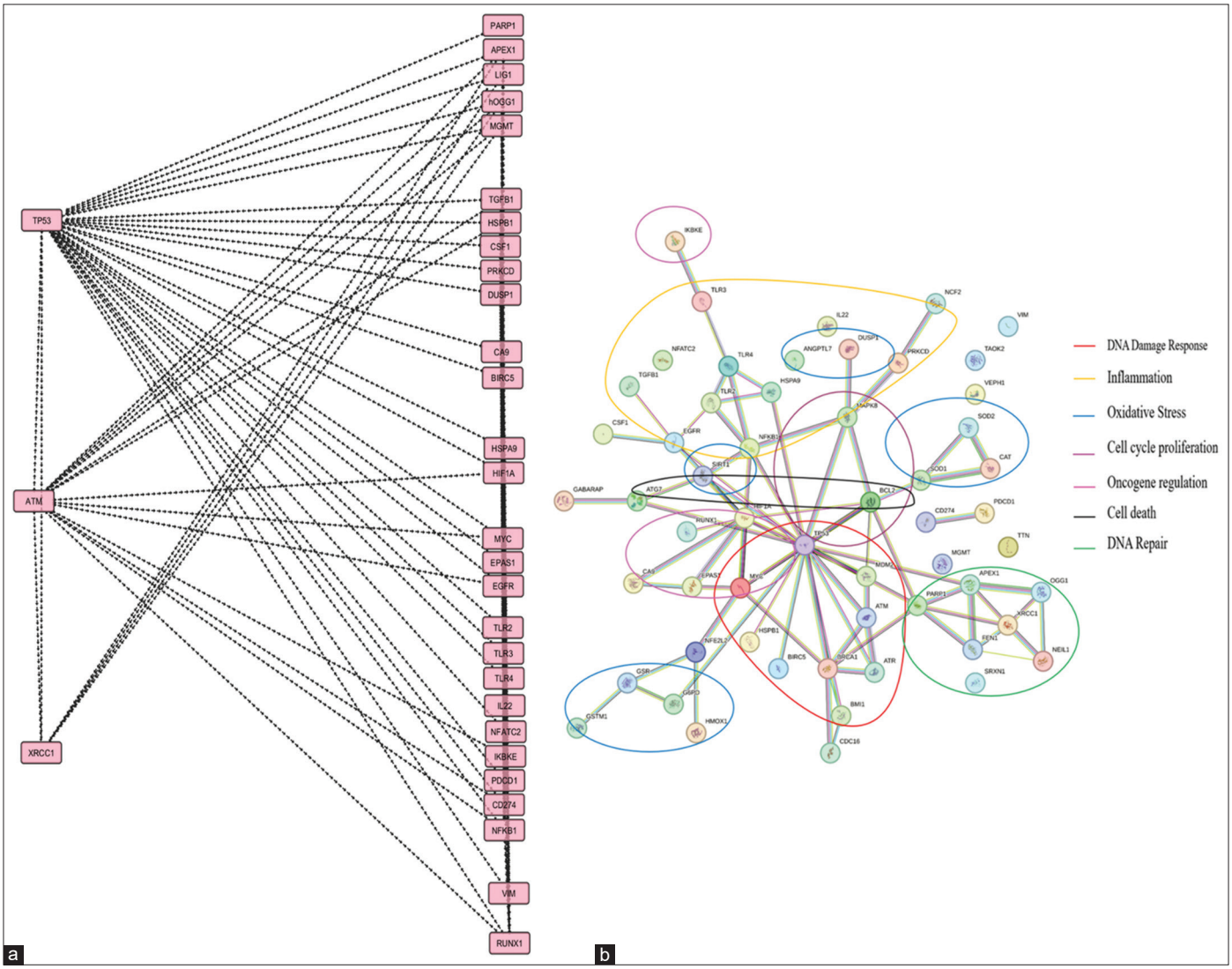

Figure 3: Gene interactions among Radiation-Induced Adaptive Response (RIAR)-associated pathway genes. (a) Cytoscape network of function-based RIAR gene interactions. (b) STRING-based functional gene interactions of RIAR genes.

further analysis. This threshold was selected to retain high-confidence interactions while reducing false positives. Similarly, for predictions from miRDB, only targets with a confidence score ≥80 were included. To account for multiple hypothesis testing, we applied the Benjamini-Hochberg False Discovery Rate (FDR) correction [13]. This method controls the expected proportion of false positives among the declared significant results. Adjusted *P*-values were calculated, and results with FDR-adjusted *P*-values (<0.05) were considered statistically significant. For the miRNA enrichment analysis, every miRNA had interactions with multiple genes as revealed in the form of gene overlaps.

3. RESULTS

3.1. Retrieval of RIAR-associated Pathway Genes

The existence of the RIAR response among various experiment models has been confirmed using the endpoints such as cell viability, clonogenic ability, DNA damages, chromosomal abnormalities, cell cycle arrest, and apoptosis. Expression of those changes is known to be regulated by different signaling molecules/pathways. The NCBI database was adopted to retrieve genes that are reported to be involved in mediating the response. The output data comprising 69 genes associated with several pathways have been found to be associated with pathways like DNA repair, DNA damage response, oxidative stress, hypoxia, ferroptosis, cellular and metabolic stress, heat shock process, apoptosis, autophagy, cell cycle regulation, cell proliferation, metastasis, inflammation, immune regulation, tumor oncogene or suppressor functions, cell survival, cell proliferation, glutathione metabolism, cell migration, and genomic stability [Supplementary Table 1]. From the earlier studies [7], RIAR-associated pathways related genes with $P < 0.05$ and logFC $|>1|$ were considered. Few genes also have radioprotective effect. The genes associated with various mechanisms/pathways that are found to alter the RIAR phenomenon are represented in Figure 1.

3.2. GSEA

GSEA was performed to derive the functional relationship among the genes involved in RIAR. There are 35 gene-set libraries in Enrichr with six categories, namely, transcription, pathways, ontologies, diseases/drugs, cell types, and miscellaneous; some of these libraries were built exclusively for Enrichr, while others have been obtained from other

Table 1: Enriched miRNAs as targets for Radiation-Induced Adaptive Response evaluation during breast cancer radiotherapy.

miRNAs	5'-3' Sequence	Overlap	Corresponding genes
hsa-miR-124-3p	UAAGGCACGCGGUGAAUGCCAA	8	*ANGPTL7, CD274, HMOX1, IKBKE, MGMT, NCF2, SIRT1, VIM*
hsa-miR-17-5p	CAAAGUGCUUACAGUGCAGGUAG	8	*APEX1, EPAS1, HIF1A, MDM2, MYC, RUNX1, SOD2, TGFB1*
hsa-miR-335-5p	UCAAGAGCAAUAACGAAAAAUGU	8	*BRCA1, EGFR, G6PD, HMOX1, MDM2, MYC, NEIL1, PARP1*
hsa-miR-128-3p	UCACAGUGAACCGGUCUCUUU	7	*APEX1, BMI1, EGFR, HMOX1, PARP1, RUNX1, SIRT1*
hsa-miR-155-5p	UUAAUGCUAAUCGUGAUAGGGGUU	7	*CAT, EGFR, HIF1A, IKBKE, MYC, NFKB1, SIRT1*
hsa-miR-185-5p	UGGAGAGAAAGGCAGUUCCUGA	7	*APEX1, ATR, EPAS1, MDM2, MYC, SOD2, TGFB1*
hsa-miR-106a-5p	AAAAGUGCUUACAGUGCAGGUAG	7	*ATG7, ATM, HIF1A, MDM2, RUNX1, SOD2, TGFB1*
hsa-miR-15a-5p	UAGCAGCACAUAAUGGUUUGUG	6	*BMI1, BRCA1, CD274, NFKB1, PDCD1, PRKCD*
hsa-miR-20a-5p	UAAAGUGCUUAUAGUGCAGGUAG	6	*EPAS1, HIF1A, MDM2, MYC, RUNX1, SOD2*
hsa-miR-218-5p	UUGUGCUUGAUCUAACCAUGU	6	*ATM, ATR, BMI1, EGFR, HMOX1, MDM2*
hsa-miR-27a-3p	UUCACAGUGGCUAAGUUCCGC	6	*APEX1, BMI1, EGFR, HIF1A, NFE2L2, RUNX1*
hsa-miR-34a-5p	UGGCAGUGUCUUAGCUGGUUGU	7	*ATG7, BRCA1, FEN1, MYC, NFKB1, SIRT1, XRCC1*
hsa-miR-93-5p	CAAAGUGCUGUUCGUGCAGGUAG	6	*CDC16, HIF1A, MDM2, MYC, SOD2, TGFB1*
hsa-let-7a-5p	UGAGGUAGUAGGUUGUAUAGUU	5	*EGFR, MYC, NFKB1, PARP1, SOD2*
hsa-miR-145-5p	GUCCAGUUUUCCCAGGAAUCCCU	5	*EGFR, EPAS1, HIF1A, MDM2, MYC*
hsa-miR-181a-5p	AACAUUCAACGCUGUCGGUGAGU	5	*ATM, BRCA1, PRKCD, RUNX1, SIRT1*
hsa-miR-24-3p	UGGCUCAGUUCAGCAGGAACAG	5	*BRCA1, FEN1, HMOX1, MYC, TGFB1*
hsa-miR-9-5p	UCUUUGGUUAUCUAGCUGUAUGA	5	*NFKB1, RUNX1, SIRT1, SOD2, VIM*
hsa-miR-92a-3p	UAUUGCACUUGUCCCGGCCUGU	5	*ATM, MDM2, MYC, NFKB1, SIRT1*

tools. The gene enrichment results with respect to genes and pathways are represented in Figure 2a-c. Figure 2a represents the common genes intersecting among the RIAR-related pathways. The order of highly enriched genes involved in the major pathways associated with RIAR is represented in Figure 2b. The enriched genes pertaining to their pathways based on "WikiPathways 2024 Human" with their combined scores are presented as Figure 2c. These genes that are found to be involved with increased combined scores. A maximum number of enriched genes are involved in oxidative stress followed by DNA repair pathways, immune regulation, miRNA-transcription factor regulation, *TP53* network, ATM signaling, and genes involved in cellular response by *ATM*, *ATR*, and *DNA* damage response pathway.

3.3. Gene Interactions Involved in RIAR

Cytoscape remains an efficient tool for retrieving data from different networks via web-service application programming interfaces, hence a more suitable approach for studying gene interactions as they aid in better visualization and analysis of interacting networks, also help us in grouping genes based on their functionality. The gene interactions were grouped into networks based on their functions using Cytoscape (v 3.10.2) and are represented [Figure 3a]. Genes such as *PARP1*, *APEX1*, *LIG1*, *hOGG1*, and *MGMT* have been found to be involved in DNA repair mechanisms that repair double-strand breaks caused by IR exposure. This repair is one of the prime mechanisms leading to radioresistance, contributing to RIAR. The adaptation involves cells to survive for the long term; hence, genes such as *TGFβ-1*, *HSPB1*, *CSF1*, *PRKCD*, and *DUSP1* are involved in RIAR. *HSPA9* and *HIF1A* are involved in oxidative stress-induced RIAR. *MYC*, *EPAS1*, and *EGFR* expression are altered and regulate the cell cycle upon pre-exposure to the priming dose. The inflammatory genes such as *TLR2*, *TLR3*, *TLR4*, *IL22*, *NFATC2*, *IKBKE*, *PDCD1*, *CD274*, *NFκB1* are responsible for mediating RIAR. *VIM* mediates cell migration hence known to induce RIAR. The gene interactions performed by the STRING database are represented [Figure 3b]. *p53* plays the primary role in interacting with the first set of interactors such as *ATM, HIF1A, MYC, MDM2, BCL2, SIRT1, MAPK,* and *BRCA1*. These are some evolutionary well-conserved defense mechanisms found to play a role in RIAR induction. The second highest interactors are DNA repair genes followed by inflammation-associated genes, DNA damage response genes, and finally genes involved in oxidative stress with an interaction score of 0.999. The other genes involved in similar pathways either directly or indirectly had an interaction score of 0.98. Since most of the genes of the vital pathways were highly interacting, the genes with at least a score of 0.98 were considered.

3.4. miRNAs among Genes of RIAR-associated Pathway and Breast Cancer

The miRNAs associated with breast carcinoma with estrogen-positive receptor and triple-negative breast cancer types in humans were included in the search criteria of miTARBASE, miRDB, and miRNet. The miRNAs of target genes were shortlisted with the genes that tend to have a common role in RIAR. About 242 miRNAs were retrieved from the above-mentioned databases and were involved in breast cancer. The miRNAs of RIAR-associated pathway genes were found to be 177. These miRNAs were collectively analyzed for enrichment.

3.5. miRNA Enrichment Analysis in Breast Cancer and RIAR

The list of predicted 19 miRNA targets of genes involved in RIAR during breast cancer RT with their 5'-3' structure and number of interactions/overlaps are tabulated as Table 1. The range of miRNA overlaps in genes ranged from 8 to 5. Single/multiple miRNAs have been found to be involved in transcript regulation.

4. DISCUSSION

Breast cancer is the most common type of cancer in women diagnosed as of 2020, and it is the leading cause of cancer morbidity, disability, and

mortality in women globally [14]. The RT remains a standard palliative therapy for curing breast cancer. The types of gene interactions may be categorized into gene regulatory networks, protein-protein interactions, regulation of gene expression, and crosstalk among pathways [15-17]. The coordinated cellular response to radiation depends on these gene-gene interactions, which affect the cancer patient's response to RT. The genes represented in Figure 1 are known to be reported in previous literature, among which DNA repair and oxidative stress pathways are highly involved in RIAR. Also, the GSEA results comprising the genes and highly enriched pathways such as DNA repair and oxidative stress [Figure 2a-c] were in correlation with the gene-gene interaction results from Cytoscape (v 3.10.2) [Figure 3a] and STRING databases [Figure 3b]. *p53* plays a main role in regulating other genes and is a major contributor for the occurrence of RIAR via seven major pathways [Figure 3b].

miRNAs serve as prognostic biomarkers for radio-resistance as reported in the literature [18]. miRNAs in this study can more effectively serve as potential targets for targeting genes involved in RIAR-associated mechanisms to enhance the effectiveness of RT. They are vital in controlling gene expression to enable the cells to respond to stress and cellular damage induced by radiation. Thus, miRNAs make cells adapt to radiation exposure, thereby promoting cell survival and maintaining genomic integrity; these correspond to the radio-adaptive response phenomenon. These can target genes in the homologous recombination and non-homologous end-joining pathway, thereby repairing the radiation-induced damages efficiently. miRNAs regulate genes involved in the oxidative stress response pathways, such as superoxide dismutase (*SOD*) and nuclear factor erythroid 2-related factor 2 such that cells have increased production of reactive oxygen species during radiation exposure [19].

Enrichr is a web-based enrichment analysis tool, which is simple to use and intuitive, providing an assortment of visualization summaries of the collective purposes of gene lists. Another noteworthy feature of the online analysis tool Enrichr is its comprehensiveness, ease of use, and interactive visualization of results. Enrichr tool predicts disease-specific signatures or transcriptional signatures. The tools used in this study identify disease-specific signatures via a comparison of gene expression patterns between the normal and diseased tissues [20]. Studies have reported the alterations in genes in various other cancers, i.e., about 381 genes have been associated with RT response in rectal cancers [21]. Genes such as *SELP, PIM2, CCL19, SDS, NRP1,* and *SF3A2* were associated with RT sensitivity in cervical cancers [22].

miRNAs are long non-coding RNAs that target genes and have regulatory functions. miRNA microarray analysis in AG01522 normal human fibroblasts upon exposure to 5 cGy followed by 2 Gy has revealed the trigger of miRNAs upon low-dose-induced RIAR. Earlier *in silico* and *in vitro* studies have analyzed miRNAs as potential targets in atherosclerosis [23]. Two separate miRNA signatures comprising 4 up-regulated and 5 down-regulated miRNAs in breast tumors were identified from 1165 breast cancer patients retrieved from the Cancer Genome Atlas (TCGA-BRCA) [24]. miRNAs have oncogenic or tumour-suppressive functions and hence remain dysregulated in several cancers [25]. Alterations in the circulating miRNAs in blood plasma or serum can reflect the patient's response to RT.

The miTarBase and miRNet enhance data usability and accessibility; thus, these are regarded as authentic databases for miRNA information. The enrichMiR tool seems to be highly advanced for interpretation and visualization of enrichment results due to its routine updates, also it uses the input data via integration with high-confidence and authentic databases like ScanMiR, TargetScan, miRTarBase & oRNAment. The enrichMiR tool provides results based on various choices such as overlap features of miRNA, and it also performs a comprehensive prediction based on miRNAs functionally relevant to target genes apart from statistically significant miRNAs [26]. The role of miRNAs associated with those 69 genes and breast cancer are discussed in Table 1. A network of miRNAs, namely miR-21, miR-155, and miR-205, has been identified as potential biomarkers for monitoring the radiation response in cancer patients [27-29]. miR-124 contributes to RIAR by either enhancing or suppressing inflammation and immune responses [27]. miR-17 downregulates *p21* expression, leading to radio-resistance of cancer cells, thereby facilitating cell survival and proliferation [30]. miR-335 is involved in the DNA damage response pathway [31]. Overexpression of miR-106a leads to increased cell proliferation, conferring radio-resistance, and miR-128 can either enhance or suppress DNA damage response genes, leading to RIAR [32]. miR-185 enhances radiation-induced apoptosis and inhibits cell proliferation via targeting *ATM* and *ATR* [33]. miR-15a regulates the *SMPD1,* which is involved in apoptosis and inflammation upon radiation response [34]. miR-20a-5p targets *Rab27b* involved in apoptosis, altering radio-resistance [35]. miR-218 targets stress response pathways involved in radiation-induced stress [36]. miR-27a targets genes involved in DNA repair and cell survival [37]. miR-34a downregulates factors involved in cancer stem cell maintenance and thereby reduces cell survival, proliferation, and alters RIAR [38]. miR-93 serves to be a promising therapeutic target for enhancing RT outcomes in cancer treatment by increasing tumor radiosensitivity [39]. hsa-let-7a-5p regulates the balance between DNA repair-mediated radio-resistance and apoptosis-mediated radiosensitivity following radiation exposure [40]. miR-145 contributes to enhanced tumor radio-resistance; hence, targeting this can be more beneficial towards RT outcomes. miR-181a targets *ATM* and *RAD52*; hence, altering miR-181a can enhance DNA damage thereby reducing radio-resistance [41]. miR-24 enhances radiosensitivity by enhancing apoptosis and inhibiting cell proliferation [19]. miR-9-5p targets *Suppressor of Cytokine Signaling 5,* thereby exploring miR-9-5p can alter angiogenesis and cell survival, thereby altering RIAR [42]. miR-92a has been found to be involved in oxidative stress influencing RIAR by producing reactive oxygen species [43]. Hence, we found that about 19 miRNAs were found to be enriched in our study; these were majorly involved in the regulation of pathways such as oxidative stress, inflammation, DNA damage response, DNA repair, apoptosis, cell proliferation, and cell survival. Therefore, these 19 miRNAs can serve as predictive therapeutic targets to be explored more via further molecular approaches for the occurrence of RIAR in breast cancer. Exploring and monitoring the miRNAs in this study, i.e., the miRNAs associated with the RIAR phenomenon and those important in breast cancer may help in the dynamic assessment of RT efficacy in breast cancer patients. This can maximize the therapeutic efficacy in spite of the non-targeted effects of radiation.

miRNAs that regulate genes upon radiation exposure (also known as radio-miRs) act as gatekeepers in regulating the cellular mechanisms and pathways that lead to radio-resistance or RIAR [44]. This study has witnessed the involvement of about 19 miRNAs that modify several radio-adaptive response pathways notably, DNA repair, DNA damage response, cell survival, proliferation, and apoptosis via targeting RIAR genes and carcinoma of the breast. The tools used in this study remain advantageous due to the advancement in software/databases. A deeper understanding of these mechanisms and targeting miRNAs via molecules involved in these mechanisms can lead to more precise combination treatments. This is an *in silico* study based on the genes and miRNA targets available from databases. However,

the targets mentioned in this study have to be evaluated using *in vitro* studies, either in blood samples of cancer patients or in cancer cell lines for analyzing and confirmation of targets being involved in RIAR during RT. The targets can be validated in breast cancer cell lines in *in vitro* by methods such as qRT-PCR, northern blotting, microarray analysis, next-generation sequencing, luciferase reporter assay, in situ hybridization, and specifically, western blot and flow cytometry for analysing the expression of proteins targeted by miRNAs. Combining these methods will give an understanding of miRNA roles in RIAR during RT, or therapy resistance. Evaluating *in vitro* will further confirm the effectiveness of the identified targets. Hence, this study serves as a preliminary basis for more futuristic lab studies for improving therapeutic efficacy.

5. CONCLUSION

The molecules and their expression patterns can be altered by the radiation treatment approaches (conventional or fractionated clinical dose regimes). Therefore, targeting these molecules may decrease tumor resistance and improve patient outcomes in RT. A better knowledge of these mechanisms via these *in silico* approaches will improve our understanding and alter the tumor prognosis due to RIAR alterations in breast cancer therapy. Although this study has found the possible miRNA targets of genes involved in RIAR during breast cancer RT via *in silico* approaches, more extensive validation via *in vitro approaches* on the found miRNA targets is required for them to be proven for their roles in RIAR. Hence, this was the limitation of this study. The dysregulation of miRNAs targeting these RIAR genes in breast cancer can be regulated by altering the radiation doses during RT, which will further help in regulating or normalizing the gene expression patterns of RIAR genes that may clinically correlate for the betterment of cancer cure. Analysing these enriched miRNAs resulting from this study may enhance the possibilities of exploring novel RT strategies in breast cancers for the advancement of therapeutic efficacy in the future.

6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

7. FUNDING

The authors acknowledge the fellowship (Reg. No: U02B210734) support to Aishwarya Thathamangalam Ananthanarayanan, from Founder Chancellor Shri N.P.V Ramaswamy Udayar Research Fellowship, Sri Ramachandra Institute of Higher Education and Research (Deemed to be University), Porur, Chennai, Tamil Nadu, India.

8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

The datasets of retrieved genes and miRNAs of Homo sapiens presented in this study can be found in online repositories.

11. PUBLISHER'S NOTE

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12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that we have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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How to cite this article:
Ananthanarayanan AT, Narayanan R, Kondaveeti SS, Ramachandran I, Gnanasekaran TS, Madhavan T, Perumal V. *In silico* analyses to identify the potential genes and microRNA targets in pathways mediating radiation-induced adaptive response in breast cancer. J Appl Biol Biotech 2025;13(6):80-87. DOI: 10.7324/JABB.2025.v13.i6.9