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Research Article

# Gene Expression Profiling in Breast Cancer: Responses to Radiation Therapy

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## Abstract

**Objectives:** This study aims to investigate the gene expression in breast cancer (BC) in response to radiation therapy using GEO2R analysis of datasets from the Gene Expression Omnibus database.

**Methods:** The data used in the research was obtained from the GEO of the GSE 59732 and GSE 59734 gene expression datasets. These datasets included various breast cancer treated with radiation therapy. DEGs (Differentially expressed genes) were identified using GEO2R of p-value less than 0.05 and a log FC threshold of 1.2. STRING was used to generate PPI (protein-protein interaction) networks, while Cytoscape software was used to identify important hub genes. The Enrichr tool was used to analyze Gene Ontology and KEGG pathway and network analyst tool was used to study gene-disease association.

**Results:** A total of 61 upregulated DEGs common in both datasets were observed in the study. Gene ontology analysis revealed that biological processes mostly enriched in regulation of peptidyl-tyrosin phosphorylation, cellular components mainly enriched in phosphatase binding. Proteoglycans in cancer found in both KEGG pathway and gene-disease association pathways.

**Conclusion:** The study identified DEGs in breast cancer in response to radiation therapy. Key hub genes, particularly EGFR and MAPK3, were linked to many cancer-related pathways including breast cancer.

**Keywords:** Breast Cancer, DEGs, gene ontology, hub genes, radiation therapy, gene expression omnibus

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Cancer in breast is the most common malignant tumor in females altogether, accounting for around 36% of all cancer incidences.[1] Breast cancer is a disease with diverse etiology and clinical diversity.[2] Various factors, including genetics, environmental influences, and hormonal status, have a substantial impact on the risk of breast cancer. While female sex and advancing age are the most common risk factors, about 10% of cases of breast cancer are caused by genetic mutations, specifically those related to BRCA1 and BRCA2.[1, 3] Invasive ductal carcinoma, which affects 50–75% of patients, is the most common histology for breast cancer. It is followed by invasive lobular carcinoma, and the remaining histologies comprise rarer ones.[4] Based on the expression of the ER, PR, and HER2, four

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major subtypes of invasive breast cancer can be distinguished using immunohistological techniques.[5] A combination of surgery, radiotherapy, chemotherapy, targeted therapies, and endocrine therapies are commonly used in the treatment plan, which is usually based on the findings of the diagnostic process.[6,7] After breast cancer is surgically removed, radiation therapy is used to get rid of any microscopic disease that may still be present.[8,6] Radiation treatment is certainly helpful in oncology, as evidenced by its ability to reduce the risk of local tumor relapse and raise total survival rates for a variety of cancer types.[9] Although there has been substantial progress in our understanding of how radiation therapy (RT) affects breast cancer, there are still many unanswered questions, especially about gene expression. Different cells with unique genetic and epigenetic profiles make up breast tumors. Different breast cancer subtypes respond differently to radiation therapy in terms of gene expression, which has an impact on several types of biological pathways and clinical outcomes. It is still unclear how radiation affects various tumor subpopulations and the gene expression patterns associated with them. The molecular effects of radiation therapy can be better understood by combining the expression of gene data with other omics data, such as proteomics and metabolomics. Treatments can be customized for each patient according to their cancer subtype by identifying a particular change in gene expression that occurs in response to radiation therapy. Additionally, the prediction of treatment outcomes can be enhanced by identifying biomarkers linked to RT response. To fill these knowledge gaps and improve the understanding of how radiation therapy affects gene expression, collaborative efforts combining computational analysis and advanced molecular biology techniques are needed.

## Methods

### Data Sources

Gene Expression Omnibus, or GEO, is a national genetic data repository that includes information from next-generation sequencing and microarray analysis. Using GEO2R, differentially expressed genes (DEGs) can be found by comparing two or more GEO datasets.[10] The GEO platform (httpwww.ncbi.nlm.nih.govgeo) provided the research data used in this study.

### Dataset Screening

The following measures were used to select GEO datasets studies involving subtypes of BC, descriptions of the technology and platforms utilized, expression profiling by array, and analysis with GEO2R Analyzer. In this study, two gene expression profiles (GSE 59732 and GSE 59734) were retrieved from the GEO database. GSE 59734, which uses platform GPL571, and GSE 59732, which includes 96 samples of breast cancer cell lines treated with radiation therapy, were selected (Fig. 1).

### Differential Expression Gene Extraction

In both datasets, samples were defined as luminal HER2 positive, luminal HER2 negative, basal, and basal HER2 positive. With a value p less than 0.05 and a log FC threshold of 1.2, DEGs were found. For GSE 59732, 4955 expressed genes were observed, and for GSE 59734, 7196 expressed genes were observed (Fig. 2). A Venn diagram was made using the tool FunRich. Data can be presented graphically using the FunRich tool (httpwww.funrich.org) in the form of Venn diagrams, pie charts, bar graphs, columns, heatmaps, and doughnuts. Each data can have its font, scale, and color modified.[11]

### PPI Network Visualization

Using software STRING (httpwww.string-db.org), a protein-protein interaction (PPI) network—which is defined by dynamic and complicated interactions between two or more proteins—was built for the identified DEGs.[12] DEGs were uploaded with the organism named Homo sapiens to model various proteins. Disconnected nodes were eliminated based on an interaction score of more than 0.04 which was used to estimate the statistical relevance of the network interaction connection. The gene interaction network was then generated using the Cytoscape tool. The hub genes in the protein-protein interaction were identified using the cytoHubba plugin, and these hub genes were subsequently selected for their potential DEGs.[13]

### Gene Ontology and KEGG Pathway Analysis

The Enrichr tool (httpmaayanlab.cloudEnrichr) was used for the analysis and identification of KEGG paths (httpwww.genome.jp) and there are three categories according to the Gene Ontology terms Biological Process, Cellular Component, and Molecular Function. Using substantial molecular datasets generated by high-throughput experimental methodologies, KEGG pathway analysis contributes to the understanding of signaling pathways and provides information on the interactions and co-regulation of several proteins involved in metabolic and cellular functions.[14]

### Construction of a Regulatory Network of Hub Genes

Gene regulation, gene co-expression, drug-gene interaction networks, and other general networks can all be investigated using the visual analytics platform Network Analyst tool (httpnetworkanalyst.ca).[15] In this study, we used network analyst to analyze the gene-disease association of hub genes.

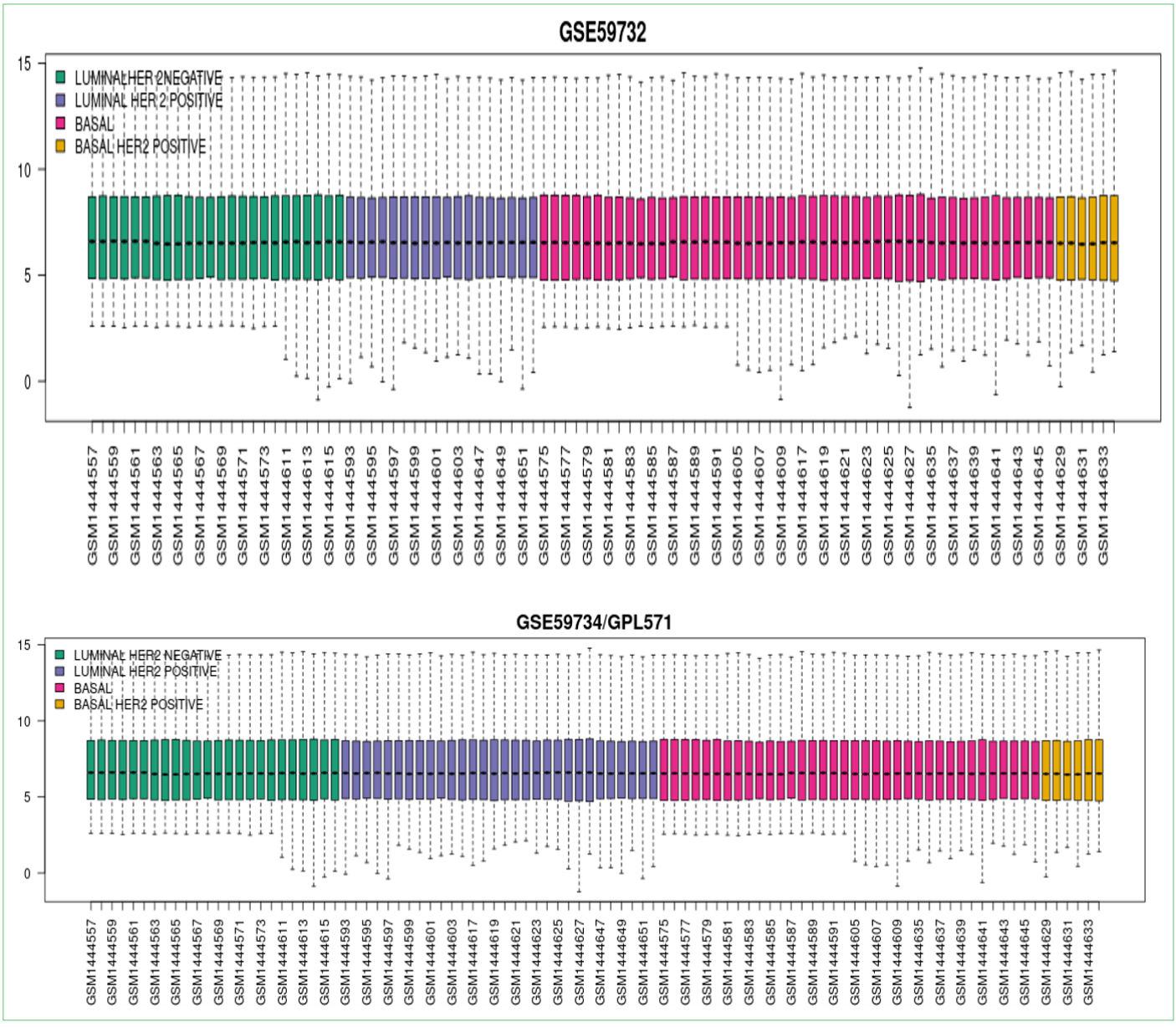


**Figure 1.** Distribution of gene expression values for the GSE 59732 and GSE 59734 datasets. The gene expression value of a single patient sample is shown by each box plot. The y axis does not have any units.

## Results

Using the GEO2R tool, two gene expression profiles—GSE 59732 and GSE 59734—were studied. The list of upregulated genes was downloaded, the p-value was 0.05, and the cutoff limit was a log FC value of 1.2. Using the Funrich tool, genes that fulfilled the cutoff standards were selected for Venn diagram analysis (Fig. 3). In the two mentioned datasets, 61 genes were shown to have significantly differing expression levels (Table 1).

Upregulated DEGs from both datasets were uploaded to the online String tool. The lowest needed interaction score option was chosen with the medium confidence option selected, and the disconnected genes were hidden. The network consists of 788 edges and 311 nodes in total (Fig. 4). After the submission of the list of upregulated expressed genes, Enrichr's ontology option presented several options, including GO-CC, GO-MF, and GO-BP. Gene Ontology study showed that the upregulated DEGs were enriched in GO-BP such as regulation of peptidyl-tyrosine phosphorylation, positive regulation of cell migration, response to EGF (epidermal growth factor), endodermal cell differentiation, positive regulation of protein phosphorylation, endoderm formation, positive regulation of cell motility, regulation of protein ERK1 and ERK 2 cascade, regulation of protein

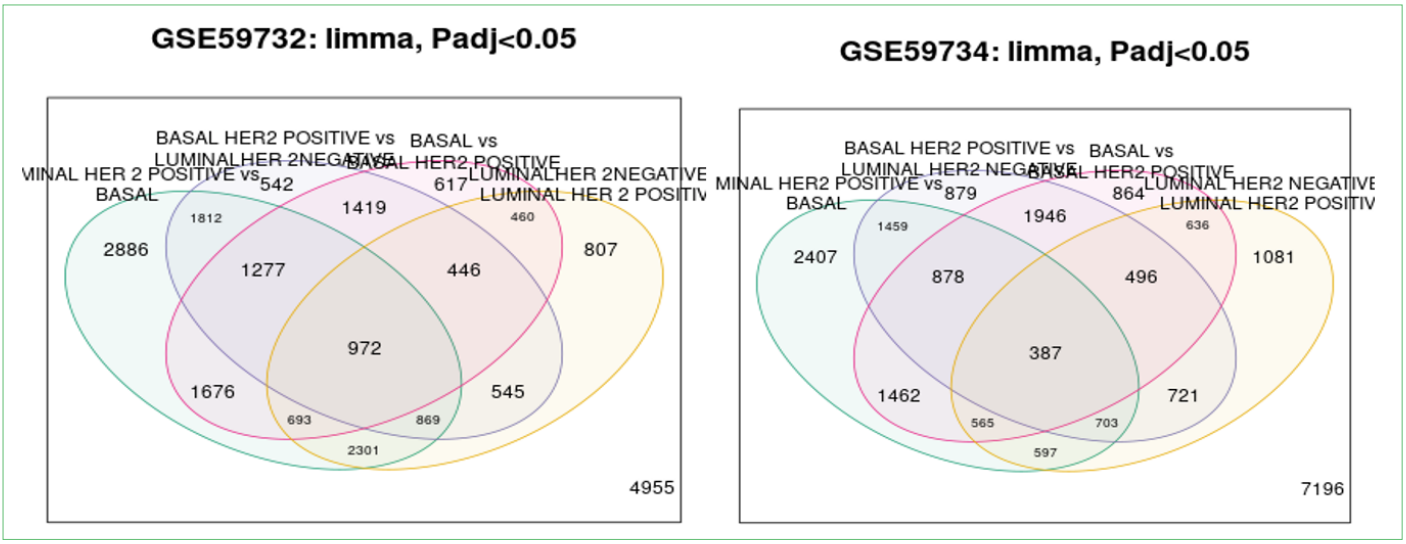


**Figure 2.** Gene expression in GSE59732 and GSE59734.

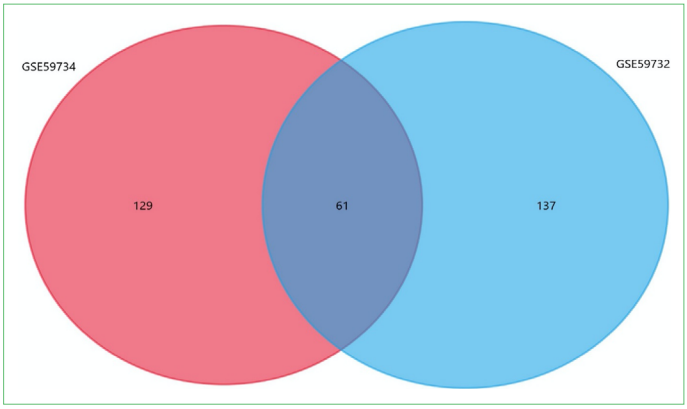


**Figure 3.** Venn diagram showing 61 genes in the GSE 59732 and GSE 59734 datasets that were expressed differentially.

**Table 1.** Dataset information of GSE 59732 and GSE 59734 Microarray datasets obtained from GEO

| Datasets | Luminal HER2 negative | Luminal HER2 positive | Basal | Basal HER2 positive |
|---|---|---|---|---|
| GSE 59732 | 24 | 18 | 48 | 6 |
| GSE 59734 | 24 | 30 | 36 | 6 |

phosphorylation and peptidyl-tyrosine autophosphorylation (Fig. 5A). For Gene Ontology Cellular Component analysis, the upregulated DEGs were mainly enriched in focal adhesion, cell-substrate junction, platelet alpha granules, intracellular organelle lumen, endoplasmic reticulum lumen, platelet alpha granule lumen, intermediate filament cytoskeleton, cytoplasmic vesicle, and cytoskeleton (Fig. 5B). Gene Ontology Molecular Function study showed that upregulated DEGs were enriched in phosphatase binding, protease binding, keratin filament binding, vascular endothelial growth factor 2 binding, MAP kinase activity, vascular endothelial growth factor receptor binding, platelet-derived growth factor receptor binding, metalloendopeptidase inhibitor activity, hyaluronic acid binding, and metalloaminopeptidase activity (Fig. 5C). The KEGG pathway study revealed that DEGs are enriched in pathways that are related to proteoglycans in cancer, microRNAs in cancer, focal adhesion, regulation of actin cytoskeleton, shigellosis, adhere junction, ECM-receptor interaction, GnRH signaling pathway, human papillomavirus infection, parathyroid hormone synthesis, secretion and action (Fig. 5D). To find out the hub genes, genes selected by the top 10 nodes ranked by degree and displayed the shortest path were selected by using the offline tool Cytoscape (Fig. 6). In the cytohubba plug-in, it was found that the epidermal growth factor receptor gene (EGFR, degree=134) has the maximum connectivity with other genes followed by fibronectin 1 (FN1, degree= 110), CD44 (degree= 88), snail family transcriptional repressor 2 (SNAI 2, degree= 64), mitogen-activated protein kinase 3 (MAPK3, degree= 64), matrix metalloproteinase 14 (MMP14, degree= 58), TIMP metallopeptidase inhibitor 1 (TIMP1, degree= 56), integrin subunit alpha 5 (ITGA5, degree= 56), vimentin (VIM, degree= 56), platelet and endothelial cell adhesion molecule 1 (PECAM1, degree=52). The network following hub gene enrichment is seen in Figure 7. P value in statistics suggests that certain factors are significant. Strongly linked results with the intended disease are indicated by a P value of less than 0.05. Table 3 of the Network Enrichment (www.NetworkAnaylst.ca) lists cancer diseases amongst the several common diseases that are listed and studied. The FDR, P value, expected values, and number of hits are shown. The

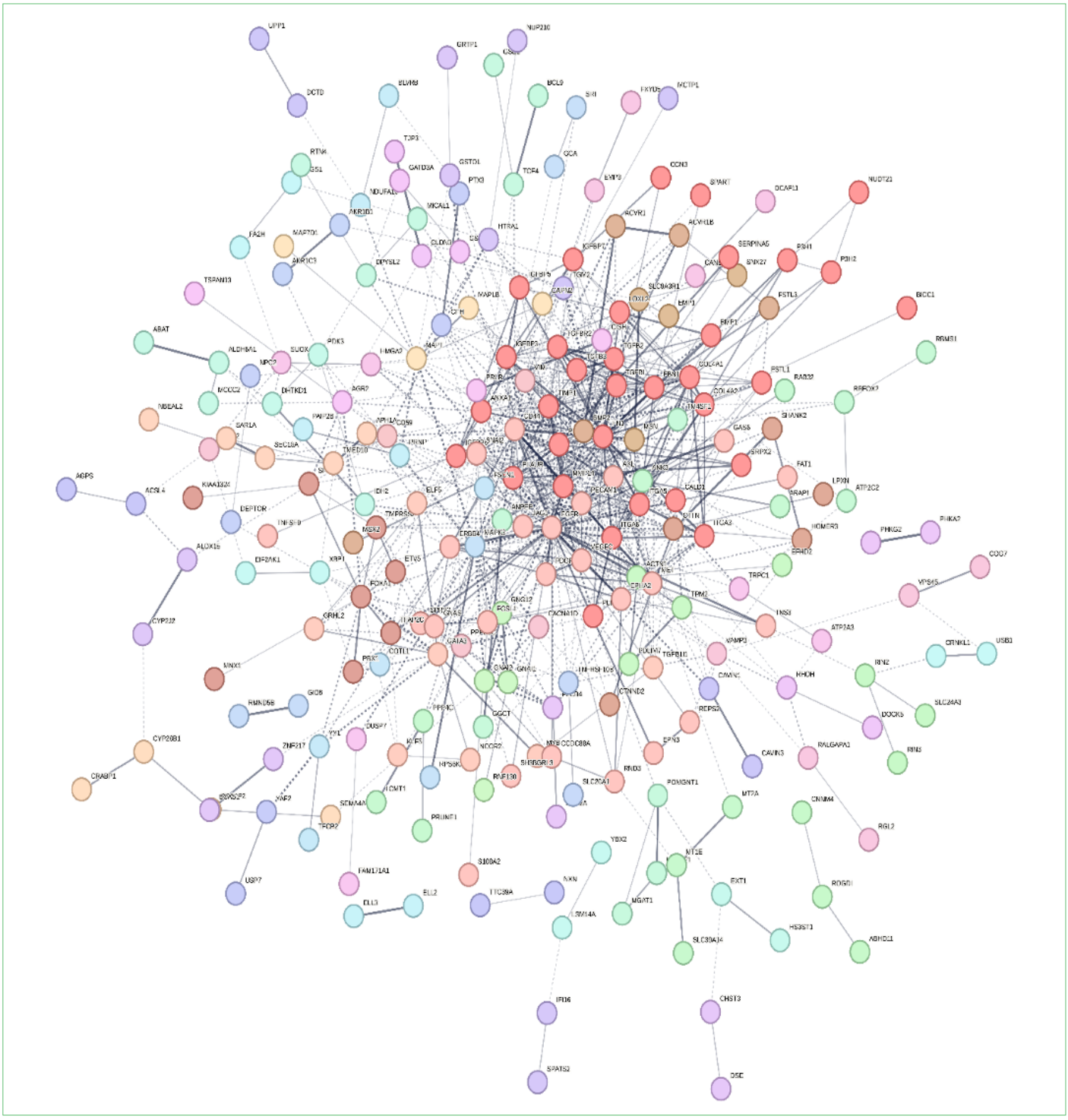

**Figure 4.** STRING protein protein interaction network.788 edges and 311 nodes in this network. Genes are symbolised by circles, while interaction is symbolised by lines.

fact that almost every single one of the FDR values is very small and all of the P values are less than 0.05 suggests that hub genes are also associated with the disease that has been observed. EGFR and MAPK3 are two genes that are expressed in most of the cancers listed in Table 3 including breast cancer.

## Discussion

Radiation therapy (RT) is a significant treatment for many cancer types, including breast cancer (BC). It can be used alone or in combination with other therapies.[16] Following a mastectomy, radiotherapy treatment is currently provid-

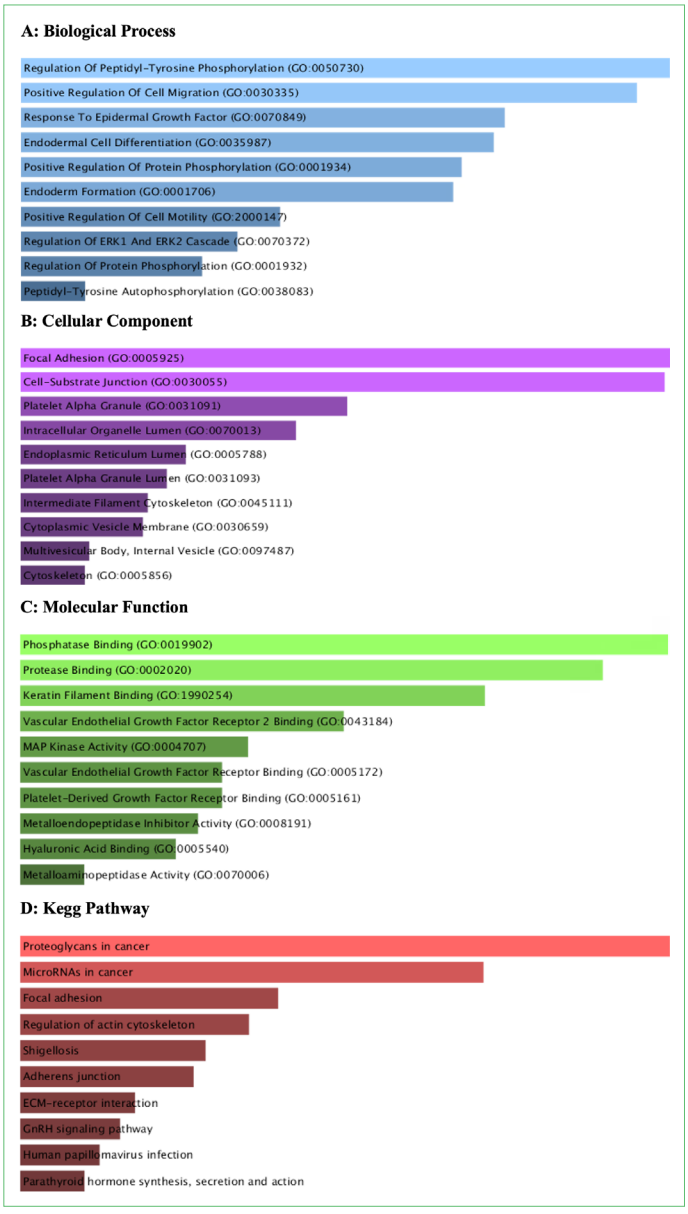


**Figure 5.** Enrichr tool is used for the study of GO and KEGG pathway enrichment. **(a)** Top 10 biological processes that are enriched in upregulated DEGs. The number of genes is shown on the x axis, while the biological process is shown on the y axis. **(b)** Cellular function in upregulated DEGs was enriched at the top. Gene number is shown by the x axis, while cellular function is shown by the y axis. **(c)** Top eniched molecular function in DEGs that are upregulated. Gene number is shown by the x axis, while molecular function is shown by the y axis. **(d)** Top enriched KEGG pathways for DEGs that are upregulated. Gene number is shown by the x axis, while KEGG pathway names are shown by the y axis.

ed based on the patient's clinicopathologic criteria, which determine their risk of local or regional recurrence (LRR), rather than their likelihood of benefiting from the therapy.[17] This study investigated the upregulated gene expression by using bioinformatics tools in two datasets (GSE 59732 and GSE 59734) having microarray data of gene expression in breast cancer cell lines which is treated with radiation therapy. Genes that were found to be differentially expressed (DEGs) had a log FC value of 1.2 and a p-value less than 0.05. Venn diagram revealed 61 genes that were significantly upregulated DEGs in both datasets (Table 2). The upregulated expressed genes were subjected to GO and KEGG pathway enrichment analysis by the Enrichr tool. The GO analysis indicated significant enrichment in biological processes such as regulation of peptidyl-tyrosine phosphorylation, favorable regulation of cell migration, and response to epidermal growth factor. These pathways are essential in many physiological and pathological situations, such as the proliferation and metastasis of cancer. In terms of cellular components, the differentially expressed genes were enriched in focal adhesion, cell-substrate junction, and platelet alpha granules. This suggests that these genes play vital roles in cell adhesion, signaling, and intracellular trafficking, which are crucial in maintaining cellular integrity and function. The molecular function analysis highlighted significant enrichment in activities such as phosphatase binding, protease binding, and keratin filament binding. These functions are essential for various signaling pathways and structural components within the cell. KEGG pathway analysis identified several pathways associated with the upregulated DEGs. Notably, pathways involved in proteoglycans in cancer, microRNAs in cancer, focal adhesion, and regulation of the actin cytoskeleton were significantly enriched. These pathways are known to be critical in cancer growth and progression, emphasizing the potential role of these DEGs in cancer.

Using Cytoscape and the cytoHubba plugin, the top 10 hub genes were identified on their degree of connectivity. EGFR (Epidermal growth factor receptor) emerged as the gene with the highest connectivity, followed by fibronectin 1 (FN1), CD44, SNAI2, MAPK3, MMP14, TIMP1, ITGA5, VIM, and PECAM1. These hub genes play an important role in many cellular processes, particularly those that are connected to cancer. Numerous intracellular signals that control cell growth, proliferation, survival, migration, and differentiation are triggered by the EGFR.[18] The EGFR is widely expressed in many cancer types and has a major part in treatment resistance, metastasis, and proliferation, among other key aspects of cancer development. The EGFR pathway can become dysregulated due to overexpression or persistent activation, which is linked to a poor prognosis in many human malignancies and can encourage tumor activities including angiogenesis and metastasis.[19,20] Numerous cell types express the fibronectin (FN) family, which is involved in blood coagulation, wound healing, host defense, cell prolifera-

**Table 2.** List of 61 upregulated DEGs common in both the dataset GSE 59732 and GSE 59734

| Serial No | Gene Symbol | Gene Title |
|---|---|---|
| 1 | GSTP1 | glutathione S-transferase pi 1 |
| 2 | AKR1B1 | aldo-keto reductase family 1 member B |
| 3 | TTC39A | tetratricopeptide repeat domain 39A |
| 4 | MSN | moesin |
| 5 | CLDN3 | claudin 3 |
| 6 | IFI16 | interferon gamma inducible protein 16 |
| 7 | LOC101928916///NNMT | uncharacterized LOC101928916///nicotinamide N-methyltransferase |
| 8 | IGFBP5 | insulin like growth factor binding protein 5 |
| 9 | GSE1 | Gse1 coiled-coil protein |
| 10 | COL4A2 | collagen type IV alpha 2 chain |
| 11 | COTL1 | coactosin like F-actin binding protein 1 |
| 12 | PRKCDBP | protein kinase C delta binding protein |
| 13 | EMP3 | epithelial membrane protein 3 |
| 14 | DFNA5 | DFNA5, deafness associated tumor suppressor |
| 15 | FOXA1 | forkhead box A1 |
| 16 | XBP1 | X-box binding protein 1 |
| 17 | TGFB1I1 | transforming growth factor beta 1 induced transcript 1 |
| 18 | EXT1 | exostosin glycosyltransferase 1 |
| 19 | GLIPR1 | GLI pathogenesis related 1 |
| 20 | S100A2 | S100 calcium binding protein A2 |
| 21 | SNAI2 | snail family transcriptional repressor 2 |
| 22 | SLC24A3 | solute carrier family 24 member 3 |
| 23 | KIAA1324 | KIAA1324 |
| 24 | LARGE1 | LARGE xylosyl- and glucuronyltransferase 1 |
| 25 | HTRA1 | HtrA serine peptidase 1 |
| 26 | ITGA6 | integrin subunit alpha 6 |
| 27 | ANXA9 | annexin A9 |
| 28 | SRPX | sushi repeat containing protein, X-linked 2 |
| 29 | POPDC3 | popeye domain containing 3 |
| 30 | HMGA2 | high mobility group AT-hook 2 |
| 31 | TGM2 | transglutaminase 2 |
| 32 | ITGA3 | integrin subunit alpha 3 |
| 33 | JAG1 | jagged 1 |
| 34 | CHST3 | carbohydrate sulfotransferase 3 |
| 35 | RMND5B | required for meiotic nuclear division 5 homolog B |
| 36 | SHOX2 | short stature homeobox 2 |
| 37 | SLC9A3R1 | SLC9A3 regulator 1 |
| 38 | PRNP | prion protein |
| 39 | SLC39A14 | solute carrier family 39 member 14 |
| 40 | CTTN | cortactin |
| 41 | ALDH6A1 | aldehyde dehydrogenase 6 family member A1 |
| 42 | NDUFA10 | NADH:ubiquinone oxidoreductase subunit A10 |
| 43 | IDH2 | isocitrate dehydrogenase (NADP(+)) 2, mitochondrial |
| 44 | BLVRB | biliverdin reductase B |
| 45 | UPP1 | uridine phosphorylase 1 |
| 46 | MT1E | metallothionein 1E |
| 47 | PLAU | plasminogen activator, urokinase |
| 48 | RGL2 | ral guanine nucleotide dissociation stimulator like 2 |
| 49 | EMP1 | epithelial membrane protein 1 |
| 50 | CNNM4 | cyclin and CBS domain divalent metal cation transport mediator 4 |
| 51 | GRHL2 | grainyhead like transcription factor 2 |
| 52 | BMP1 | bone morphogenetic protein 1 |
| 53 | MAP7D1 | MAP7 domain containing 1 |
| 54 | VPS45 | vacuolar protein sorting 45 homolog |
| 55 | TNFRSF10B | TNF receptor superfamily member 10b |
| 56 | GNAS | GNAS complex locus |
| 57 | ACVR1B | activin A receptor type 1B |
| 58 | GAS6 | growth arrest specific 6 |
| 59 | LDHB | lactate dehydrogenase B |
| 60 | GRTP1 | growth hormone regulated TBC protein 1 |
| 61 | EMP2 | epithelial membrane protein 2 |

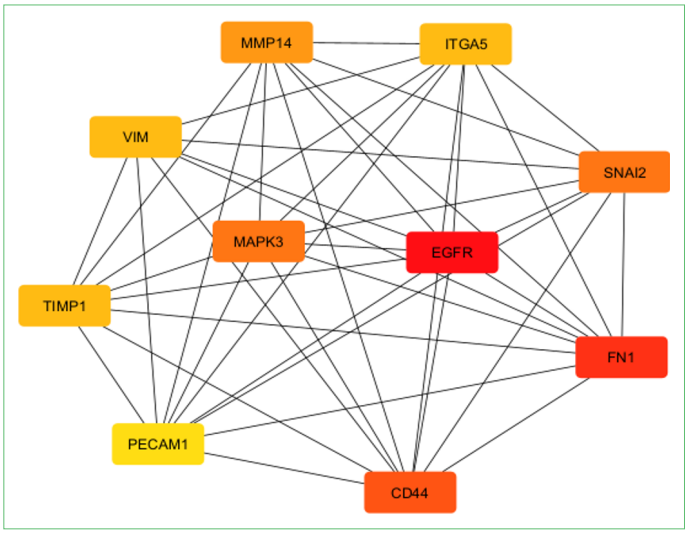


**Figure 6.** Top ten hub gene networks using the Cytoscape tool. The color indicates the degree of connection; the lowest degree is represented by yellow, the intermediate degree by orange, and the highest degree by red.

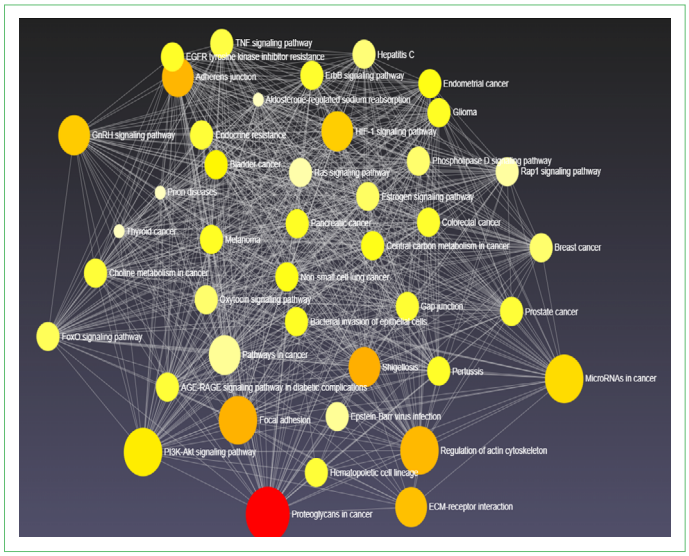


**Figure 7.** Network with ten hub gene enrichment – EGFR, FN1, CD44, SNAI2, MAPK3, MMP14, TIMP1, ITGA5, VIM, PECAM1.

**Table 3.** For ten hub genes, network enriched values and associated cancer disease

| Pathway | Total | Expected | Hits | p | FDR | Genes |
|---|---|---|---|---|---|---|
| Proteoglycans in cancer | 201 | 0.26 | 5 | 2.55E-06 | 0.000812 | EGFR, FN1, CD44, MAPK3, ITGA5 |
| MicroRNAs in cancer | 299 | 0.386 | 4 | 0.000382 | 0.0135 | EGFR, CD44, ITGA5, VIM |
| Bladder cancer | 41 | 0.053 | 2 | 0.0012 | 0.0347 | EGFR, MAPK3 |
| Endometrial cancer | 58 | 0.075 | 2 | 0.00239 | 0.0634 | EGFR, MAPK3 |
| Central carbon metabolism in cancer | 65 | 0.084 | 2 | 0.00299 | 0.0682 | EGFR, MAPK3 |
| Non-small cell lung cancer | 66 | 0.0853 | 2 | 0.00309 | 0.0682 | EGFR, MAPK3 |
| Melanoma | 72 | 0.0931 | 2 | 0.00366 | 0.0682 | EGFR, MAPK3 |
| Pancreatic cancer | 75 | 0.0969 | 2 | 0.00397 | 0.0682 | EGFR, MAPK3 |
| Glioma | 75 | 0.0969 | 2 | 0.00397 | 0.0682 | EGFR, MAPK3 |
| Colorectal cancer | 86 | 0.111 | 2 | 0.00519 | 0.075 | EGFR, MAPK3 |
| Prostate cancer | 97 | 0.125 | 2 | 0.00656 | 0.079 | EGFR, MAPK3 |
| Choline metabolism in cancer | 99 | 0.128 | 2 | 0.00682 | 0.079 | EGFR, MAPK3 |
| Breast cancer | 147 | 0.19 | 2 | 0.0146 | 0.143 | EGFR, MAPK3 |
| Pathways in cancer | 530 | 0.685 | 3 | 0.0267 | 0.229 | EGFR, FN1, MAPK3 |
| Thyroid cancer | 37 | 0.0478 | 1 | 0.0468 | 0.355 | MAPK3 |
| Acute myeloid leukemia | 66 | 0.0853 | 1 | 0.0821 | 0.511 | MAPK3 |
| Renal cell carcinoma | 69 | 0.0892 | 1 | 0.0857 | 0.511 | MAPK3 |
| Chronic myeloid leukemia | 76 | 0.0982 | 1 | 0.0941 | 0.516 | MAPK3 |
| Chronic myeloid leukemia | 76 | 0.0982 | 1 | 0.0941 | 0.516 | MAPK3 |
| Viral carcinogenesis | 201 | 0.26 | 1 | 0.232 | 0.652 | MAPK3 |

tion, and adhesion and migration. It was found that FN1 levels were higher in BRCA, or breast, tissues than in normal tissues.[21] CD44 plays an in the regulation of several signaling pathways, including those involving protein kinases, cytoskeletal alterations, intracellular pathways, proteinases, and transcription factors. Angiogenesis, invasion, division, proliferation, and changes in metabolism in cancer cells are all enhanced by these pathways.[22] The behaviour of cancer stem cells (CSCs) may be impacted by SNAI2's role in normal tissues, where it enhances stem cell function. In CD44+CD24– CSCs from breast tumors, SNAI 2 is highly expressed. Overexpression of SNAI2 has been shown to generate a highly regenerative population of breast cancer cells capable of initiating tumor

spheres.[23] Cell survival and proliferation are facilitated by MAPK3's activation of many nuclear transcription factors, including c-Jun and c-fos, through the phosphorylation of downstream cytoplasmic proteins. The overexpression of MAPK3 has been related to the development, spread, metastasis, and therapeutic resistance of certain carcinomas.[24] The membrane-type MMP matrix metalloproteinase 14 is involved in both the prognosis and development of tumors. According to earlier studies, MMP 14 is significantly expressed in several cancer types and, via changing the extracellular matrix, facilitates tumor invasion and metastasis. But recently, there haven't been many findings on the roles of MMP 14 and BCSC 1 in human breast cancer.[25] TIMP-1, or tissue inhibitor of metalloproteinases-1, has been identified as a prognostic sign for BC. After being shown to be a growth factor, TIMP-1 was later demonstrated to inhibit matrix metalloproteinases (MMPs).[26] It has been documented that ITDGA5 increases drug resistance, metastasis, and tumor progression.[27] Vimentin (VIM) is a type III intermediate filament that functions as a mesenchymal marker and is found in numerous tissues during development. It helps to maintain the integrity of cells and tissues. Its expression is thought to be a hallmark of the epithelial-to-mesenchymal transition (EMT), which denotes advanced tumor dedifferentiation and a strong likelihood of tumor invasion in malignancies, including breast cancer.[28] PECAM-1 mediates several essential biological processes, such as platelet aggregation, T cell activation, leukocyte emigration at inflammatory sites, vascular development, and maintenance of the vascular endothelial barrier function. Additionally crucial to the angiogenesis of human tumors is PECAM-1.[29]

The Network Analyst tool was used to conduct network enrichment analysis, which identified several diseases connected to the hub genes, with cancer being a prominent finding. The analysis indicated significant associations between the hub genes and various cancer types, including breast, endometrial, bladder, and non-small cell lung, among others. EGFR and MAPK3 were notably associated with most of the cancer types listed, highlighting their essential roles in cancer biology.

Identifying hub genes in breast cancer cell lines treated with radiation therapy provides insights into the cellular mechanisms involved in the response to radiation. The hub genes identified in response to radiation therapy can serve as biomarkers to predict radiosensitivity. Understanding how radiation interacts with hub genes can help develop combination drugs that target several pathways at once, decreasing the risk of resistance and improving treatment results.

## Limitations

Incomplete or biased datasets can lead to incorrect identification of hub genes, significantly impacting research and therapeutic development. Furthermore, studies on cell lines may fail to capture the complexity of tumors in patients, potentially yielding inaccurate results. The use of bioinformatics tools can also result in false positives or false negatives.

### Disclosures

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**Ethics Committee Approval:** Ethical approval was not required for this study as it involved bioinformatics analysis. No wet lab experimental procedures involving human or animal subjects were conducted. Our research utilized publicly available datasets and computational methods to analyze the data, adhering to all relevant guidelines and standards for bioinformatics research.

**Peer-review:** Externally peer-reviewed.

**Conflict of Interest:** The authors confirm that there is no conflict of interest in their decision to publish this work.

**Declaration of Interest:** The corresponding author can provide the datasets used and or/ analyzed in the current work upon reasonable request.

**Authorship Contributions:** Concept – M.U., Su.S., S.K., S.S., R.S.; Design – M.U., Su.S., S.K., S.S., R.S.; Supervision – M.U., Su.S., S.K., S.S., R.S.; Materials – M.U., Su.S., S.K., S.S., R.S.; Data collection and/or processing – M.U., Su.S., S.K., S.S., R.S.; Analysis and/or interpretation – M.U., Su.S., S.K., S.S., R.S.; Literature search – M.U., Su.S., S.K., S.S., R.S.; Writing – M.U; Critical review – M.U., Su.S., S.K., S.S., R.S.

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