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REVIEW ARTICLE

Distinct microRNA expression pattern in breast cancer cells following anti-neoplastic treatment: A systematic review and functional analysis of microRNA target genes

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Abstract

Breast cancer remains a significant cause of mortality in females worldwide, despite advances in technology and treatment. MicroRNA expression in breast cancer is studied both as potential biomarkers and for therapeutic purposes. Accumulated evidence revealed microRNA profile of various types of cancer cells following antineoplastic treatment. The progression of research in this area provides better understanding on the anti-cancer mechanism of various natural compounds and drugs specifically on the microRNA regulation. Hence, we aim to systematically review differentially expressed microRNA in MCF-7, a commonly studied breast cancer cell line, after treatment with anti-neoplastic agents. Relevant keywords were used to screen for research articles that reported on the differentially expressed microRNAs in experimental models of MCF-7 before and after anti-neoplastic treatment. Target genes of microRNAs were identified from MiRTarbase and further *in silico* functional analysis of the target genes were performed using DAVID bioinformatic resources. Two upregulated microRNAs (mir-200c and let-7d) and 3 downregulated microRNAs (mir-27a, mir-27b and mir-203) were identified by highest number of studies. Three microRNAs (let-7a, mir-23a and mir-7) showed inconsistent direction of expression. Genes functional analysis revealed the regulatory effect of microRNA on genes related to angiogenesis, hypoxia, P53, FoxO and PI3K-AKT signalling. Clusters of genes associated to the pathway of angiogenesis, cancers, cell proliferation and apoptosis were noted through protein-protein interaction analysis. MicroRNAs, especially the mir-200c, let-7d, mir-27a, mir-27b and mir-203 from this review could be further validated experimentally to serve as molecular target or biomarkers for anti-neoplastic therapy.

Keywords: Breast cancer; MCF-7; miRNA; gene expression profiling; pathway anaysis

INTRODUCTION

Breast cancer is one of the most prevalent cancers and the primary cause of mortality in females worldwide. About 2.09 million new breast cancer cases and 626,679 deaths were diagnosed in 2018, with almost 50% of the incidence and mortality in Asia.[1] According to the Malaysia National Cancer Registry 2012-2016 published in 2019, breast cancer topped the ten most common cancers among Malaysians with a total of 21,925 cases in both sexes and accounted for 34.1% of total cancer incidence among females.[2] According to the World Health Organization (WHO), an increase in life expectancy and urbanisation are among the factors contributing to the high incidence of breast cancer in developing countries. At the same time, late diagnosis is a significant problem in low- and middle- income countries.[3]

The progression and metastasis of breast cancer are responsible for the increased number

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of deaths it caused.[4] The mechanism by which the cancer cells spread were reported to be via 1) dissociation from extra cellular matrix (ECM) and invasion into local surrounding tissue,[5] 2) invasion,[6] and circulation,[7] into the lymph and blood vessels, 3) adhesion to capillary walls of target organ and extravasation into the organ,[8] and 4) proliferation of cancer cells to form new tumours.[9] It is important to note that all these processes are controlled by molecular signalling of genes that eventually correspond to protein expressions. Advances in breast cancer research have discovered the regulatory involvement of miRNAs in the development of breast cancer.[10]

miRNA is a small non-coding RNA gene product composed of approximately 22 nucleotides which was first discovered in nematode *Caenorhabditis elegans*.[11] Accumulated evidence revealed its significant role as an oncogene or tumour suppressor gene depending on the binding site of a mature miRNA to its target messenger RNA (mRNA). This protein-coding gene determines the mRNA translational repression or degradation.[12] Hence, miRNAs' upregulation is correlated to downregulation of genes while its downregulation indicates no change or increased baseline expression of the miRNAs' target genes. Therefore, miRNA expression profiling has gained much interest in research and clinical application due to its involvement in cancer progression. Over- or under-expression of miRNAs would suggest the therapeutic strategies for breast cancer treatment. Growing information on miRNA network disclosed new opportunity for diagnostics and therapy of breast cancer. For example, profiling of miRNA expression following treatment with potential anti-cancer agent could guide on the establishment of molecular biomarkers that indicate the key regulator of cancer progression and suppression. At the same time, miRNAs could also be useful markers to evaluate the anti-cancer effects of drugs and natural products.

Despite extensive breast cancer treatment available nowadays, research on certain drugs or anti-cancer activity of natural products is continuously growing.[13,14] The studies include the effects of potential antineoplastic agents in natural products such as pomegranate extract,[15] *Thymus vulgaris*[16] and Turkish propolis[17] on miRNA expression level in breast cancer. Emerging data on global miRNA expression in breast cancer however is varied among miRNAs profiling studies.[18] Thus, we aimed to systematically review published reports on miRNAs signature following anti-cancer treatment in commonly studied human breast cancer cell line, MCF-7. Further bioinformatics analysis on gathered data could bring better understanding on the pathways that could be affected by the antineoplastic treatment on MCF-7. In future research, signature miRNAs and genes from this analysis could be further explored as potential molecular targets in anticancer treatment or serve as biomarkers.

MATERIAL AND METHODS

Search strategy

Relevant published reports of miRNA expression profile in MCF-7 cells were searched through the electronic databases Ovid, Ebscohost, Pubmed, Scopus and Web of Science (WOS). A combination of subject headings (MeSH terms) and keywords were created based on population, indicator, comparator and outcome (PICOS) approach. The search string is presented in Table 1. The same search strategy was adapted to each database.

Inclusion and exclusion criteria

The inclusion criteria for this systematic review were as follows: 1) study that investigates miRNA expression profile of MCF-7 cells treated with antineoplastic agents, 2) study that compares miRNA profile of MCF-7 with and without the treatment of antineoplastic agent and 3) the miRNA profiling approaches used are microarray

Table 1: List of subject headings and keywords

Population	MCF-7 (MeSH) or MCF7 or "michigan cancer foundation 7" or "breast neoplasms" (MeSH) or "breast cancer*" or "breast neopl*"
Indicator	"gene expression profiling" (MeSH) or expression or profil* or microarray or sequencing
Outcome	microRNA* (MeSH) or miRNA* or miR*
Study design	"anti-neoplastic agents" (MeSH) or antineopl* or anticancer or antitumour or cytotoxic* or anti-neopl* or anti-cancer or anti-tumour

or sequencing. Meanwhile, the exclusion criteria were 1) general study of miRNA profiling in MCF-7 cells (no antineoplastic treatment), 2) the study of antineoplastic treatment on chemo resistant MCF-7 cells or epithelial-mesenchymal transition model, 3) the study of miRNA expression using methods other than microarray and sequencing or *in silico* through online databases. Review papers were not included.

Data extraction

Author, year of study, the platform used for microarray/ sequencing analysis, types of antineoplastic treatment and number of differentially expressed miRNA were extracted from each selected study.

Ranking

A list of differentially expressed miRNA after treatment with antineoplastic agents was extracted from each study. The miRNAs were ranked based on Griffith's and Chan's ranking method.[19-20] This ranking system was based on the following order of importance: 1) the differentially expressed miRNA was consistently reported and in a consistent direction of change and 2) the frequency of the reported miRNA in the expression profiling studies.

Identifying miRNA target genes

An established database, miRTarbase (http://mirtarbase.mbc.nctu.edu.tw/) was used to identify the potential target genes of the top ranked miRNA. Only target genes validated by reporter assay, Western blot and qPCR were considered solid evidence and listed in the table. Meanwhile, other relevant information such as chromosomal localisation and mature sequences of the miRNA was extracted from miRBase database (www.mirbase.org/).

Functional analysis of target genes

DAVID Bioinformatics Resources 6.8 (https://david.ncifcrf.gov/tools.jsp), a web-based functional annotation tool was used to analyse the list of miRNAs' potential target genes. It systematically mapped the genes into several biological annotations such as gene ontology terms and statistically highlighted the most enriched biological terms or functions. It also provided pathways analysis generated by the Kyoto Encyclopedia of Genes and Genome (KEGG) and BioCarta. All listed terms were the top 10 most significant terms ranked according to the modified Fisher exact p-value or EASE score. The terms with a p-value equal to or less than 0.05 ($p \leq 0.05$) were considered strongly enriched in the annotation categories.

Protein–Protein Interaction (PPI) Network

We selected target genes of 3 up- (mir-27a-5p, mir-27b-5p, mir-203a-5p) and 3 downregulated (mir-200c-5p, mir-143-5p, let-7d-5p) miRNAs for computational analysis of PPI using STRING (Protein-Protein Interaction Network Functional Enrichment Analysis) (https://string-db.org/). Results from STRING were then explored using Cytoscape to visualise molecular interaction networks and identify clusters of protein interaction that are highly related to the MCF-7 miRNA and gene expression profile. The gene interaction relationship was downloaded in the "TSV" format file and was imported into the Cytoscape software (http://www.cytoscape.org/) for analysis and clustering. The Cytoscape MCODE plug-in was utilised to perform module analysis of the target network and protein clustering. The module selection criteria include degree cut-off = 2, node score cut-off = 0.2, node density cut-off = 0.1, K-score = 2 and max depth = 100. The list of genes in the cluster was then analysed again using DAVID for significantly enriched ontology terms.

RESULTS

Study selection

The electronic databases search identified 3,132 articles (PubMed: 648, Ovid: 183, Ebscohost: 547, Scopus: 1,007, Web of Science: 747). First screening was performed on the title and abstract of the remaining articles according to the inclusion and exclusion criteria after duplicates were removed. From 34 articles that were eligible for full-text review, only 11 independent studies met the criteria and were included in further analysis. The other 23 articles were excluded for reasons; 11 articles have no microarray/ sequencing analysis; 8 articles used different breast cancer cell lines; 4 irrelevant articles studied gene/DNA methylation instead of miRNA. Figure 1 (FIG. 1) shows the careful selection of the studies.

Differentially expressed miRNA

The findings revealed the expressions of 441 miRNAs in 11 studies. The number of up- and down-regulated miRNAs were 94 and 247 respectively. Among the 441 differentially expressed miRNAs, only 35 (7.9%) were

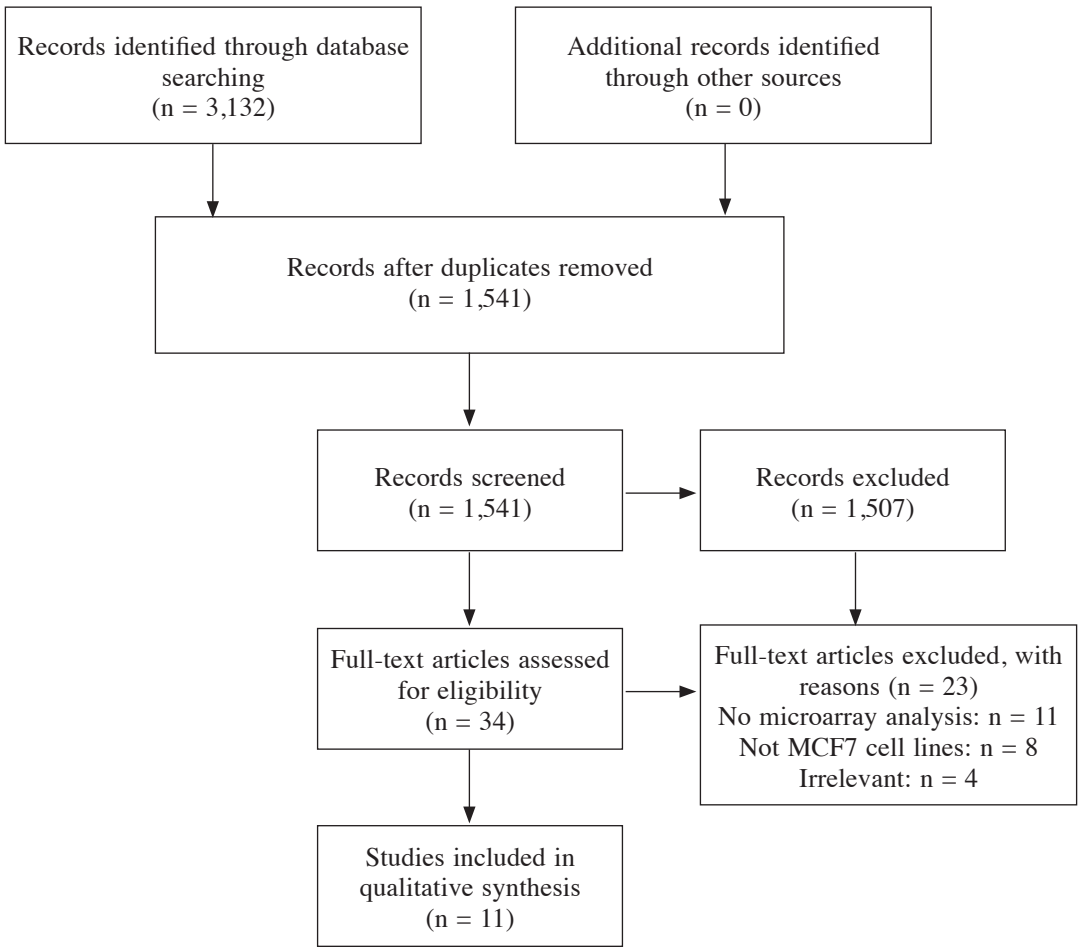

FIG. 1: Flow diagram of the selection study.

reported in at least 2 or more studies. From these, 28 (80%) miRNAs were consistently reported as upregulated or downregulated. Meanwhile, 3 (8.6%) miRNAs have an inconsistent pattern of expression. From the upregulated miRNAs, 2 miRNAs (mir-200c and let-7d) were reported in 3 studies. Another 10 upregulated miRNAs were reported in 2 studies (Table 2). Four studies revealed the same downregulation of mir-27a, mir-27b and mir-203. Another 8 and 4 miRNAs were reported to be downregulated in 3 and 2 studies respectively (Table 4). Mir-1826, mir-1979, mir-1280 and mir-720 appeared to be a fragment of RNA and were removed from the miRNA databases (e.g., miRbase and PubMed). Three miRNAs (let-7a, mir-23a, and mir-7) were reported with inconsistent expression in more than 2 studies (Table 5).

Gene ontology and pathway analysis by DAVID Bioinformatic Resources

Figure 2 and 3 (FIG. 2 and 3) show the top 10 most significant biological processes and the Biocarta and KEGG pathway analysis of 312 upregulated and 613 downregulated miRNAs' target genes. The regulation of RNA polymerase II promoter, apoptotic process, gene expression, and cell proliferation were among the positive or negatively regulated processes. It was noted that, angiogenesis and response to drugs were regulated by upregulated and downregulated miRNAs respectively.

Influence of Ras and Rho proteins on G to S1 transition, signal transduction through ILR, p53 signalling, cell cycle and role of ERBB2 in signal transduction and oncology were found in both Biocarta results. VEGF, hypoxia and angiogenesis were more enriched compared to p53 signalling for upregulated miRNA.

Table 2: MicroRNA profiling studies identified from the systematic review

Study	Year	Platform	Treatment	Upregulated	Downregulated
Fanale, D *et al.*[21]	2016	Taqman Low Density Array A Human MicroRNA v2.0	10 uM zoledronic acid, 24h	9	12
Fix, L *et al.*[22]	2010	LC Science, µParaFlo® microfluidics chip	10 ug/ml green tea polyphenon-60, 48h	7	16
Hannafon, B *et al.*[23]	2015	MiSeq Next Generation Sequencer (Illumina)	100 uM docosahexaenoic acid (DHA), 24h	19	7
Ilisso, C *et al.*[24]	2018	Taqman MiRNA Array	500 uM S-adenosylmethionine, 72h	24	4
Lee, S *et al.*[25]	2011	Roche NimbleGen Human Whole Genome 12 Plex Array	1 nM and 100 nM melatonin, 72h	12	10
Maillot, G *et al.*[26]	2009	Affymetrix Exon Array	10 nmol 17 β- estradiol	0	23
Peng, F *et al.*[27]	2017	Affymetrix GeneChip Human Gene 2.0 ST array	25 uM isoliquiritigenin, 24h	2	22
Shah, M *et al.*[28]	2011	LC Science, µParaFlo™ microfluidics chip	10 nM 5-fluorouracil, 24h	23	19
Tang, B *et al.*[29]	2012	LC Science, µParaFlo™ microfluidics chip	17.1 umol/L marine fungal metabolite 1386A	21	23
Tormo, E *et al.*[30]	2015	Affymetrix GeneChip miRNA 2.0 Array	5 uM doxorubicin, 48h	32	56
Zhang, H *et al.*[31]	2011	LC Science, µParaFlo™ microfluidics chip	60 mg/mL Aidi injection, 48h	45	55

Table 3: Upregulated miRNA reported in at least 2 studies

miRNA	Chromosomal localisation	Mature sequence (5'-3')	Potential target genes	Number of studies (references)
Mir-200c-3p	12p13.31	44-UAAUACUGCCGGGUAAUGAUGGA-66	TUBB3, BMI1, GEMIN2, BAP1, ZEB2, ZEB1, FN1, ZFPM2, UBE21, JAG1, PTPN13, RNF2, RCOR3, BRD7, ACVR2B, MSN, NTRK2, ERRFI1, CCNE2, XIAP, BCL2, TIMP2, FBLN5, VEGFA, NCAM1, VLDLR, RELN, IKBKB, FLT1, KLF9, TBK1, PMAIP1, NTF3, LPAR1, EDNRA, RHOA, KLHL20, PTPRD, ELMO2, ERBIN, WDR37, VAC14, TCF7L1, RASSF2, HOXB5, RIN2, KLF11, SEPT7, SHC1, MYB, ETS1, DUSP1, USP25, EFNA1, RND3, DNMT3A, DNMT3B, SP1, CFL2, CDH11, SEC23A, KDR, HFE, DLC1, ATRX, ZNF217, TRPS1, NOG, HOXA5, BTC, ZFPM1, PIN1, KRAS, NOTCH1, GATA4, SUZ12, ROCK2, UBQLN1, E2F3, GL13, OSR1, PKD1, MALAT1, CDK2, PRKCZ, NOS3, SIRT1, FOXO1, PDCD10, HNF1B, ADAM12, PTEN, E2F3, LEPR, HIF1A, CDH1, CRKL, MYLK, SH3PXD2A, DNAJC3, JAZF1, RPS6KB1, SLC1A2, CYP1B1	3 [23, 28, 29]
Let-7d-5p	9q22.32	8-AGAGGUAGUAGGUUGCAUAGUU-29	HMGA2, APP, DICER1, SLC11A2, PDGFA, IL13, MPL, AGO1, TNFRSF10B, COL3A1, CRYGEP	3 [23, 28, 30]
*Mir-1826				3 [22, 28, 29]
Mir-142-3p	17q22	52-UGUAGUGUUCCUACUUUAUGGA-74	RAC1, ARNTL, IL6, TGFBR1, BOD1, PROM1, ROCK2, CCNT2, TAB2, Wasl, HMGA1, PTPN23, EGR2, IL1A, HSPA1B, APC, RAB2A, C18orf25, TIPARP, KAT2B, LPP, MGLL, USPN6NL, CCDC6, HECTD1, WASL, TWF1, SUCO, ABCG2, LGR5, HOXA10, HOXA7, HOXA9, LRRC32, HMGB1, DOCK6, SOCS6, XRCC1, PRKCA, THBS4	2 [21, 24]
Mir-143	5q32.33	61-UGAGAUGAAGCACUGUAGCUCA-82	KRAS, MYO6, DNMT3A, FNDC3B, MAPK7, COL1A1, HRAS, FSCN1, HK2, SERPINE1, MACC1, PTGS2, JAG1, AKT1, BCL2, SDC1, RREB1, CD44, KLF5, BRAF, COX2, IGF1R, GABARAPL1, NR2C2, IL13RA1, DDX6, CTGF, NFATC1, CYP2C9, DTNB, AKT2, ERBB3, FAM83F, PIK3R1, BAG3, TLR2, MMP2, MMP9, NFKB2, MMP14, ITGB1, ITGB4, OSBPL8, TNF, NR2C2, IL13RA1, COL3A1, DDX6, LIMK1, HNF4A, COL1A1, FHIT, MDM2, MMP13	2 [21, 24]

*Mir-1979				2 [22, 28]
Mir-505	Xq27.1	51-CGUCAACACUUGCUGGUUUCCU-72	SRSF1, ATF3, TGFA, PSMD10, AMFR	2 [23, 25]
Mir-374b	Xq13.2	11-AUAUAAUACAACCUGCUAAGUG-32	RECK, VEGFA, WNT16, AKT1	2 [25, 29]
Mir-663a	20p11.1	15-AGGCGGGGCGCCGCGGGACCGC-36	JUNB, JUND, KLF4, FOSB, SLC7A5, NAV2, TCEAL1, TGFB1, HSPG2, CDK1, APC, CCND2, PIK3CD, EEF1A2, MYL9, HRAS, IGF2, TP53, CDKN1A, CXCR4, PLOD3	2[28, 29]
Mir-638	19.p13.2	16-AGGGAUCGCGGGCGGGUGGCG-GCCU-40	OSCP1, SP2, SOX2, CDK2, STARD10, DACT3, PLD1, TP53INP2, PTEN, TP53	2 [28, 29]
mir-1246	2q31.1	11-AAUGGAUUUUUGGAGCAGG-29	DYRK1A, NFIB, PRKAR1A, AXIN2, GSK3B	2 [28, 29]
mir-146a	5q33.3	21-UGAGAACUGAAUUCCAUGGGUU-42	CXCR4, CFH, IRAK2, TLR2, FADD, TRAF6, IRAK1, ROCK1, BRCA2, BRCA2, FAF1, CCNA2, PA2G4, CXCL8, NFKB1, CDKN1A, EGFR, MTA2, CD40LG, FAS, CDKN3, KIF22, ERBB4, SMAD4, TLR4, WASF2, BGLAP, STAT1, UHRF1, CD80, SPP1, L1CAM, SMN1, CARD10, COPS8, ELAVL1, NUMB, ICAM1, SMAD2, PTGS2, CCL5, S100A12, ZNF117, MY06, CCDC6, IL1RL2, IL1RAP, ROBO1, PTGES2, ERBB4, CNOT6L, SIKE1, CXCL12, PRKCE, HOXD10, RAC1, LAMC2, COX2, DUSP1, RNF11, SOS1, CASP7, BCLAF1, PTTG1, CPM, NOTCH2, SOX2, IL6, RHOA, IS2, KDM2B, LFNG, TGFB1, MIF, FANCM, NOTCH1, LIN52, LRP2, CCND2, CCND1, NFAT5, RHO, RARB, CXCR4, NOS1, PLAUR	2 [24, 30]
mir-302a	4q25	44-UAAGUGCUUCCAUGUUUUGGUGA-66	CDK4, CCND1, CDKN1A, LEFTY1, LEFTY2, DAZAP2, SLAIN1, TOB2, NRF2, AKT1, TAC1, CDK2, BMI1, MBD2, NANOG, CDK1, MAP3K1, PTEN, MMP9, MMP2, SDC1, GAB2, ABCA1,	2 [24, 30]
mir-1915	10p12.31	47-CCCCAGGGCGACGCGGCGGG-66	BCL2	2 [28, 30]

*Human mir-923 and mir-1979 appears to be a fragment of the 5.8S rRNA and Y RNMA respectively, and so is removed from the miRNA database.

Table 4: Downregulated miRNA reported in at least 2 studies

miRNA	Chromosomal localisation	Mature sequence (5'-3')	Potential target genes	Studies (references)
Mir-27a-3p	19p13.12	51-UUCACAGUGGCUAAGUUCCGC-71	FOXO1, PHB, ZBTB10, MYT1, SP4, SP3, SP1, HIPK2, SPRY2, WEE1, APC, FBXW7, IGF1, PAX3, MMP13, MSTN, WDR77, ABCA1, PDS5B, NFE2L2, EGFR, PLAG1, RMND5A, TP53, SEMA6A, MXI1, MET, CCND1, YWHAZ, SLC7A11, MAP2K4, PPARG, HOXA10, LDLR, PSAP, GATA2, WNK1, PIK3CG, BNIP3, ENDOU, CRISP2, LPIN1, CCNYL1, CAB39L, CPPED1, CNN3, IFNG, GRB2, SEMA7A, DPYD, AKT1, KRAS, IFNR, PPARA, BTG2, SMAD4, SMAD2, PHLPP2, GATA3, LAMP2, MAPK14, SLC6AB, DKK2, PRKAA2, HIF1A, GSK3B, YAP1, FSTL1, SFRP1, MRC1, EGFR, RARA, RXRA, EZH2, SOCS6, TFPI, TBC1D9, GREM1, CDC27, HMGCR, SNAP25, TXN2, PINK1, SFRP1, PEBP1, SMAD5, SIGLEC1, TRIM27	4 [22, 26, 28, 30]
Mir-27b-3p	9p22.32	61-UUCACAGUGGCUAAGUUCUGC-81	NOTCH1, ST14, MMP13, ADORA2B, CYP1B1, TRAPPC2B, PPARG, EDNRA, EYA4, WEE1, VDR, CYP3A4, PAX3, CCNT1, KHSRP, PAX7, SEMA61, VEGFC, CREB1, ABCA1, PSAP, MFF, LDLR, WNK1, TGFBR1, ENDOU, BNIP3, RMND5A, CRISP2, LPIN1, CCNYL1, CAB39L, CPPED1, CNN3, DPYD, SHC1, THBS2, THBS1, FOXO1, RET, PHB, CCNA2, NR2F2, RUNX1, SMAD2, HIP1R, PLK2, NR5A2, ROR1, CCNG1, FZD7, OSBPL6, HMGB3, CDH11, EGFR, MET, ARFGEF1, PXN, RAB14, ADAM19, CX3CL1, SOCS6, UCA1, PINK1, CDH5	4 [22, 26, 28, 30]
Mir-203a-3p	14q32.33	65-GUGAAAUGUUUAGGACCACUAG-86	UVRAG, ABL1, TP63, ABCE1, CDK6, SOCS3, BCL2L2, PPM1D, GDAP1, EDNRA, EYA4, ZEB2, BMI1, RUNX2, SMAD4, E2F1, MMP10, DLX5, IL6, MMP1, AKT2, BIRC5, E2F3, ZNF148, CREB1, TJP2, SOCS6, TNF, IL24, SNAI2, CBLL1, MYD88, LIFR, LASP1, JUN, PRKCA, VEGFA, ASAP1, CAV1, SRC, TCF4, RAN, RAPH1, CASK, PLD2, KIF5B, ATM, TYMS, PIK3CA, IFIT1, ALOX15, KIF2A, SNAI1, KHDRBS1, CXCL8, LIN28B, WIF1, BANF1, SRP4, TM4SF1, TBK1, HOXD3, NR2C2, STAT1, CDH1, CDKN1A, CDKN1B, CCND2, CDK6, MMP2, MMP7, MMP9, RAP1A, ZNF217, RAB22A, FGF2, FZD2	4 [22, 26, 28, 30]

Let-7c	21q21.1	11-UGAGGUAGUAGGUUGUAUGGUU-32	CDC25A, TGFBR1, TRIM71, HMGA2, MYC, DICER1, BCL2L1, NRAS, MPL, COPS8, GPS1, COPS6, AGO1, IGF1R, ITGB3, MAP4K3, IL10, MTOR, RICTOR, CEBPB, IL6, PBX2, TNFRSF10B, TRIB2, NUMB, HSPA4, STAT3, IL6R, MTPN, CASP3	3 [22, 26, 29]
Let-7e	19q13.41	8-UGAGGUAGGAGGUUGUAUAGUU-29	HMGA2, EIF3J, SMC1A, WNT1, CCND1, MPL, COPS8, GPS1, COPS6, MYCN, AGO1, IGF1R, MMP9, IGF1, LIN28A, AURKB, EZH2, TNFRSF10B, ARID3A, FASLG, PLK1, TNFAIP3	3[22, 28, 29]
Let-7g	3p21.2	5-UGAGGUAGUAGUUUGUACAGUU-26	KRAS, COL1A2, MYC, HMGA2, IGF2BP1, CDKN2A, BCL2L1, GAB2, FN1, IL13, TBC1D9, BMI1, AGO1, CASP3, THBS1, TGFBR1, SMAD2, AKT2, TNFRSF10B, CCL2, CCL5, DDR1, MYBPC3	3 [22, 26, 28]
Mir-21	17q23.1	8-UAGCUUAUCAGACUGAUGUUGA-29	MAT2B, MAT2A, SORBS2, RBPMS, STAT3, RCBTB1, ZNF608, PDCD4, HPGD, FASLG, L1CAM, PTEN, ZNF350, RASGRP1, CDC25A, BCL2, TM9SF3, RTN4, RPS7, PLOD3, NCAPG, DERL1, BASP1, JAG1, REST, SPRY2, TIMP3, SOX5, MTAP, RECK, E2F2, FMOD, TGFBR2, E2F1, TGFBI, LRRFIP1, MARCKS, TPM1, NFIB, APAF1, BTG2, HIPK3, RHOB, SERPINB5, BMPR2, RASA1, TGIF1, NCOA3, MYC, ERBB2, PCBP1, JMY, TOPORS, HNRNPK, DAXX, TP53BP2, TP63, TGFBR3, PPIF, MSH2, MSH6, TIAM1, ISCU, MEF2C, EIF4A2, ANKRD46, EGFR, IL1B, ICAM1, PLAT, PTX3, TNFAIP3, CCR1, CDK2AP1, CCL20, SP1, PIAS3, DOCK4, DOCK5, DOCK7, DUSP10, PPARA, ANP32A, SMARCA4, NTF3, COL4A1, FASLG, SOD3, TCF21, SMAD7, SOX2, RMND5A, MMP2, VEGFA, SASH1, SERPINI1, DDAH1, TGFB2, PIK3R1, MMP9, PTPN14, AKT2, TOR1AIP2, FBXO11, PELI1, YOD1, STAT3, BCL6, SATB1, WWP1, SMN1, HPGD, MYD88, IRAK1, VHL, GDF5, MAP2K3, IL12A, SECISBP2L, RFFL, NFIA, SETD2, CXCL10, CLU, TNFRSF10B, IGF1R, SIRT2, GAS5, RHO, CBX4, CASC2, DNM1L, LATS1, ICOSLG, TICAM2, UBE2N, BCL10, CEBPB, HMGB1, TAP1, TLR3, NAV3, STUB1, LRP6, EGLN1, CADM1, PSMD9, COX2, ABCB1, FZD6, PCGF2, BMI1, SOCS6, OXTR, FOXP3, MSLN, FASL, SMAD7, SOCS1, FOXO1, CASP8, TRAF7	3 [22, 26, 28]
Mir-26b	2q35	12-UUCAAGUAAUUCAGGAUAGGU-32	PTGS2, EPHA2, CDK6, CCNE1, ABCA1, ARL4C, GATA4, CHORDC1, PLOD2, NR2C2, TAB1, EZH2, USP9X, KPNA2, RB1, NAMPT, IGF1R, PTEN, COX2, MIEN1, ULK2, TRAF5, SMAD1, HAS2, ESR1, COL1A2, CTGF, TLR4, IGF1, ST8SIA4, COX2, PDE4A, SOCS6, FH, HGF, JAG1, LARP1	3 [22, 26, 28]

Mir-125a-5p	19q13.41	15-UCCCUGAGACCCUUUAACCUGUGA-38	CDKN1A, LIN28A, NTRK3, CD34, TP53, VEGFA, ERBB3, ERBB2, KLF13, BAK1, ARID3B, ELAVL1, TNFAIP3, SMAD4, MMP11, HK2, EIF4EBP1, TEF, LIFR, MASP1, RTN2, UBE2L3, ADAM9, EGFR, BCL2L12, BCL2, ETNK2, IL1RN, PRDM1, B4GALT1, TRPS1, FAM129B, RIT1, DOCK3, MEGF9, LFNG, IFNG, ESRRA, TRAF6, VPS51, SIRT7, CLEC5A, GALNT14, STAT3, MCL1, NAIF1, MAPK14, EDN1, CASP2, DLL4, ABL2, TAZ, HAX1, ZBTB7A, HDAC4, PIK3CG, AKT1, MTOR, MEG3, HDAC5, RAF1, E2F3, JAK2, SMAD2, ARID3A	3 [22, 28, 29]
*Mir-1280				3 [23, 28, 29]
*Mir-720				3 [23, 29, 30]
Mir-25	7q22.1	52-CAUUGCACUUGUCUCGGUCUGA-73	PRMT5, BCL2L11, KLF4, CDKN1C, KAT2B, TP53, WDR4, CDH1, CCL26, MDM2, PTEN, EZH2, SMAD7, MAP2K4, DSC2, ATP2A2, RECK, ERBB2, TCEAL1, LATS2, REV3L, FBXW7, HAND2, MYC, CPEB1, TNFSF10, CCNE1, CYP2B6, RGS3, COL1A2, PRKCZ, DHFR, SEMA4C	3 [22, 28, 30]
Mir-222	Xp11.3	69-AGCUACAUCUGGCUACUGGGU-89	STAT5A, CDKN1B, SOD2, MMP1, FOXO3, CDKN1C, KIT, TMED7, PPP2R2A, PTEN, SELE, TP53, SSSCA1, CORO1A, TCEAL1, DIRAS3, ETS1, DICER1, RECK, TRPS1, CERS2, GRB10, GJA1, SSX2IP, DKKK2, ARID1A, PHACTR4, TIPARP, ADAM1A, MGMT, VGLL4, GNAI3, GAS5, PRDM1, GNAI2, SMAD5, RUNX2, CDKN1C, ABCG2, FOXO1, BMF, PLXNC1, SSSCA1	3 [25, 27, 30]
Mir-200b	1p36.33	57-UAAUACUGCCUGGUAAUGAUGA-78	PTPN12, ZEB2, BAP1, ZEB1, RERE, ETS1, GATA4, FN1, WASF3, ZFPM2, MATR3, RNF2, BMI1, E2F3, VEGFA, FLT1, KDR, RND3, CCNE2, BCL2, XIAP, SMAD2, CREB1, KLHL20, ELMO2, PTPRD, ERBIN, WDR37, TCF7L1, VAC14, HOXB5, RIN2, RASSF2, KLF11, SEPT7, SHC1, MYB, QRSL1, SUZ12, WNT1, DNMT3A, DNMT3B, SPP1, MSN, DNMT1, EZH2, HFE, HNRNPA3, FERMT2, RAB21, RAB3B, RAB18, RAB23, OXR1, FOXG1, NOTCH1, BTC, CDKN1B, LOX, PHLPP1, SEC23A, ROCK2, PIN1, DDX53, JUN, MAPK7, RHOA, PROM1, ADAM12, ETV5, ATG12, CD274, IKBKB, ATG12, LDHA, CRKL, MALAT1, FSCN1	2 [22, 28]
Mir-454	17q22	64-UAGUGCAAUAUUGCUUAUAGGGU-86	SMAD4, CXCL12	2 [22, 28]

Mir-24	9q22.32	44-UGGCUCAGUUCAGCAGGAACAG-65	FEN1, CDK4, CCNA2, AURKB, MYC, E2F2, NOTCH1, DHFR, MAPK14, TRIB3, HNF4A, ACVR1B, MLEC, CDKN2A, BRCA1, POLD1, CDKN1B, NFAT5, DND1, TGFB1, FURIN, FAF1, BCL2, ZNF217, ST7L, MCM10, H2AFX, PAK4, BCL2L12, MBD6, PER2, EIF2S3, CDK1, BCL2L11, TMED7, E2F3, DHFRP1, CD34, E2F1, CORO1A, UBC, MCM4, PCNA, PTPN9, PTPRF, FURIN, S100A8, XIAP, NOS3, MEN1, CYP11B2, REG4, LDHA, LDHB, MAP3K9, HMOX1, PSAP, SP1, WNT4, IFNG, FOXM1, CCND1, IFNR, SPRY2, FSCN1	2 [26, 28]
Mir-23b	9q22.32	58-AUCACAUUGCCAGGGAUUACCAC-80	PRAP1, NOTCH1, PLAU, MET, PRODH, PRKACB, PTK2B, VHL, TAB3, TAB2, CHUK, SRC, GJA1, ZEB1, TUSC7, RUNX2, CA2, MARCKS, RRAS2, HMGA2, ETS1, NOTCH2, HMGB2, ATG12, TMEM64, SIRT1, CCNG1, SMAD3, G3BP2, ACVR2B, PRDX3, RGS5, TGFB2, TGFBR2, VCAN, TGIF1	2 [26, 30]

*Human mir-1280 and mir-720 appears to be a fragment of tRNA, and so is removed from the miRNA database.

Meanwhile, p53 signalling was the most enriched term for downregulated miRNA.

Eight out of 10 enriched terms generated by the KEGG pathway were similar in both results. However, focal adhesion was enriched in upregulated miRNAs only, while FoxO signalling was enriched in downregulated miRNAs. PI3K-AKT signalling seemed the most likely involved pathway in this review (FIG. 4).

Protein–Protein Interaction (PPI) Network

From the analysis of 3 upregulated target genes of miRNAs, we found 4 clusters of protein interaction: cluster 1 (score=15.63, nodes=28, edges=211), cluster 2 (score=12.89, nodes=19, edges=116), cluster 3 (score=3.33, nodes=7, edges=10) and cluster 4 (score=3, nodes=3, edges=3) (FIG. 5). Whereas, PPI analysis of the other 3 downregulated miRNAs' target genes revealed 5 clusters of protein interaction; cluster 1 (score=29.65, nodes=35, edges=504), cluster 2 (score=8.91, nodes=23, edges=98), cluster 3 (score=3, nodes=7, edges=9), cluster 4 (score=3, nodes=3, edges=3) and cluster 5 (score=3, nodes=3, edges=3) (FIG. 6). Cluster 1 and 2 of both up- and downregulated proteins showed high number of edges which indicate high intermolecular interactions between the nodes.

DISCUSSION

Differentially expressed miRNAs

According to our findings, 2 miRNAs (mir-200c and let-7d)[23,28,29] were consistently upregulated in 3 studies while 11 miRNAs (mir-27a, mir-27b, mir-203a, let-7c, let-7e, let-7g, mir-21, mir-125a-5p, mir-25, and mir-222) were consistently downregulated in 4 and 3 studies, respectively.[22,26,28,30] From the results, half of the let-7 family members (let-7d, let-7c, let-7e, let-7g including let-7a which were found in an inconsistent direction) were differentially expressed in antineoplastic agent-treated MCF7.[22,26,28] Accumulating evidence reported that let-7 family were downregulated in many cancers such as lung cancer[32] and ovarian cancer[33] when compared to normal tissue. These findings support the important role of the let-7 family in cancer[34] and especially in breast cancer.[35]

From this systematic review, let-7d was upregulated after antineoplastic treatment on MCF7 which might suggest its function in cell proliferation inhibition. Studies performed on renal cell carcinoma[36] and breast cancer cell[37]

Table 5: The differentially expressed miRNA with an inconsistent direction between studies

miRNA	Chromosomal localisation	Mature sequence (5'-3')	Potential target genes	Direction of expression	Studies (references)
Let-7a	9q22.32	6-UGAGGUAGUAGGUUGUAUAGUU-27	CDK6, MYC, BCL2, NKIRAS2, ITGB3, NF2, NRAS, KRAS, PRDM1, LIN-41, TRIM71, FOXA1, NR1I2, VDR, RAVER2, HMGA2, HMGA1, AGO4, APP, E2F1, UHRF2, DICER1, HRAS, IGF2, LIN28A, NFKB1, ZFP36L1, THBS1, TUSC2, SLC20A1, NEFM, MEIS1, EGR3, HNRNPDL, AMMECR1, CASP3, CASP8, CASP9, IL6, E2F2, CCND2, IGF2BP1, CDC34, EWSR1, MPL, CCR7, TMED7, CDKN1A, HAS2, EGFR, RRM2, AGO1, EZH2, UHRF1, PAK1, AURKB, RAB40C, ARG2, TNFRSF10B, TGFBR3, APOBEC3A, LIN28B, PARP1, PKM, CCND1, WNT1, STAT3, MAP4K4, TNFAIP3	↑	2 [22, 28]
				↓	3 [26, 29, 30]
Mir-23a	19p13.12	45-AUCACAUUGCCAGGGAUUUCC-65	CXCL12, HES1, POU4F2, CELF1, ATAT1, IL6R, PPARGC1A, G6PC, FOXO3, FANCG, MYH1, MYH2, MYH4, PTEN, PTPN11, HMGN2, LDHA, CDH1, KLF3, IRF1, FAS, MT2A, PPP2R5E, LDHB, CXCL8, APAF1, NEK6, LPAR1, FZD5, TOP1, HOXB4, LAMP1, HMGB2, SPRY2, MEF2C, HIP1R, XIAP, LRP5, TMEM64, HSP90AA1, HNF1B, FOXM1, TSC1, SMAD3, STAT3, GJA1, FOXA1, RUNX2, SOCS6, TRIM63, SMAD5, CHUK, ST7L, TERF2, RGS5, TGFB2, TLR2, GLS	↑	2 [23, 28]
				↓	2 [26, 30]
Mir-7	9q21.32	24-UGGAAGACUAGUGAUUUUGUUGUU-47	SNCA, PAK1, EGFR, ABCC1, IRS1, IRS2, RAF1, PSME3, HELLS, SRSF1, SLC7A5, IGF1R, PIK3CD, HOXB3, PTK2, HOXB3, HOXB5, BCL2, RELA, ILF3, KMT5A, NDUFA4, KEAP1, SIRT2, SMARCD1, YY1, SKP2, PIK3R3, XRCC2, KLF4, XIAP, MYC, GFI1, CUL5, PAX6, PIK3CG, TET2, VDAC1, SERPINB5, FOS, BAX, REL, GDF5, ILF2, RGS5, UBE2A, RNF183	↑	2 [25, 29]
				↓	2 [23, 28]

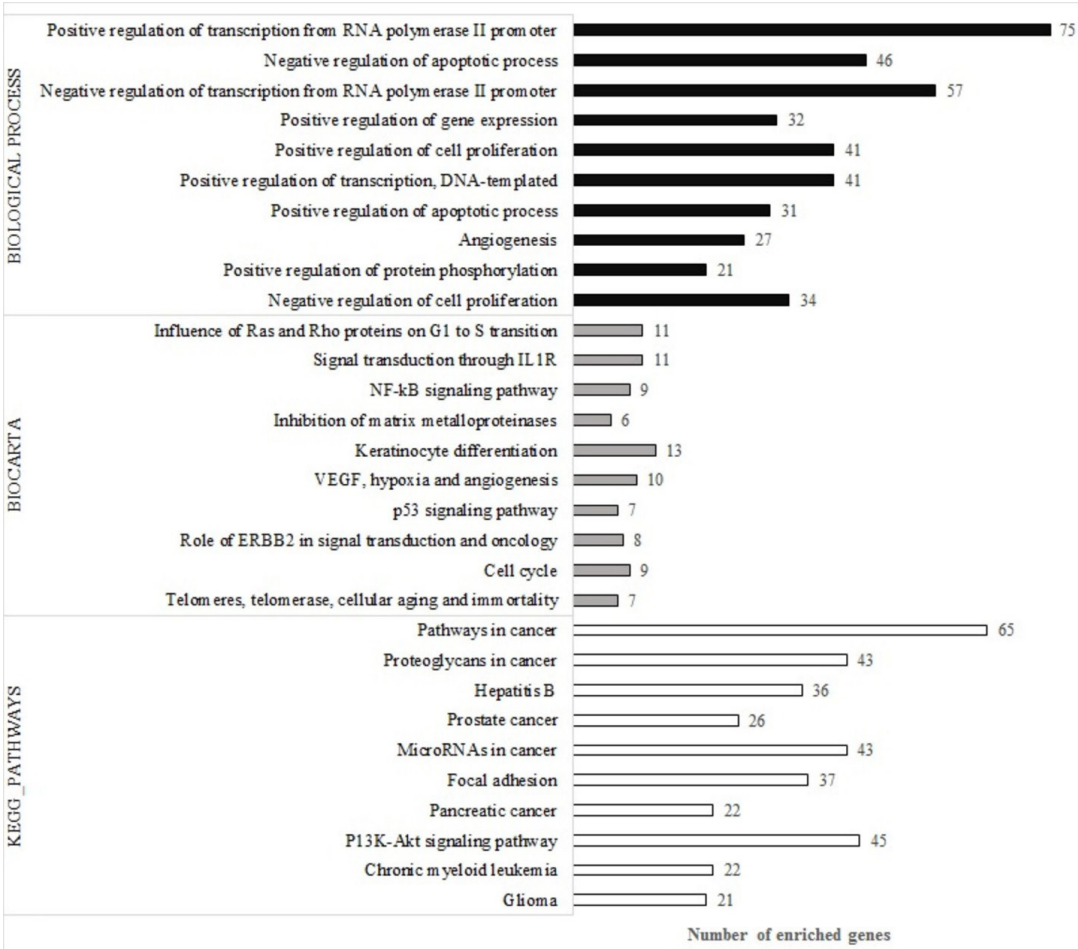

FIG. 2: GO terms, BIOCARTA and KEGG pathways analysis of potential target genes regulated by 12 upregulated miRNAs. *Direction of miRNA expression = ↑ upregulated, ↓ downregulated

found that let-7d was downregulated when compared with normal tissue. However, after transfecting the cells with let-7d mimic, both studies showed a significant reduction in cell proliferation and invasion. This concludes that overexpression of let-7d suppresses the cancer progression which is consistent with our findings.

Similar to let-7d, mir-200c was downregulated in several malignancies such as renal cell carcinoma[38] and gastric carcinoma.[39] A study done by Song *et al*. (2015) revealed almost 80% (33 of 41 breast cancer patients) and 11 mammary cell lines (human and mouse) showed a reduction in mir-200c expression compared to normal tissues.[40] Furthermore, decreased expression of mir-200c was correlated with drug resistance such as paclitaxel[41] and tamoxifen[42] in breast cancer therapy. However, overexpression of mir-200c enhance the breast cancer treatment, as shown in a radiotherapy study done by Lin and colleague.[43] Therefore, these findings proved that upregulation of mir-200c in this study was associated with MCF7 cell inhibition.

Mir-23-27-24 family consists of 2 paralogs located at different genomic loci. Mir-23a cluster (mir-23a, mir-27a, mir-24-2) localised at chromosome 19p13.13 while mir-23b clusters (mir-23b, mir-27b, mir-24-1) localised at chromosome 9q22.32.[44] Interestingly, all mir-23-27-24 families were found downregulated in this study except for mir-23a which showed inconsistent expression. Mir-27a and mir-27b were downregulated in 4 studies while mir-23b and mir-24 were downregulated in 2 studies. According to a study done by Liang *et al*. (2014), mir-24-1 and mir-24-2 can yield the same mature mir-24 sequence.[45] Deregulated expression of mir-23-27-24 family was reported in various cancers such as hepatocellular carcinoma,[46] prostate cancer,[47] bladder cancer,[48]

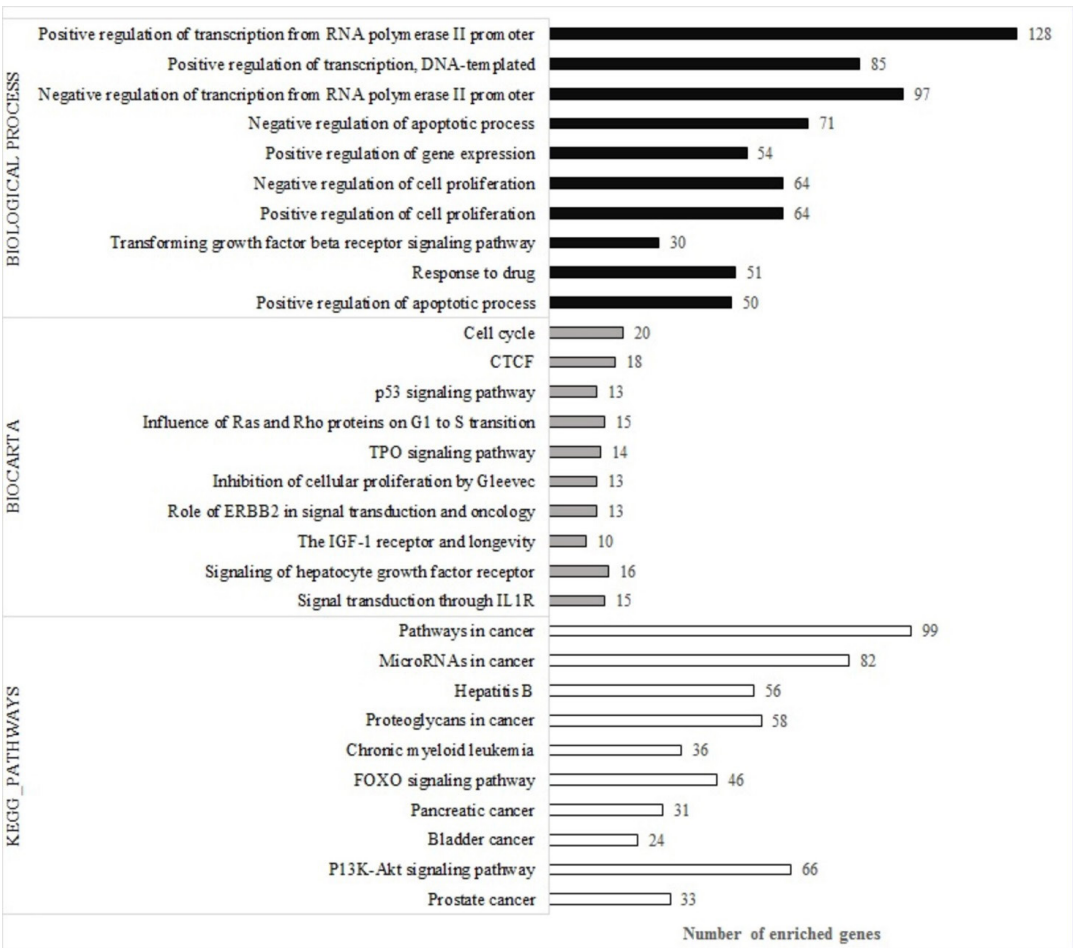

FIG. 3: GO terms, BIOCARTA and KEGG pathway analysis of potential target genes regulated by 15 downregulated miRNA.

osteosarcoma,[49] colorectal cancer[50] and gastric cancer.[51,52] A study done by Wang *et al*. (2016) found that mir-23a, mir-27a, mir-24, mir-273b, mir-27b were consistently upregulated in 25 human breast cancer tissue compared to noncancerous tissue.[53] Moreover, knockdown studies of mir-23b and mir-27b on breast cancer reported a substantially repressed breast cancer cell growth which further support that high expression of these miRNAs is associated to the breast cancer progression.[54,55] Downregulation of mir-27a, mir-27b, mir-23b and mir-24 in this study suggest their involvement in breast cancer inhibition.

PI3K-AKT signaling pathway

In physiologic conditions, Phosphoinositide 3-kinases (PI3K)-AKT signalling pathway is activated by many types of extracellular stimuli such as growth factor, chemokine, and hormone (Figure 4). Upon activation, PI3K isoform class IA is activated by receptor tyrosine kinase (RTK) or oncogenes such as RAS while PI3K isoform class IB is activated exclusively by G-protein-coupled receptor (GPCR).[56] Phosphorylation of phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol (3,4,5)-trisphosphate (PIP3) by PI3K activates signaling protein such as kinase AKT which in turn phosphorylates substrates involved in protein synthesis, cell cycle, apoptosis and metabolism.[57]

PI3K-AKT signalling was reported to be deregulated in numerous cancers including prostate cancer,[58] renal carcinoma[59] and gynaecological cancer.[60] It has been reported that the activation of PI3K-AKT signalling contributes to tumour development and therapy resistance while its inhibition results in reduced cell proliferation and increased cell death.[61,62] Furthermore, *in silico* analysis of target genes for miRNAs from this systematic review revealed that PI3K-AKT signalling was one of the most

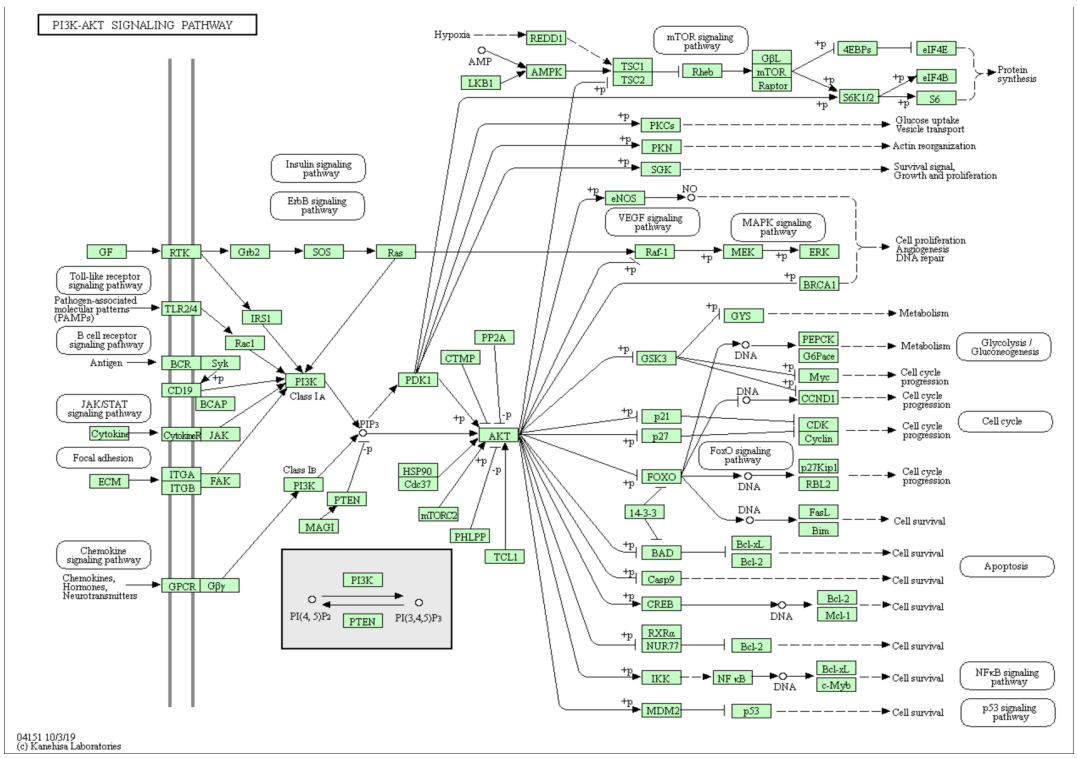

FIG. 4: PI3K-Akt signaling pathway generated by Kyoto Encyclopedia of Genes and Genome (KEGG).

enriched pathways. Hence antineoplastic effect on MCF7 could possibly be due to regulation of miRNAs which targeting the PI3K-AKT signalling.

Zhang and colleagues (2017) found that the anti-cancer effect of metformin on breast cancer cells was associated with the upregulation of mir-200c which led to suppression of AKT2 and BCL2.[63] This was supported by a study done on non-small cell lung cancer (NSCLC)

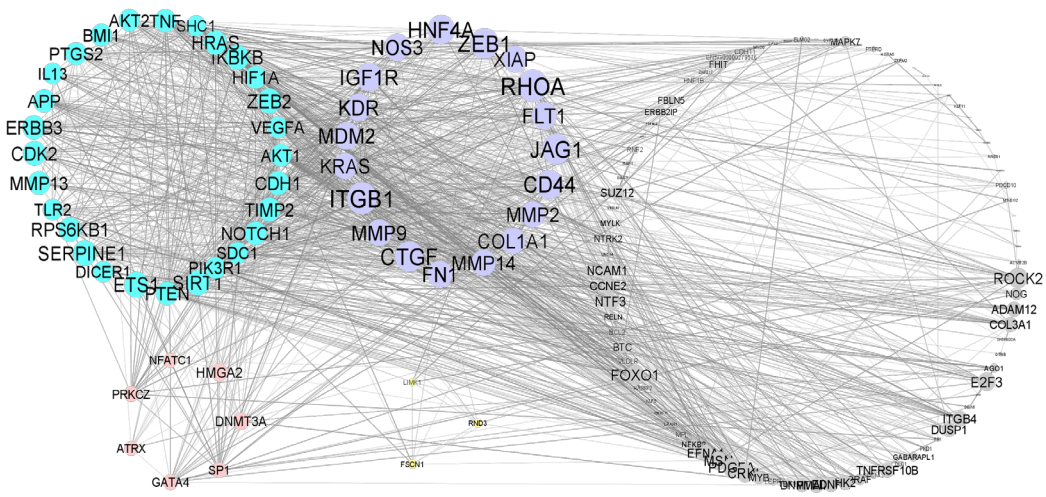

FIG. 5: STRING protein-protein analysis of up-regulated miRNAs's (mir-27a-5p, mir-27b-5p, mir-203a-5p) target genes. A high number of edges indicates high intermolecular interactions between the nodes. Genes that appear bigger involved in higher and stronger interaction. Darker line shows a stronger interaction. Node fill colours are indicated as blue (cluster 1), purple (cluster 2), peach (cluster 3), yellow (cluster 4) and grey (unclustered).

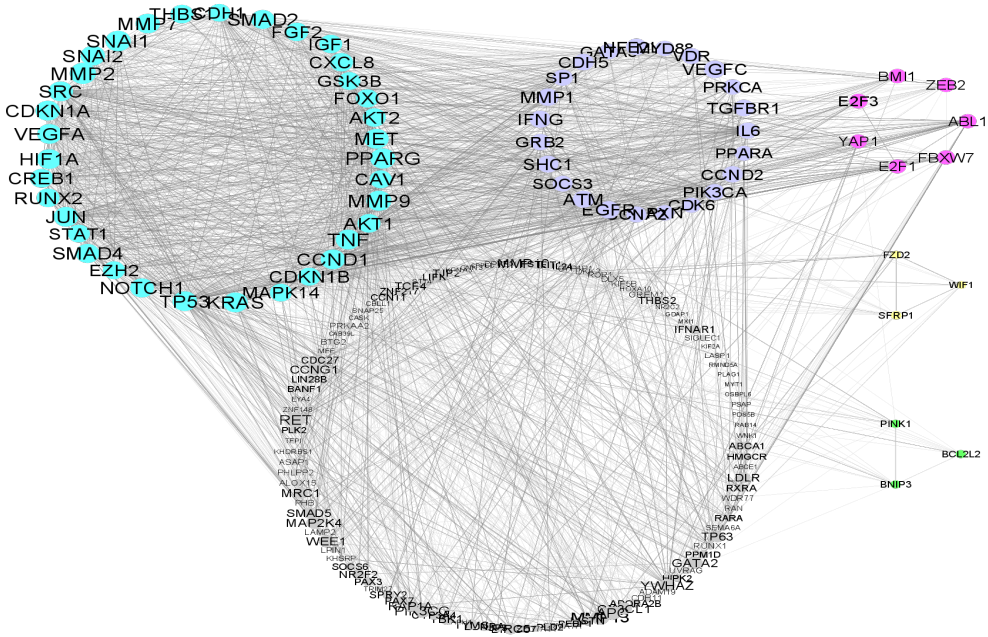

FIG. 6: STRING protein-protein analysis of down-regulated miRNAs's (mir-200c-5p, mir-143-5p, let-7d-5p) target genes. A high number of edges indicate high intermolecular interactions between the nodes. Genes that appear bigger involved in higher and stronger interaction. Darker line shows a stronger interaction. Node fill colours are indicated as blue (cluster 1), purple (cluster 2), pink (cluster 3), yellow (cluster 4), green (cluster 5) and grey (unclustered).

treated with Gefitinib where the upregulation of mir-200c significantly induced cell apoptosis by decreasing AKT and BCL2 expression and increasing BAX and CASPASE-3 expressions.[64] Another study demonstrated that upregulation of mir-200 suppressed AKT expression which in turn led to an inhibitory activity of prostate cancer[65] and also suppressed KRAS expression by directly targeting the anti-apoptotic gene, BCL2 in breast cancer.[66] Meanwhile, another study on triple-negative breast cancer showed overexpression of mir-200c increased focal adhesion kinase and AKT expression by secreting VEGFA which in turn significantly decreased cell growth but increased migration and invasion.[34] This finding might suggest a reason why focal adhesion and VEGF, hypoxia and angiogenesis categories were enriched by upregulated miRNA in this study (FIG. 2).

To control activation of downstream kinase, phosphatase and tensin homolog (PTEN) is the most important regulator to dephosphorylate PIP3 to PIP2. A study on gallbladder cancer found that 3 out of 73 patients (4.1%) with a complete absence of PTEN died before 10 months, while the remaining patients had a survival rate of 53% at 10 months.[67] This proves that loss or inactivation of PTEN consequently increases PI3K-AKT signalling and tumourigenesis. Moreover, a significantly low PTEN expression in breast cancer has been demonstrated to be associated with a larger tumour size, lymph node metastasis, advanced stage and poor differentiation.[68,69]

Several studies have reported an alteration of PTEN expression through the mir-23 family. A study done on 29 renal cell carcinoma (RCC) patients found that 15 patients with higher mir-23b-3p expression had a lower 5-year survival rate with only 50% survived. Further study revealed that inhibition of mir-23b-3p which could directly target the PTEN gene induced cell apoptosis in RCC cell line.[70] Another study demonstrated the overexpression of mir-27a in triple-negative breast cancer cells promoted proliferation and inhibited apoptosis by regulating CASPASE 3/7 and BCL2 expression. In this study, PTEN and AKT expressions were also altered.[71] In conclusion, upregulation of mir-200c and downregulation of mir-23 family contribute to antineoplastic effects on MCF7 by suppressing the cell proliferation and inducing cell apoptosis through involvement of PI3K-AKT signalling.

Protein-protein interaction network
The upregulated miRNAs' target genes from clusters with higher edges showed involvement in pathways such as microRNAs and pathways in cancer, proteoglycans in cancer and PI3K-Akt signalling. The topmost biological processes for these genes were angiogenesis, positive regulation of apoptosis and leucocyte migration. The genes with higher interactions were ITGB1, FLT1, JAG1, NOS3, MMP2, FN1, XIAP, MMP9, RHOA, CTGF, IGF1R, COL1A1, MMP14, ZEB1, HNF4A, MDM2, KDR, KRAS and CD4. Meanwhile, most genes targeted by the downregulated miRNAs in cluster 1 and 2 of the PPI were involved in pathways related to cancer, mainly bladder, prostate, melanoma, colorectal and pancreatic. Genes with bigger size in the figure such as VEGFA, HIF1A, CREB1, RUNX2, SMAD4, NOTCH1, MET, PPARG, MMP9 and CCND1 were analysed to have higher and stronger interactions. These findings suggest that antineoplastic agents could regulate miRNAs that control the progression of cancer cells by regulating genes with higher interactions as reported in the PPI analysis.

CONCLUSIONS

The expression of 20 miRNAs were found to be influenced by antineoplastic treatment on MCF-7. Further *in silico* analysis confirmed the association of these miRNAs with genes that regulate cancer-related pathways, angiogenesis, hypoxia, P53, FoxO, PI3K-AKT signalling, apoptosis and cell proliferation. Hence, MCF-7 profiles of miRNAs and its target genes consolidated from this review are beneficial for future investigation on the effect of anti-cancer agent including natural extracts. These could be further developed as biomarkers for progression of breast cancer or indicators of cancer treatment efficacy. With advanced investigation of the functional mechanism, novel cancer therapeutic agents could also be designed to act on potential miRNAs as documented in this systematic review.

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Consent for publication: Permission to publish PI3K-Akt signaling pathway in this manuscript was granted from KEGG.

Availability of data and materials: All data were outlined in the result section of the manuscripts. Original articles were properly cited next to analysed data.

Competing interests: The authors declare that they have no competing interests

Authors' contributions: QND and MML data collection, analysis and writing of the manuscript. NFMM involved in data collection, analysis, writing and review of the manuscript. NA is the expert in systematic review who confirm the design and protocol of the systematic review. MM, SGP, MHE, HAR, FAF, AAH involved in validation and interpretation of the presented data as well as in the manuscript writing. All authors of this paper have read and approved the final version submitted.

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