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Unraveling Roles of miR-27b-3p as a Potential Biomarker for Breast Cancer in Malay Women via Bioinformatics Analysis

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ABSTRACT

Abnormal miRNA expression has been associated with breast cancer. Knowing miRNA and its target genes gives a better understanding of the biological mechanism behind the development of breast cancer. Here, we evaluated the potential prognostic and predictive values of miRNAs in breast cancer development by analyzing Malay women with breast cancer expression profiles. Seven differentially expressed miRNAs (DEMs) were subjected to miRNA−target interaction network analysis (MTIN). A comprehensive MTIN was developed by integrating the information on miRNA and target gene interactions from five independent databases, including DIANA-TarBase, miRTarBase, miRNet, miRDB, and DIANA-microT. To understand the role of miRNAs in the progress of breast cancer, functional enrichment analysis of the miRNA target genes was conducted, followed by survival analysis to assess the prognostic values of the miRNAs and their target genes. In total, 1416 interactions were discovered among seven DEMs and 1274 target genes with a confidence score (CS) $\geq$ 0.8. The overall survival analysis of the three most DEMs revealed a significant association of miR-27b-3p with poor prognosis in the TCGA breast cancer patient cohort. Further functional analysis of 606 miR-27b-3p target genes revealed their involvement in cancer-related processes and pathways, including the progesterone receptor signaling pathway, PI3K-Akt pathway, and EGFR transactivation. Notably, six high-confidence target genes (BTG2, DNAJC13, GRB2, GSK3B, KRAS, and UBR5) were discovered to be associated with worse overall survival in breast cancer patients, underscoring their essential roles in breast cancer development. Thus, we suggest that miR-27b-3p has significant potential as a biomarker for detecting breast cancer and can provide valuable understanding regarding the molecular mechanisms of the disease.

Keywords: Breast cancer, miRNA, biomarkers, differentially expressed, bioinformatics, Malay women

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Introduction

Breast cancer is the most commonly diagnosed cancer in women, contributing to a substantial public health and economic burden to the nation. It is projected that 2.3 million new cases of breast cancer will be diagnosed among women, accounting for 11.7% of all cancer cases worldwide. Breast cancer has a higher incidence than any other type of cancer and is considered the fifth leading cause of cancer-related deaths (6.9%), behind stomach (7.7%), liver (8.3%), colorectal (9.4%), and lung cancer (18%) (1). An increased incidence and mortality rate of breast cancer has significantly impacted the economic burden of a middle-income country such as Malaysia. The costs associated with diagnosing, treating, and managing the disease can be substantial, placing financial and resource strains on individuals and healthcare systems. One study on financial catastrophes was conducted on 1,294 cancer survivors who were receiving medical treatment in government-funded and private hospitals in Malaysia. Nirmala *et al.* (2) reported that 18% of respondents incurred out-of-pocket (OOP) expenses solely for medical treatment. The incidence is expected to rise to 51% once the cost of goods and services associated with cancer are considered. The study revealed that 33% of the participants were individuals diagnosed with breast cancer, constituting the largest proportion of all the observed cancer cases. Therefore, early detection of breast cancer is essential for effective disease management and to mitigate the expenses associated with the treatment, especially among middle- and lower-income households.

MicroRNAs (miRNAs) are short, single-stranded RNAs consisting of 19-23 nucleotides that can potentially assist as biomarkers for early cancer detection. miRNAs play a crucial role in the posttranscriptional regulation of gene expression in various biological systems (3),(4). Numerous studies have uncovered an association between the dysregulation of miRNA expression and breast cancer progression (5). For instance, overexpression of miR-365 has been related to increased cell proliferation and migration of breast cancer cells (6), while miR-30 acts as a tumor suppressor by inhibiting several genes related to breast cancer bone metastasis, such as CTGF, ITGB3, ITGA5, IL8, TWIF1/IL11, DKK-1, RUNX2, and CDH11 (7). Cuk *et al.* (8) proposed that miRNAs have the potential to serve as noninvasive and early detection markers for breast cancer, while Fkih *et al*., (9) suggested that miR-10b, miR-26a, and miR-153 can be considered as biomarkers for triple-negative breast cancer (TNBC). Various subtypes of breast cancer exhibit distinct microRNA expression signatures. For example, luminal type A is characterized by the expression of Let-7c, miR-10a, and Let-7f, whereas basal type breast cancer is related to the expression of miR-18a, miR-135b, miR-93, and miR-155 (10).

To date, few studies have been carried out to investigate the prognosis-related network of miRNAs in search of potential biomarkers in breast cancer. Liu *et al.* (11) identified a miR-622 target gene, RNF8, related to poor prognosis by performing target prediction and in vitro validation of miR-622. In another study, RACGAP1 was identified as a target gene for miR-517a and miR-4784. RACGAP1 has been reported as a prognostic biomarker for breast cancer (12). However, there is a lack of studies specifically examining breast cancer patients among women of Malay ethnicity. In our previous study, the miRNA expression profiles of the blood samples were obtained from Hospital Universiti Sains Malaysia (USM), comprising eight breast cancer patients and nine healthy control individuals of female Malay ethnicity. We found seven miRNAs were significantly expressed in the plasma of breast cancer patients as compared to healthy

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individuals. The seven miRNAs are miR-125b-5p, miR-142-3p, miR-145-5p, miR-193a-5p, miR-27b-3p, miR-22-5p and miR-423-5p (13). To gain deeper insights into the roles of the identified miRNAs, the present study was conducted by analyzing the miRNA–target gene interactions from DIANA-TarBase, miRTarBase, miRNet, miRDB, and DIANA-microT with CS calculation. In a nutshell, this study revealed that high expression of miR-27b-3p and its oncogenic target genes could influence various cancer-related processes and pathways, including the progesterone receptor signaling pathway, PI3K-Akt pathway, and EGFR transactivation, potentially contributing to the poor prognosis of breast cancer.

Materials and methods

Network-based analysis of functional miRNA–target interactions

Seven significant differentially expressed miRNAs (DEMs) were selected based on the results of our previous study (13). To identify the posttranscriptional regulatory roles of the seven DEMs in early-stage breast cancer, the miRNA–target interaction network (MTIN) was constructed by integrating the miRNA–target interaction (MTI) information from publicly available resources. The bulk data of MTI information were downloaded from five independent databases, known as DIANA-TarBase v7.0 (http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=tarbase/index, accessed on 7 February 2023) and v9.0 (https://dianalab.e-ce.uth.gr/tarbasev9; accessed on 17 January 2024) (14), miRTarBase v9.0 (https://mirtarbase.cuhk.edu.cn/~miRTarBase/miRTarBase_2022/php/index.php, accessed on 7 February 2023) (15), miRNet v2.0 (https://www.mirnet.ca, accessed on 7 February 2023) (16), miRDB v1.0 (https://mirdb.org, accessed on 7 February 2023) (17), and DIANA-microT v3.0 (https://web.archive.org/web/ 20101208180159/http://diana.cslab.ece.ntua.gr/microT/, accessed on 7 February 2023) and DIANA-microT 2023 (https://dianalab.e-ce.uth.gr/microt_webserver/#/; accessed on 17 January 2024) (18). The MTI information of the seven targeted DEMs was then filtered out. We selected all interactions regardless of the score value or experiments between the selected miRNAs and their target genes. The confidence score (CS) of MTI was further evaluated by calculating the average interaction score range from 0 – 1, where the closer it is to 1, the more reliable the interaction between the miRNAs and target genes. To calculate the CS of MTI, the relations between MTI and the database were expressed with a binary matrix (denoted as M). $M_{ab} = 1$ means that the MTI (a) is identified in one of the databases (b), and $M_{ab} = 0$ represents that is not found in any b. The CS of the MTI is calculated based on $CS = (a_1 + a_2 + a_5)/b$. The database, b, was set to 5 due to the total number of independent databases used in this study. Finally, only MTIs with CS> 0.8 were considered and visualized using Cytoscape v3.9.1 (19).

Functional enrichment analysis of cancer-related pathways and Gene Ontology (GO)

MiRNAs play a vital function in regulating the expression of messenger RNAs (mRNAs) in the progression of various cancers. Therefore, functional enrichment analysis was conducted using a Cytoscape plug-in called ClueGO (20) to discover the overrepresented cancer-related pathways and molecular functions among the miRNAs' target genes in breast cancer. In ClueGO, the functional enrichment of GO (molecular function, cellular component, and biological process) and biological pathways (Kyoto Encyclopedia of Genes and Genomes and Reactome) were conducted on the target gene sets of miRNAs. Two-sided (enrichment/depletion) tests based on hypergeometric distribution were used to calculate discreetness and conservatism effects (21). The Bonferroni method was applied to control the type I error

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rate (false positive) and correct the p-values during multiple testing (22). ClueGO adopts kappa statistics to calculate the gene-term matrix between ontology terms and associated genes. The kappa score threshold used in this study was 0.4, which can be adjusted from 0 to 1 to control network connectivity. The size of the nodes represents the significant enrichment terms based on their percentage or the total number of genes involved.

Overall survival analysis

The overall survival analysis of miR-145-5p, miR-22-5p, and miR-27b-3p was conducted using the UALCAN data portal (https://ualcan.path.uab.edu/index.html, accessed on 7 March 2023) (23, 24). The UALCAN data portal allows users to perform various analyses, such as gene expression, survival, and clinical data correlation analysis, across 31 different cancer types. In this study, Kaplan–Meier survival analysis and overall survival plots were generated using the available breast cancer patient survival data from the Cancer Genome Atlas (TCGA). The log-rank test was used to compare the survival curves between samples with high and low/medium expression. A Kaplan–Meier analysis was also conducted for 40 enriched target genes associated with miR-27b-3p to evaluate their expression level effects on breast cancer survival. To simplify the overview of this research, Fig. 1 illustrates the flow chart of this study.

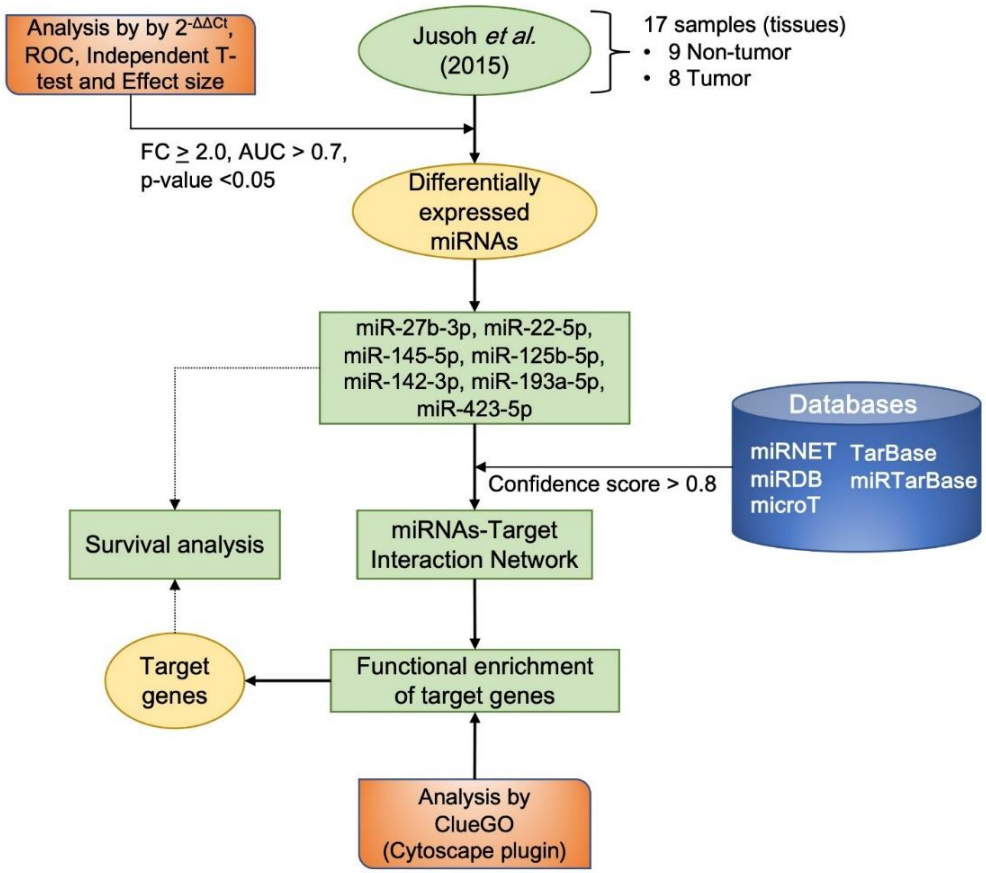

Fig. 1. A schematic diagram representing the overview of this study.

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Results

Network-based analysis of miRNA–target interactions

The miRNA–target interaction network (MTIN) was constructed by connecting differentially expressed miRNAs (DEMs) with their target genes (Fig. 2A). A total of 1274 target genes were identified to interact with seven miRNAs: miR-22-5p, miR-423-5p, miR-145-5p, miR-125b-5p, miR-27b-3p, miR-142-3p, and miR-193a-5p, based on the amount and reliability of evidence (CS) $\geq$ 0.8. miR-27b-3p had the highest number of interacting target genes (TG = 606), followed by miR-142-3p (TG = 233), miR-125b-5p (TG = 202), miR-423-5p (TG = 188), miR-145-5p (TG = 122), miR-22-5p (TG = 54), and miR-193a-5p (TG = 11). Furthermore, several genes were also found to be regulated by multiple miRNAs (Figure 2B). For example, the two most significant miRNAs ($p < 0.05$), miR-27b-3p and miR-142-3p, were found to co-regulate 36 target genes, whereas 24 target genes were regulated by both miR-27b-3p and miR-125b-5p, followed by miR-27b-3p and miR-145-5p (TG = 15). TAOK1, RAB14, and NR1D2 were discovered to be

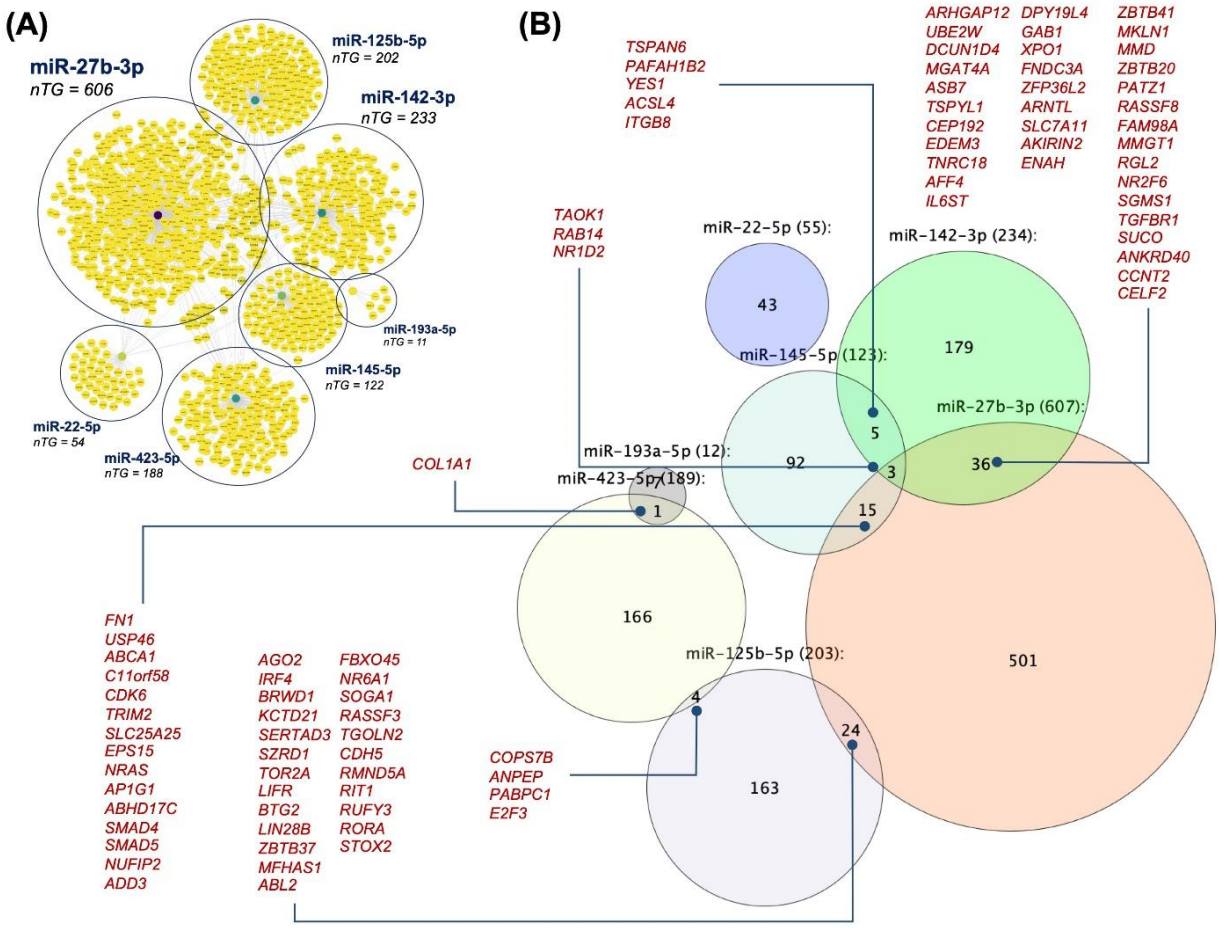

Fig. 2. miRNA–target interaction network. (A) The network consisted of seven DEMs: miR-22-5p, miR-423-5p, miR-145-5p, miR-125b-5p, miR-27b-3p, miR-142-3p and 193a-5p. (B) The Venn diagram shows the overlapping target genes shared by the miRNAs. Each node indicates a single target gene or DEM, whereas the edge represents the interaction between DEM and its target gene (TG). Yellow nodes denote target genes, while centered nodes represent miRNAs.

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regulated by miR-27b-3p, miR-142-3p, and miR-145-5p, indicating a potential synergistic combination of miR-27b-3p, miR-142-3p, and miR-145-5p in regulating specific pathways.

Functional enrichment analysis of GO and pathways

The GO and pathway enrichment analysis of the target genes for miRNA-27b-3p, miRNA-145-3p, and miRNA-22-5p revealed 32, 39, and 17 enriched terms, respectively, comprising molecular function (MF), cellular components (CC), and biological processes (BP) and pathways. In total, 32 enriched GO terms were discovered among the 606 target genes of miRNA-27b-3p (Fig 3A & 3B). Nine significantly enriched BP terms were discovered to be associated with breast cancer: superior temporal gyrus development (CNTNAP2 & GSK3B), gamma-aminobutyric acid metabolic process (ABAT & ALDH5A1), progesterone receptor signaling pathway (NEDD4, PHB1, UBR5 & YAP1), regulation of early endosome to recycling endosome transport (DNAJC13 & SORL1), regulation of protein kinase C activity (CEMIP, EGFR & NRXN1), positive regulation of nuclear-transcribed mRNA poly(A) tail shortening (BTG2, CNOT1, CNOT7, CPEB3, TNRC6B & ZFP36), intestinal epithelial cell differentiation (GATA6, IL6ST & YAP1), phosphorylation of RNA polymerase II C- terminal domain serine 2 residues (CCNK & CCNT1), and

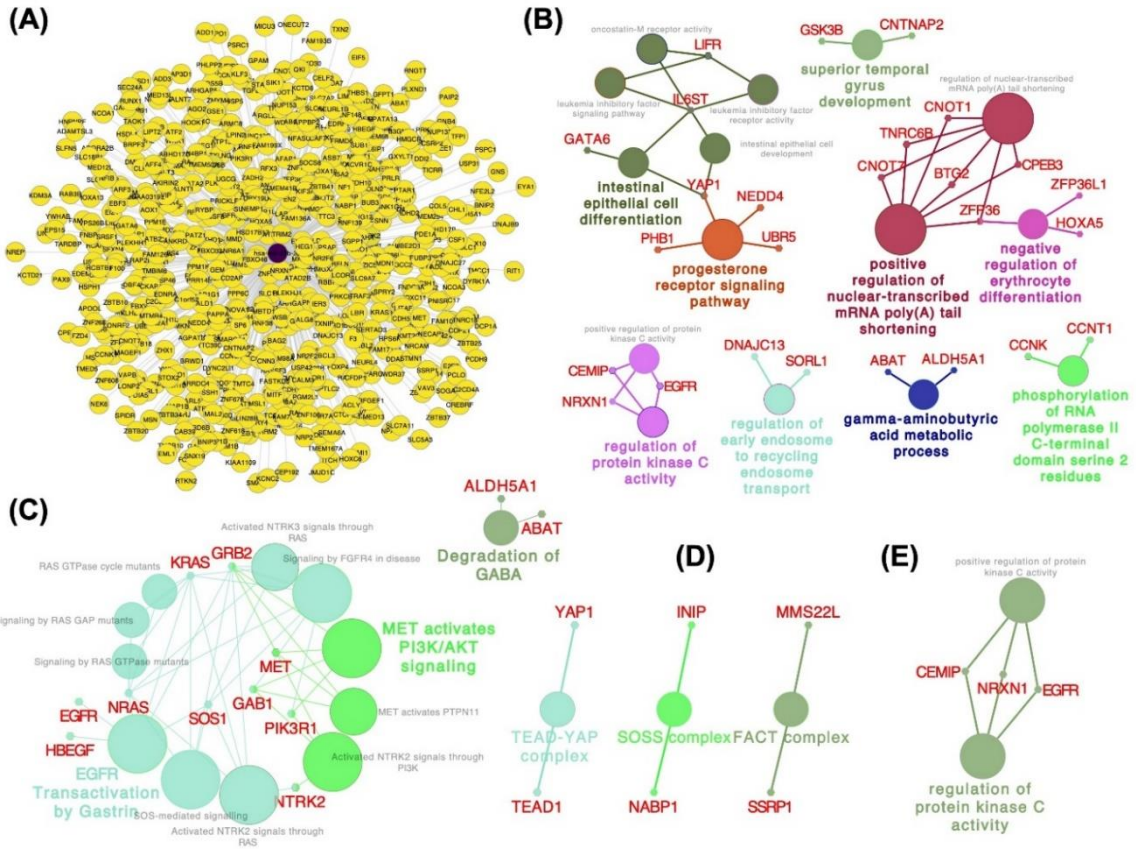

Fig. 3. GO functional enrichment of miR-27b-3p target genes. The significant GO terms are denoted as nodes based on the GO tree interval of minimum level = 7 and maximum level = 15 for detailed network specificity with the p-value ≤ 0.05. (A) miR-27b-3p target gene interactions and functional enrichment of (B) Biological process (BP), (C) KEGG and Reactome pathway, (D) Cellular component (CC), and (E) Molecular function (MF).

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negative regulation of erythrocyte differentiation (HOXA5, ZFP36 & ZFP36L1) (Figure 3B). Furthermore, three highly significant pathways, such as EGFR Transactivation by Gastrin (SOS1, NRAS, KRAS, HBEGF, GRB2 & EGFR), degradation of GABA (ALDH5A1 & ABAT), and MET activates PI3K/AKT signaling (PIK3R1, MET, GRB2 & GAB1), were also enriched among miRNA-27b-3p target genes (Figure 3C). The SOSS, FACT, and TEAD-YAP complexes were among the enriched cellular components identified (Figure 3D). Lastly for enriched MF, CEMIP, EGFR, and NRXN1 were associated with regulating protein kinase C activity (Fig 3E). For miRNA-145-5p target genes, the following genes, such as ABCA1, SERPINE1, SMAD2, SLC27A1, TGFB2, ABCE1, and CFTR, were respectively involved in enriched BP terms of apolipoprotein A-I receptor activity, positive regulation of leukotriene production involved in inflammatory response, nodal signaling pathway involved in determination of lateral mesoderm left/right asymmetry, biotin transmembrane transporter activity, negative regulation of alkaline phosphatase activity, endoribonuclease inhibitor activity, and intracellularly ATP-gated chloride channel activity (Figure 4B).

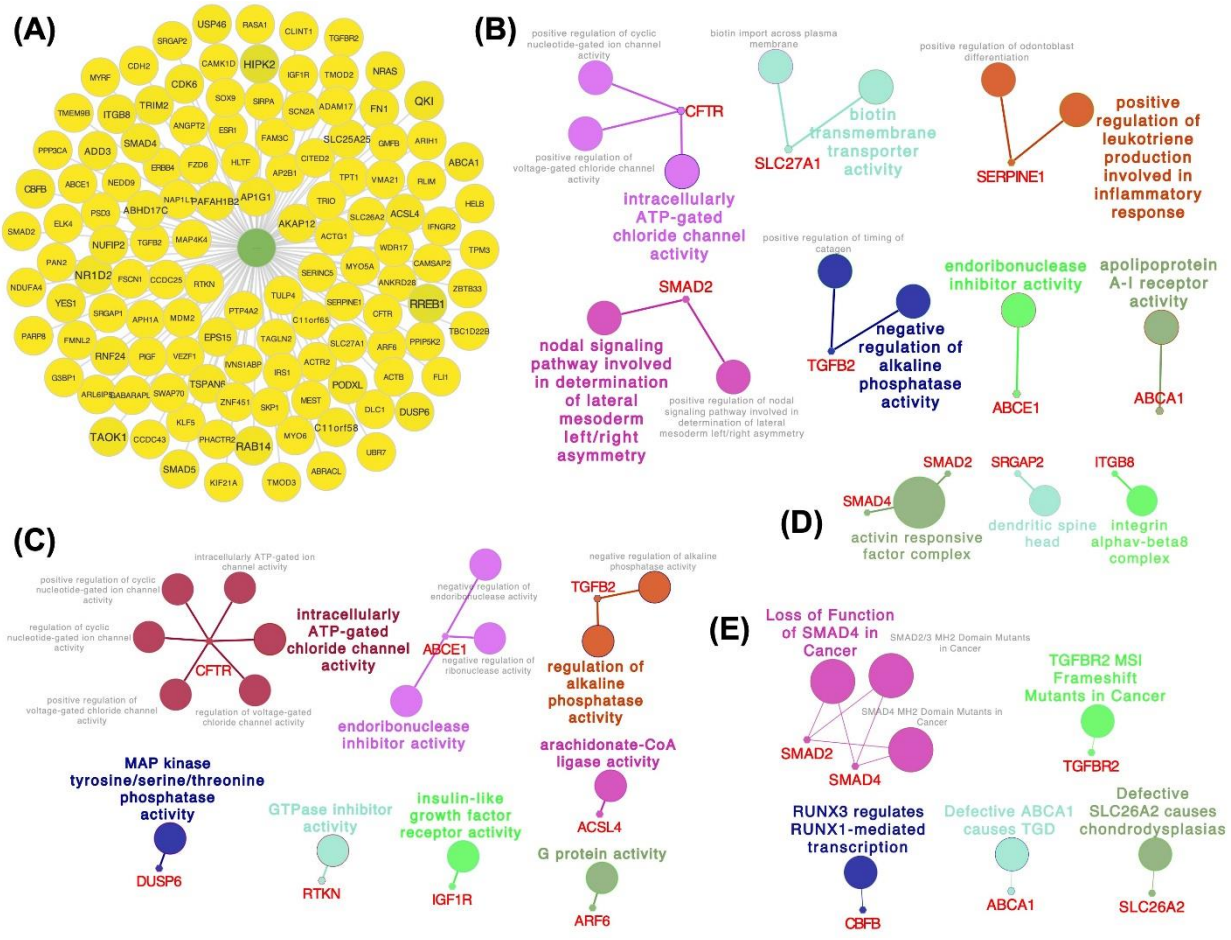

Fig. 4. GO functional enrichment of miR-145-5p target genes. The significant GO terms are denoted as nodes based on the GO tree interval of minimum level = 7 and maximum level = 15 for detailed network specificity with the p-value ≤ 0.05. (A) miR-145-5p target gene interactions and functional enrichment of (B) Biological process (BP), (C) Molecular function (MF), (D) Cellular component (CC), and (E) KEGG and Reactome pathway.

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Interestingly, several miRNA-145-5p target genes were also uncovered to be involved in several cancer-related pathways, including RUNX3 regulates RUNX1-mediated transcription (CBFB), defective SLC26A2 causes chondrodysplasias (SLC26A2), defective ABCA1 causes TGD (ABCA1), TGFBR2 MSI frameshift mutants in cancer (TGFBR2), and loss of function of SMAD4 in cancer (SMAD4 and SMAD2) (Figure 4E). miR-22-5p target genes, on the other hand, were enriched in noradrenergic neuron fate commitment (ASCL1), positive regulation of Rho guanyl-nucleotide exchange factor activity (EPHA4), histone kinase activity (H3-T6 specific) (PRKCA), positive regulation of Rho guanyl-nucleotide exchange factor activity (EPHA4), regulation of insulin secretion and integration of energy metabolism (MARCKS, KCNB1, PRKCA & GNG11) (Figure 5B-D).

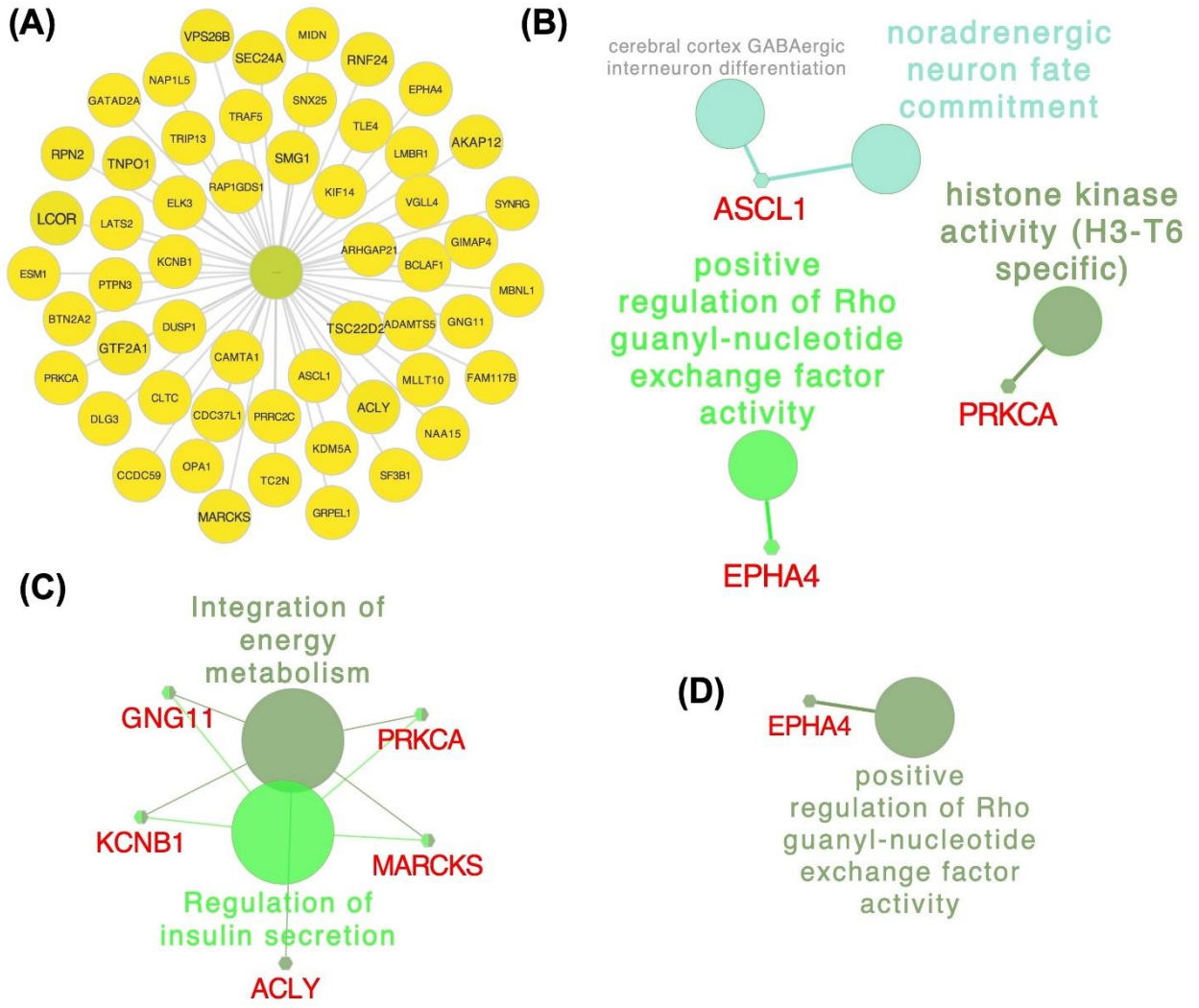

Fig. 5. GO functional enrichment of miR-22-5p target genes. The significant GO terms are denoted as nodes based on the GO tree interval of minimum level = 7 and maximum level = 15 for detailed network specificity with the p-value ≤ 0.05. (A) miR-22-5p target gene interactions and functional enrichment of (B) Biological process (BP), (C) KEGG and Reactome pathway, and (D) Molecular function (MF).

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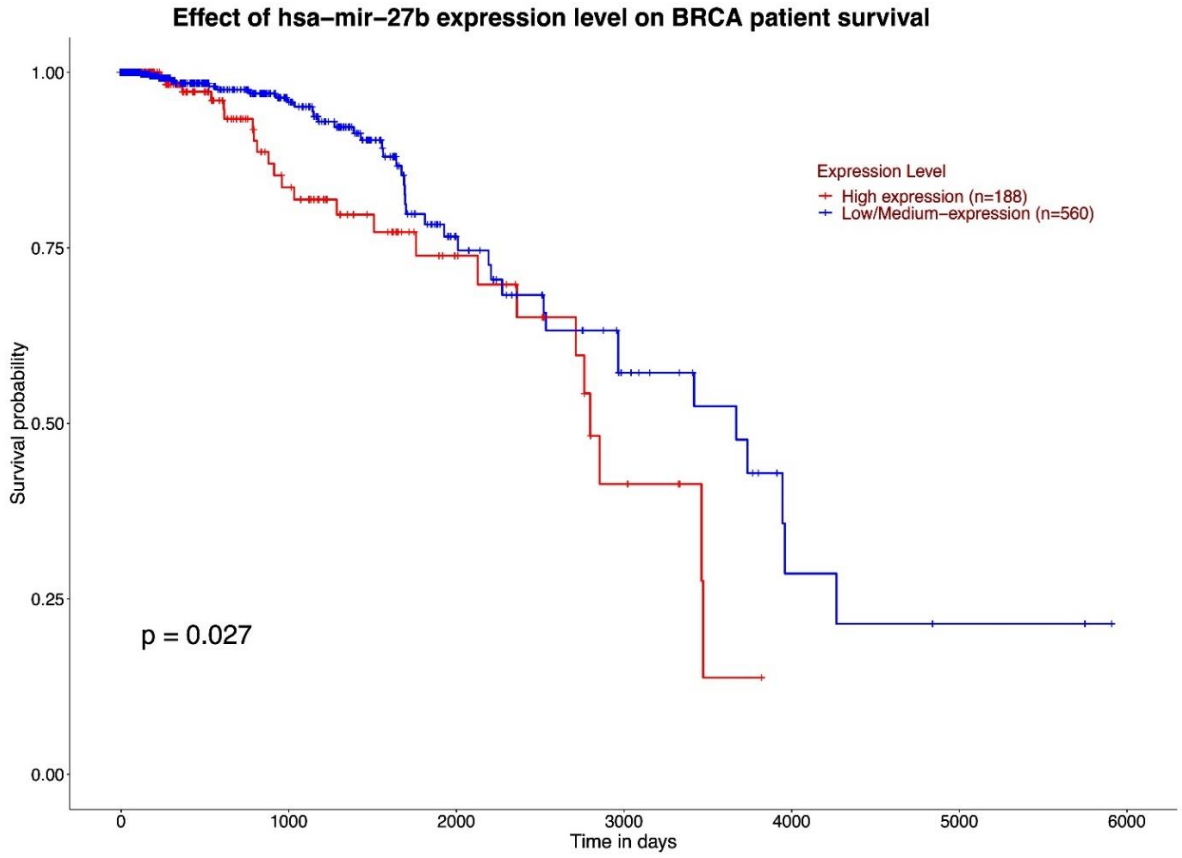

Fig. 6. Kaplan–Meier analysis shows the overall survival of breast cancer patients based on the expression levels of significantly expressed miR-27b-3p. The survival of miR-22-5p and miR-145-5p was not statistically significant, as the p-value > 0.05. The survival curves were plotted using the UALCAN web server. P-values were calculated using log-rank statistics.

Overall Survival Analysis

A Kaplan−Meier plot was generated to assess the association between DEM expression and patient survival. Based on the TCGA cohort, patients with high expression of miR-22-5p, miR-27b-3p, and miR-145-5p are likely to exhibit a shorter survival rate than those with lower expression. However, our findings suggested that the expression levels of miRNA-22-5p and miRNA-145-5p were not significantly related to the prognosis status of TCGA breast cancer patients, as reflected by a p-value > 0.05 (Figure 6).

To uphold the role of miR-27b-3p in breast cancer, we further examined the expression of target genes regulated by miR-27b-3p in the TCGA cohort. The results showed that high expression of six miR-27b-3p target genes was related to poor survival for breast cancer patients, except for BTG2. These genes include BTG2 (p = 0.019), DNAJC13 (p = 0.043), GRB2 (p = 0.018), GSK3B (p = 0.015), KRAS (p = 0.018) and UBR5 (p = 0.0084) (Figure 7A-F).

As observed, the downstream targets of miR-27b-3p have the potential to play significant roles in supporting the progression of breast cancer and are worth investigating and validating in future studies.

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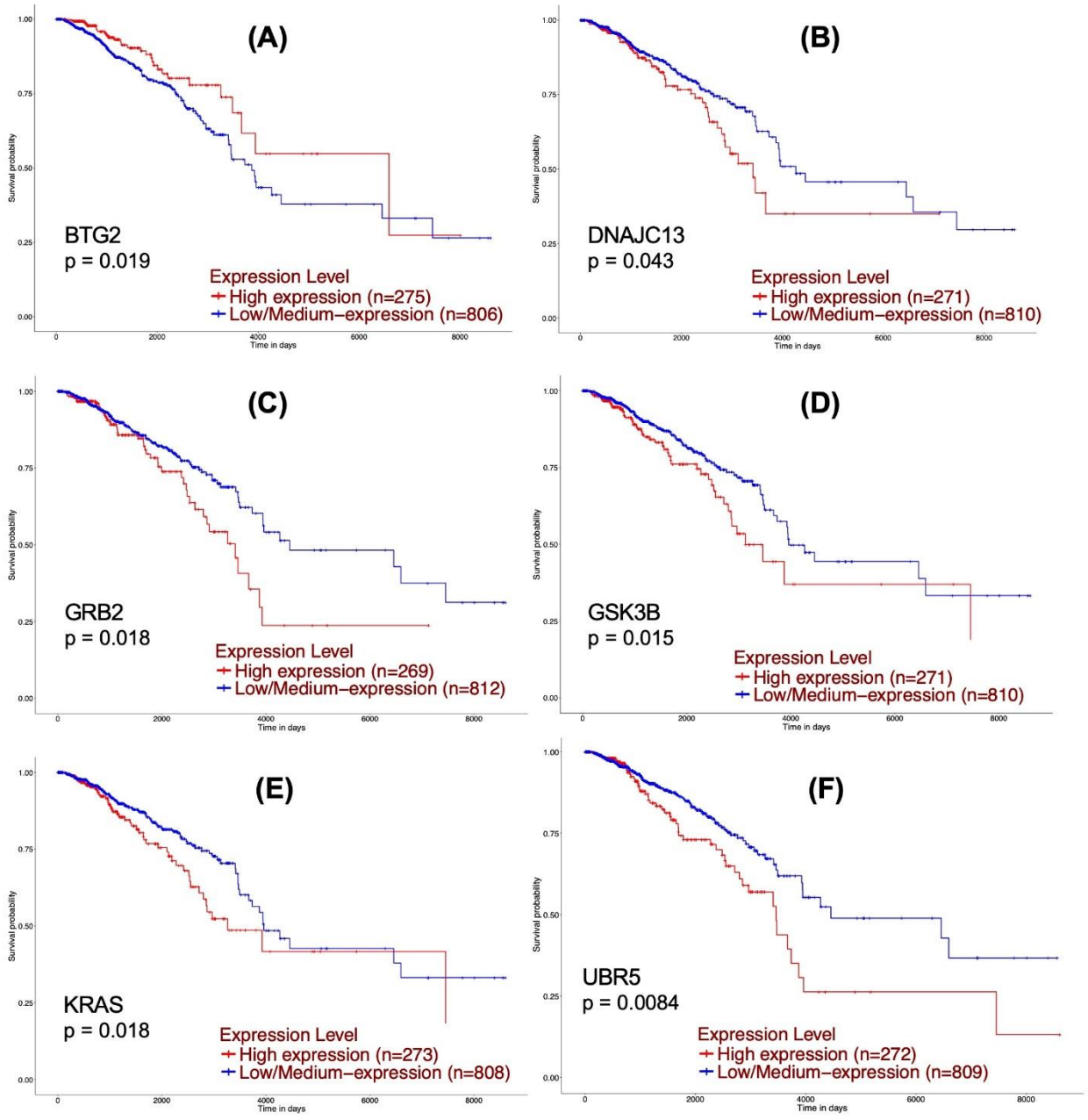

Fig. 7. Kaplan–Meier overall survival analysis on the effect of the expression levels of six miR-27b-3p target genes on breast cancer patient survival. (A) BTG2, (B) DNAJC13, (C) GRB2, (D) GSK3B, (E) KRAS, and (F) UBR5. The survival curves were plotted using the UALCAN web server.

Discussion

miRNA plays a significant role in cancer development and progression by inhibiting the translation of mRNA to protein or promoting mRNA degradation. Our previous study found that miR-145-5p, miR-22-5p, and miR-27b-3p are highly expressed in the plasma of breast cancer patients compared to healthy

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individual (12). miR-27b has been recorded to be involved in many biological processes, such as angiogenesis, proliferation, metastasis, and drug resistance. It has also been reported to have dual roles as either a tumor oncogene or a tumor suppressor (25). Hannaffon *et al.* (26) reported that miR-27b-3p had an oncogenic effect on MCF7 breast cancer cells. Demolish of miR-27b in human breast cancer cell lines inhibited breast cancer growth. However, the finding by Chen et al. opposes that fact because their study showed low expression of miRNA-27b was associated with multi-chemoresistance. Low expression of miRNA-27b will directly regulate CBLB and GRB2 genes. That gene will cause the inactivation of MAPK/Erk and P13K/Akt signaling pathway that finally causes the tumor to be more resistant to the chemo drug (27). Meanwhile, miR-22-5p and 145-5p have been reported to be related to many diseases, such as cardiovascular, neurological, inflammatory, metabolic, gastrointestinal tract, reproductive disease, and viral infection. miRNA-22-5p, on the other hand, they show significantly higher expression in patients with Hashimoto Thyroiditis compared to healthy individuals (28).

Out of the 606 miR-27b-3p target genes, BTG2, DNAJC13, GRB2, GSK3B, KRAS, and UBR5 were significantly enriched, and their high expression levels were reported to significantly affect breast cancer patient survival, which supports the critical involvement of miR-27b-3p in breast cancer. The B-cell translocation gene 2 (BTG2) comprises two preserved domains, BTG boxes A and B, and is associated with various cellular functions, including cell proliferation, acting as a tumor suppressor, and participating in DNA damage repair (29). The investigation into the roles of BTG2 in breast cancer has been elusive until now; however, recent studies by Wang et al. have revealed that the overexpression of BTG2 leads to the suppression of cell proliferation and invasion in luminal A breast cancer (30). Another study by Zubair et al. supported the role of BTG2 in cell proliferation. In this context, BTG2 is negatively regulated by protein kinase B via Erk1/2 inhibition, therefore causing an increase in cell survival and proliferation (31). Furthermore, research indicates that the upregulation of BTG2 and the activation of AKT can reduce the effectiveness of tamoxifen in managing estrogen receptor (ER)-positive breast cancer. This outcome is associated by activating the human epidermal growth factor receptor (HER) pathway, wherein reduced BTG2 expression correlates with breast cancer recurrence in patients undergoing tamoxifen treatment (32). According to Kim *et al.* (33) BTG2 protects cells from neoplastic transformation by restricting the abundance of messenger RNA at the posttranscriptional level. In our study, the involvement of BTG2 in regulating nuclear-transcribed mRNA poly (A) tail shortening further supports the role of BTG2 in modulating the frequency or rate of nuclear-transcribed mRNA production. Besides, miR-27a-3p appears to regulate cell proliferation and apoptosis in breast cancer cells by targeting BTG2 and enhancing the PI3K/Akt pathway, resulting in Adriamycin resistance in breast cancer treatments (34).

DnaJ homolog subfamily C member 13 (DNAJC13), on the other hand, is a family member of the heat-shock protein 40 that plays a vital role in early endosome trafficking (35). DNAJC13 is often associated with Parkinson's disease (36), and as of now, there have been no reported studies linking miR-27b with DNAJC13 in breast cancer. However, a study by Yang *et al.* (37) has confirmed the interaction between miR-193b and DNAJC13. The expression of DNAJC13 decreased as a result of miR-193b inhibition in breast cancer cells, implying the regulatory role of miR-193b in promoting cancer progression by targeting DNAJC13.

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In our study, Kirsten rat sarcoma virus (KRAS) and growth factor receptor bound protein 2 (GRB2) were enriched in breast cancer-associated pathways, including activated NTRK2 signals through PI3K and RAS, EGFR transactivation by gastrin, MET activates PI3K/AKT signaling and PTPN11, SOS-mediated signaling, and FGFR4 signaling. KRAS serves as a proto-oncogene, playing a significant role in the signaling pathways and regulating cell division (38). The protein-encoding KRAS gene is integral to the Ras/MAPK (mitogen-activated protein kinase) signaling pathway, which mediates the cellular response to growth signals in various cancers (39). Mutations in the KRAS gene guide the production of a hyperactive, mutated KRAS protein resistant to normal regulatory mechanisms (40). According to Waters *et al*. (41) ~20% of mutations gathered from the Catalogue of Somatic Mutations in Cancer (COSMIC) were identified in the KRAS gene, making them a focal point for research and the development of targeted therapies. Elevated KRAS signaling, frequently observed in pancreatic and non-small cell lung cancer with a high KRAS mutation rate, differs from the $\leq$ 2% occurrence of KRAS mutations found in breast cancers (42). Although mutations are not common in breast cancer, a recent review by Tang *et al.* (43) reported that the persistent activation of the EGFR/KRAS/MAPK/SIAH pathway is responsible for driving the malignancy of triple-negative breast cancer. KRAS was also identified as a crucial regulator influencing the metastatic characteristics associated with the mesenchymal features of breast cancer cells (44). In another example, miR-382-5p expedites breast cancer progression by modulating the RERG/RAS/ERK axis (45). Overexpression of miR-30c also leads to the downregulation of KRAS, resulting in the inhibition of proliferation in breast cancer cells (46). Furthermore, miR-200c exerts an inhibitory impact on tumor growth by negatively regulating KRAS (47). While it is undeniable that the participation of KRAS in breast cancer is a significant factor, no studies have reported on the associated roles between KRAS and miR-27b-3p in breast cancer thus far.

Metastasis is the prime cause of death in cancers. GRB2 is known for its role in metastasis, which facilitates the recruitment of various signaling molecules to receptors that trigger cellular responses like proliferation and invasion. As primary tumors advance, cells at the primary site undergo detachment from neighboring cells due to the Integrin-FAK-Grb2-ERK2 signaling cascade. Grb2 interacting with FAK, Shc, and other proteins activates the Ras and ERK2 pathway. The aberrant activation of matrix metalloproteinases precedes invasion into surrounding tissue. Several angiogenic signals rely on GRB2 to generate new blood vessels through growth factor receptors or integrin signaling as tumor cells reorganize cytoskeletal. Tumor cells infiltrate the systemic circulation, adhere to endothelial cells through Selectins, and rapidly proliferate or remain dormant until stimulated in the new environment (48). At the metastatic site, the Grb2-Sos-Ras-MAPK cascade plays a role in promoting proliferation and invasiveness. It begins with the binding of Grb2 to phosphorylated tyrosine residues on activated receptor tyrosine kinases (RTKs), leading to the recruitment of Son of Sevenless (Sos) to the plasma membrane (49). Sos serves as a guanine nucleotide exchange factor for Ras proteins, a molecular switch that, upon becoming GTP-bound, activates MAPK signaling pathways (50, 51).

In addition to RAS, our findings revealed that KRAS and GRB2 are important in activating neurotrophic tyrosine receptor kinase 2 (NTRK2) signals through the PI3K pathway. In recent years, the NTRK gene family has emerged as a novel predictive biomarker in breast cancer, where the frequent events

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of copy number gain and amplification in NTRK were discovered and may play a predictive role, as detected via next-generation sequencing technology (52). Activation of NTRK2 results in the engagement and stimulation of PI3K, which, in turn, phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP2) to produce phosphatidylinositol 3,4,5-trisphosphate (PIP3), a second messenger that activates Akt signaling molecules (53–55). Simultaneously, NTRK2 activation initiates the RAS/MAPK pathway, activating the RAS protein and protein kinase cascades, including RAF, MEK (MAPK/ERK kinase), and ERK (56). Akt and MAPK will ultimately influence diverse cellular processes such as cell proliferation and differentiation.

Dysregulation of E3 ubiquitin-protein ligase (UBR5) has also been involved in breast and ovarian cancer. Liao *et al.* (57) work has uncovered a distinctive function of UBR5 in the hostility of a murine TNBC model, which also enhances metastasis in ovarian cancer by facilitating the infiltration of immunosuppressive tumor-associated macrophages (TAMs) through the mediation of CCL2 (Chemokine (C-C motif) ligand 2) and CSF-1 (Colony-Stimulating Factor 1) (58). RNA-seq study by Wu *et al.* (59) shows that UBR5 affects genes in the IFN-γ-induced signaling pathway. Through its poly adenylate binding (PABC) domain, UBR5 enhances programmed death-ligand 1 (PDL1) transactivation by upregulating protein kinase RNA-activated (PKR) and downstream factors, including signal transducers and activators of transcription 1 (STAT1) and interferon regulatory factor 1 (IRF1). Restoring PD-L1 expression in UBR5-deficient tumor cells restores its *in vivo* malignancy. In a recent study, inhibiting UBR5 has been observed to impede lung metastases in postsurgical breast cancer by promoting apoptosis through cell division cycle 73 (CDC73) and tumor protein p53 (60).

Based on overall survival analysis, the expression of miR-27b and its six target genes significantly impacted breast cancer patients' survival. According to Chen *et al.* (27) downregulation of miR-27b is related to advanced tumor stage and poor survival compared to high miR-27b expression, contradicting our findings. However, the knockout of miR-27b in xenografts led to an increase in mouse survival, and immunohistochemical staining confirmed a significant inhibition of tumor xenograft growth, indicating that the depletion of miR-27b significantly reduces tumor growth *in vivo* (26). In our findings, we observed that elevated expression of target genes was linked to poor clinical survival among breast cancer patients, except for BTG2. The loss of BTG2 expression in patients with breast cancer has not only been linked to worse clinical outcomes but also to resistance to tamoxifen response (32). Bai *et al.* (61) reported that reduced BTG2 levels were associated with poor relapse-free survival across all subtypes of breast cancer, and in patients classified as luminal A, decreased BTG2 also showed worse overall survival and distant metastasis-free survival. Furthermore, breast cancer patients from the Psoriasis in Adolescents (PiA) cohort in Denmark with low levels of GRB2 exhibited better overall and progression-free survival (62). The correlation between GSK3B expression levels and clinicopathological data of breast cancer patients reveals that higher GSK3B levels were associated with shorter overall survival, specifically in TNBC and not in estrogen/progesterone receptor positive or human epidermal growth factor receptor 2 (HER2)-positive breast cancers (63,64). High expression of KRAS and UBR5 was also associated with poor clinical survival. Increased KRAS expression was linked to poorer survival, and this expression level independently served as a prognostic factor specifically affecting the luminal A subtype in breast cancer (65). On the other hand, breast cancer patients with overexpressed UBR5 exhibit a substantial decrease in survival, where UBR5 promotes tumor growth

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by interacting with the immune system, inhibiting $CD8^+$ T lymphocyte cytotoxic response, and independently facilitating metastasis through transcriptional control of epithelial–mesenchymal transition regulators (66).

In summary, the exploration of differentially expressed miR-27b-3p as a potential biomarker for breast cancer in Malay women through bioinformatics analysis has provided valuable insights into the intricate molecular landscape of this prevalent disease. The comprehensive investigation of MTIN in breast cancer has opened avenues for further research and clinical applications, with the revelation of miR-27b-3p holding promise as a diagnostic biomarker, offering a non-invasive means of early detection for breast cancer in the Malay population. Moreover, the main enriched cancer-related GO and pathways of miR-27b-3p target genes, such as NTRK2 signals through PI3K and RAS, EGFR transactivation, MET activates PI3K/AKT signaling and PTPN11, SOS-mediated signaling, and FGFR4 signaling, pave the way for a comprehensive understanding of complex mechanisms underlying breast cancer development and progression. As we continue to bridge the gap between bioinformatics and clinical practice, the identification of miR-27b-3p as a potential biomarker contributes significantly to the ongoing efforts in understanding and combating breast cancer, ultimately fostering advancements in precision medicine, and improving outcomes for Malay women at risk.

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Ethics approval and consent to participate

This study was also approved by the Ethics Committee of the Health Campus USM in Kelantan with ethical no: USM/JEPeM/16050172. All individuals involved in this study understand, agree, and sign the consent form.

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