Bioinformatic Profiling of miR-27b-3p in Breast Cancer among Malay Women: Uncovering Ethnic-Specific Molecular Signature

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Abstract – Breast cancer remains the most prevalent malignancy among women globally, with notable ethnic-specific differences in incidence and molecular characteristics. In Malaysia, Malay women represent a significant proportion of breast cancer cases, yet molecular profiling studies specific to this population are limited. MicroRNAs (miRNAs), particularly miR-27b-3p, have emerged as key regulators in cancer biology, influencing gene expression and tumour progression. This study aimed to investigate the role of miR-27b-3p in breast cancer among Malay women using a bioinformatics approach to identify its target genes, associated biological functions, and prognostic significance. Differentially expressed miRNAs were identified from plasma samples of breast cancer patients and healthy controls. miRNA target gene interactions (MTGIs) for miR-27b-3p were retrieved from five public databases, filtered by a confidence score ≥ 0.8, and visualized using Cytoscape. Functional enrichment analyses of Gene Ontology (GO) terms and KEGG cancer-related pathways were performed. Overall survival analysis was conducted using TCGA data via the UALCAN portal. A total of 606 high-confidence target genes were identified for miR-27b-3p. GO enrichment revealed significant associations with cellular components (e.g., serine/threonine protein kinase complex), molecular functions (e.g., mRNA 3'-UTR binding), and biological processes (e.g., mRNA stability, cell development). KEGG analysis highlighted enrichment in cancer-related pathways, including ErbB and thyroid hormone signalling. Survival analysis indicated that high miR-27b-3p expression correlates with poorer overall survival ($p = 0.027$). Eleven target genes, including GRB2, KRAS, and BTG2, were significantly associated with survival outcomes, with some exhibiting subtype-specific dual roles. In conclusion, miR-27b-3p plays a dual role in breast cancer, functioning as either an oncogene or tumour suppressor depending on the molecular subtype. These findings underscore its potential as a diagnostic and prognostic biomarker, warranting further investigation in subtype-specific contexts.

Keywords – Breast cancer, miRNAs, Malay women, bioinformatic, miRNA-target gene interactions

1 INTRODUCTION

Breast cancer is the most common malignancy among women globally, with notable differences in incidence and molecular profiles across ethnicities. In Malaysia, Malay women constitute a significant portion of breast cancer cases, yet molecular studies specific to this group are limited. MicroRNAs (miRNAs), small non-coding RNAs that regulate gene expression post-transcriptionally, are increasingly recognized for their roles in cancer initiation, progression, and metastasis. This study investigates the involvement of selected miRNA-27b-3p in breast cancer among Malay women using bioinformatics analysis to identify molecular signatures that can be used to support personalized medicine.

2 MATERIALS & METHODS

A bioinformatics approach was employed to analyze three differentially expressed microRNAs (miRNAs); miR-27b-3p, miR-145-5p, and miR-22-5 that were identified via PCR array from plasma samples of breast cancer patients compared to healthy individuals from previous study. miRNA–target gene interactions (MTGIs) were mapped using five publicly available databases: DIANA-TarBase, miRTarBase, miRNet, miRDB, and DIANA-microT. Only MTGIs with a confidence score (CS) ≥ 0.8 were considered for further analysis and visualized using Cytoscape v3.9.1. Target genes identified through the MTGI process were subjected to Gene Ontology (GO) enrichment analysis, covering three categories: biological processes, cellular components, and molecular functions, using the ClueGO plugin in Cytoscape. Additionally, these genes were enriched in cancer related pathways. A two-sided enrichment/depletion test based on the hypergeometric distribution was applied to assess statistical significance and conservatism. To control the Type I error rate (false positives) during multiple testing, the Bonferroni correction was used to adjust p-values. ClueGO employs kappa statistics to calculate the gene–term association matrix, with a kappa score threshold of 0.4 (adjustable between 0 and 1) to regulate network connectivity. The node size in the resulting network represents significantly enriched terms, based on either the percentage or the total number of genes involved. Finally, the overall survival analysis was conducted using the UALCAN data portal to evaluate their prognostic significance. Kaplan–Meier survival curves and overall survival plots were generated using breast cancer patient data from The Cancer Genome Atlas (TCGA). The log-rank test was employed to compare survival outcomes between patients with high versus low expression of the selected miRNAs.

3 RESULT

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

A total of 606 target genes were identified as being regulated by miR-27b-3p, based on the amount and reliability of evidence (confidence score ≥ 0.8) (Figure 1A). Functional enrichment analysis of these genes using the Gene Ontology (GO) term "cellular component" revealed significant enrichment in two categories: the serine/threonine protein kinase complex, with associated genes including ACVR1C, TGFBR1, CAB39, SBE2, MED13, CCNT2, CDK6, CCNT1, and CCNK; and the cyclin-dependent protein kinase holoenzyme complex, involving MED13, CCNT2, CDK6, CCNT1, and CCNK. Notably, five genes (MED13, CCNT2, CDK6, CCNT1, and CCNK) were shared between both complexes, indicating a functional connection (Figure 1B). Detailed GO IDs, terms, p-values, gene counts, and associated gene names are provided in supplementary section S1.

Further analysis revealed enrichment in two molecular function clusters: mRNA 3'-UTR binding, with genes such as CPEB3, SERBP1, ZFP36L2, KHSRP, ZFP36L1, ZFP36, AGO2, NUDT21, LARP4, TARDBP, and CELF1; and mRNA 3'-UTR AU-rich region binding, involving ZFP36L2, KHSRP, ZFP36L1, ZFP36, NUDT21, and AGO2. Six genes (ZFP36L2, KHSRP, ZFP36L1, ZFP36, NUDT21, and AGO2) were common to both functions (Figure 1C). Detailed GO IDs, terms, p-values, gene counts, and associated gene names are provided in supplementary Section S2.

In terms of biological processes, the genes were enriched in six functional clusters: erythrocyte differentiation, negative regulation of TOR signaling, regulation of nervous system development, peptidyl-serine modification and phosphorylation, epithelial and endothelial cell development, and mRNA stability and post-transcriptional regulation. Several genes, such as RAPGEF2, RAB18, AGO2, and CDK6, were found to be associated with multiple biological processes (Figure 1D). Detailed GO IDs, terms, p-values,

gene counts, and associated gene names are provided in supplementary section S3.

Finally, enrichment analysis of cancer-related pathways using KEGG revealed that the genes were involved in 23 distinct pathways. Among these, four pathways showed significant enrichment: the ErbB signaling pathway, thyroid hormone signalling, longevity regulation, and pathways regulating stem cell pluripotency (Figure 1E). These pathways are known to play critical roles in tumorigenesis, cellular metabolism, and therapy resistance. Detailed KEGG IDs, terms, p-values, gene counts, and associated gene names are provided in supplementary section S4.

3.2 Overall Survival analysis

Higher expression of miR-27b-3p was significantly associated with poorer overall survival among breast cancer patients compared to those with lower expression levels ($p = 0.027$, Figure 2). Further survival analysis of 116 target genes enriched for miR-27b-3p revealed that 11 genes showed a significant association with overall survival in breast cancer patients. These genes include BNIP3 ($p = 0.041$), BTG2 ($p = 0.019$), GRB2 ($p = 0.018$), GSK3B ($p = 0.015$), KRAS ($p = 0.018$), LARP4 ($p = 0.0099$), MAP3K4 ($p = 0.00075$), PAK6 ($p = 0.0016$), SOCS6 ($p = 0.0012$), UBE2W ($p = 0.012$), and ZFHX3 ($p = 0.0018$). Among these, ten genes showed that higher expression of miR-27b-3p was associated with poorer survival outcomes compared to lower expression levels. However, BTG2 exhibited the opposite trend, where lower expression of miR-27b-3p was linked to poorer overall survival compared to higher expression (Figure 3).

4 DISCUSSION

The functional enrichment analysis identified six biological process clusters significantly associated with genes targeted by miR-27b-3p. Literature evidence supports the relevance of all six clusters to breast cancer. For instance, mRNA stability and post-transcriptional regulation have been shown to play a critical role in breast cancer progression. Karner et al. (2025) reported a hidden metastasis-suppressive program driven by mRNA stability regulation, highlighting the RBMS3-TXNIP axis as a key post-transcriptional pathway. Dysregulation of this axis leads to transcript destabilization and promotes metastasis. Similarly, Qi et al. (2022) demonstrated that disruptions in mRNA translation and stability contribute to tumour development, including in breast cancer. In association with molecular function two term was significantly enriched. Both enriched molecular function terms are closely linked to breast cancer development. For example, ZFP36L1 is frequently mutated, epigenetically silenced, and under expressed in bladder and breast carcinomas. This gene facilitates mRNA degradation by binding to AU-rich sequences in the 3′ untranslated region (UTR). Increased expression of ZFP36L1 has been shown to reduce cell proliferation both in vitro and in vivo (Loh et al., 2020), whereas its silencing promotes tumour growth. The two enriched cellular component complex, serine/threonine protein kinase complex and cyclin-dependent protein kinase holoenzyme complex are also implicated in breast cancer. Notably, CDK6, a gene associated with both complexes, plays a pivotal role in breast cancer development. The CDK4/6 pathway is known to drive uncontrolled cell proliferation in breast cancer (Guo et al., 2025). This has led to several clinical trials investigating CDK4/6 inhibitors as treatments for hormone receptor-positive and HER2-negative breast cancer patients.

The KEGG pathway analysis revealed enrichment in several cancer-related pathways, which are known to influence tumorigenesis, cellular metabolism, and therapy resistance. For example, the thyroid hormone signalling pathway includes FOXO1, a member of the FOXO transcription factor family that acts as a tumour suppressor. FOXO1 regulates genes involved in cell cycle arrest, apoptosis, and oxidative stress resistance. Dysregulation of FOXO1 contributes to breast cancer progression and metastasis (Jiramongkol & Lam, 2020). Another gene, NRAS, enriched in this pathway, is significantly associated with triple-negative breast cancer (TNBC). Elevated expression of NRAS correlates with poor survival outcomes in breast cancer patients (Banys-Paluchowski et al., 2020).

In further survival study on elevated expression of miR-27b-3p, 11 target genes was found to be associated with poor overall survival among breast cancer patients, except for BTG2. This observation is supported by Takahashi et al. (2014), who reported that loss of BTG2 expression in breast cancer patients correlates with worse clinical outcomes and resistance to tamoxifen therapy. Similarly, Bai et al. (2017) demonstrated that BTG2 expression is reduced across various cancer types, including breast cancer. Their findings further revealed that low BTG2 levels are

associated with poor relapse-free survival across all breast cancer subtypes. In luminal A patients specifically, decreased BTG2 expression was linked to worse overall survival and distant metastasis-free survival. These findings suggest that BTG2 may serve as a potential diagnostic and prognostic biomarker in breast cancer. Several other genes with elevated expression were also associated with poor survival outcomes in breast cancer, including GSK3B, KRAS, and GRB2. Studies by Vijay et al. (2019) and Xu et al. (2021) showed that high levels of GSK3B were significantly correlated with shorter overall survival, particularly in triple-negative breast cancer (TNBC), but not in oestrogen/progesterone receptor-positive or HER2-positive subtypes. Similarly, increased expression of KRAS was linked to poor clinical survival and was identified as an independent prognostic factor in luminal A breast cancer (Hwang et al., 2019).

However, this finding contrasts with more recent data from Tokumaru et al. (2020), which reported that higher KRAS expression was associated with better survival in TNBC patients. These conflicting results suggest that KRAS may exhibit dual functionality either acting as an oncogene or a tumour suppressor depending on the molecular subtype of breast cancer. High expression of GRB2 was also associated with poor survival outcomes. GRB2 plays a multifaceted role in breast cancer progression, with its impact varying across molecular subtypes. In HER2-positive breast cancer, GRB2 promotes malignancy. Li et al. (2025) reported that GRB2 enhances brain metastasis by activating the Ras/MAPK pathway, facilitating tumour cell migration and invasion, and worsening prognosis. Additionally, Liu et al. (2025) identified GRB2 as an RNA-binding protein that regulates gene expression and alternative splicing, contributing to tumour aggressiveness in HER2-overexpressing cells. Conversely, in progesterone receptor (PR)-positive breast cancer, GRB2 appears to have a protective role. Wittayavimol et al. (2023) found that GRB2 interacts with the PR to inhibit EGF-mediated signalling, which correlates with lower tumour stage and reduced metastasis. These findings highlight the context-dependent role of GRB2, functioning as an oncogene in HER2-positive cancers and potentially as a tumour suppressor in PR-positive subtypes, underscoring its relevance as both a prognostic marker and therapeutic target.

5 CONCLUSION

Functional enrichment analysis of Gene Ontology (GO) terms and cancer-related pathways, along with overall survival analysis, suggests that miR-27b-3p and its associated genes are involved in the biological development and progression of breast cancer. Interestingly, miR-27b-3p appears to play a dual role, acting either as an oncogene or a tumour suppressor depending on the breast cancer subtype. Therefore, further studies are essential to elucidate its context-dependent functions across different subtypes, which could pave the way for its potential use as a biomarker for diagnosis or as a target for therapeutic intervention in breast cancer.

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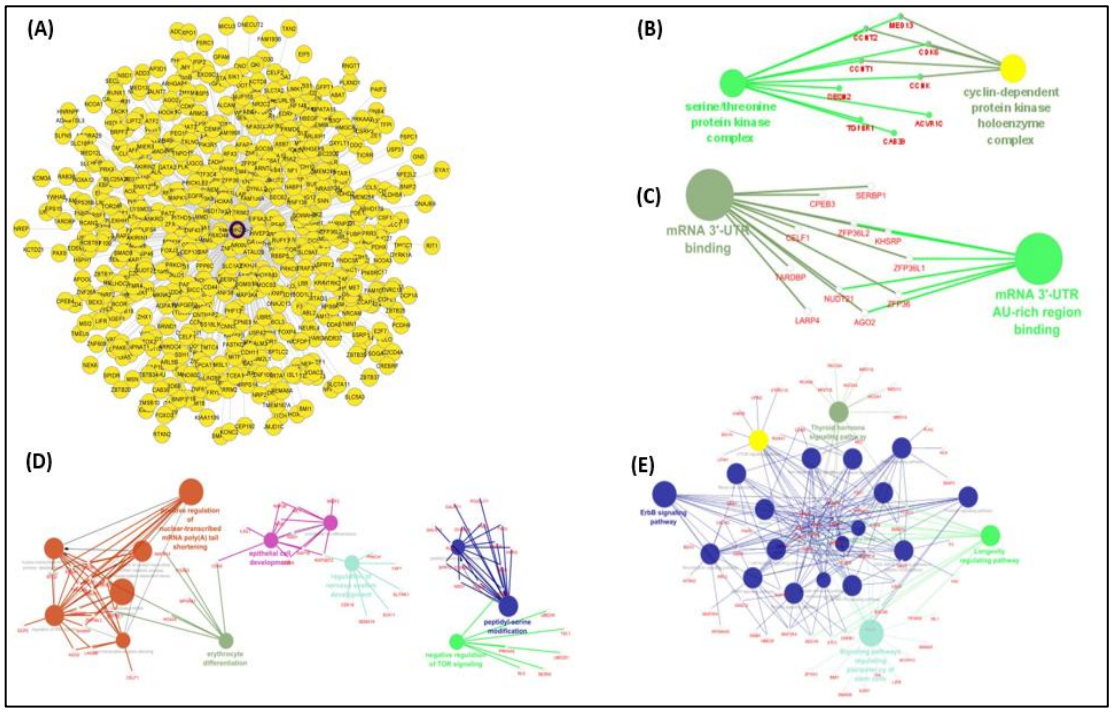

Figure 1: miRNA target interaction network (MTIN) and functional enrichment analysis (FEA) of target genes for miRNA-27b-3p. (A) MTIN, (B) network visualization of enriched cellular component, (C) network visualization of enriched molecular biology (D) network visualization of enriched biological functions, and (E) network visualization of enriched cancer-related pathway. Node size reflects term significance, and edge represent the association between terms and related genes and white nodes represent related genes.

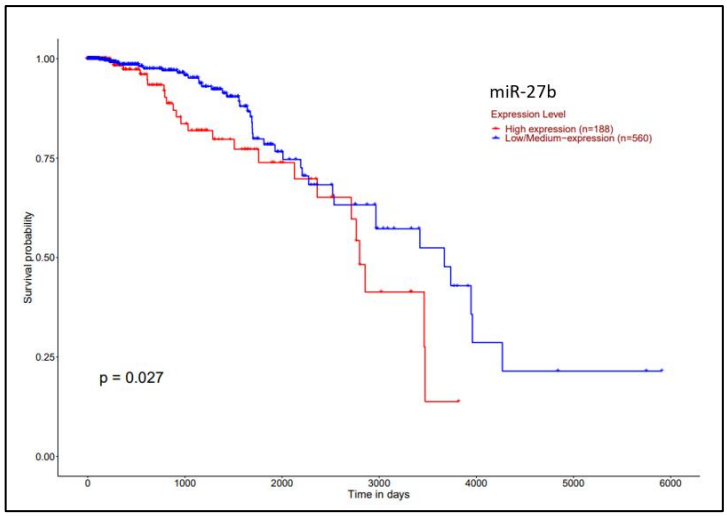

Figure 2: Kaplan–Meier overall survival analysis on the effect of the expression levels of miR-27b on breast cancer patient survival. The survival curves were plotted using the UALCAN web server. Red line indicates higher expression and blue line indicates lower/medium expression.

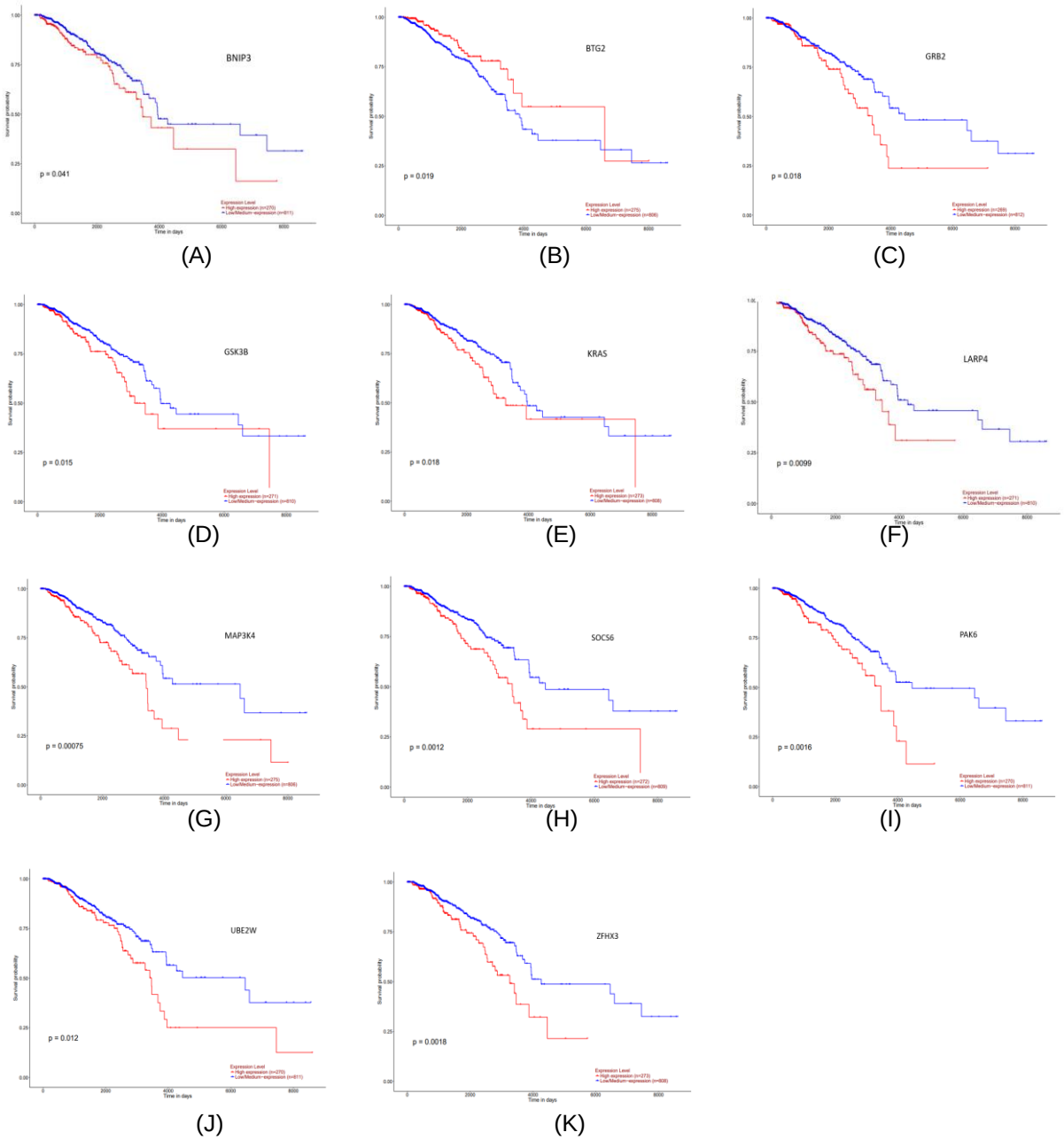

Figure 3: Kaplan–Meier overall survival analysis on the effect of the expression levels of eleven miR-27b-3p target genes on breast cancer patient survival. (A) BNIP3, (B) BTG2, (C) GRB2, (D) GSK3B, (E) KRAS, (F) LARP4 (G) MAP3K4, (H) PAK6, (I) SOC6, (J) UBE2W, and (K) ZFHX3. The survival curves were plotted using the UALCAN web server. Red line indicates higher expression and blue line indicates lower/medium expression.