ORIGINAL ARTICLE

In-Silico Data Mining Identifies Potential Methylated Genes as Candidate Saliva-Based Biomarkers for Nasopharyngeal Cancer

Almohammadin Saleh Suleiman Silmi[1,2], Rabiatul Basria S.M.N. Mydin[1], Adam Azlan[1], Gurjeet Kaur[3], Satvinder Singh Dhaliwal[3,4,5,6], Muhamad Yusri Musa[7,8,9]

[1] Department of Biomedical Science, Advanced Medical and Dental Institute, Universiti Sains Malaysia, 13200 Kepala Batas, Pulau Pinang, Malaysia.
[2] Ministry of Education, Educational Supervision Directorate, Post Office Box: 1646, Amman 11118, Jordan.
[3] Institute for Research in Molecular Medicine, Universiti Sains Malaysia, 11800 Minden, Pulau Pinang, Malaysia.
[4] Curtin Health Innovation Research Institute, Faculty of Health Sciences, Curtin University, Bentley, Australia
[5] Duke-NUS Medical School, National University of Singapore, Queenstown, Singapore
[6] Singapore University of Social Sciences, 463 Clementi Road, Clementi, 599494, Singapore
[7] Department of Clinical Medicine, Universiti Sains Malaysia, 13200 Kepala Batas, Pulau Pinang, Malaysia.
[8] Pusat Perubatan Universiti Sains Malaysia Bertam, Universiti Sains Malaysia,13200 Kepala Batas, Pulau Pinang, Malaysia
[9] Islamic Medical Association of Malaysia's (IMAM), 68100 Batu Caves, Selangor, Malaysia

ABSTRACT

Introduction: DNA methylation plays a critical role in the development of various cancers. However, current knowledge on detecting DNA methylation from saliva samples, particularly in nasopharyngeal carcinoma (NPC), remains limited. This study aims to identify key methylated genes associated with NPC using in silico approaches. **Methods:** In silico analyses were conducted utilizing publicly accessible mRNA expression and methylation datasets. Functional enrichment analyses were carried out using the GO and KEGG databases. **Results:** The analysis identified that the EGFR, PTEN, and SIK1 genes are significantly downregulated and exhibit hypermethylation. In contrast, the RCN1 gene is significantly upregulated and shows hypomethylation. Pathway enrichment analysis highlighted the significant roles of the MAPK Signaling and PI3K/Akt signaling pathways in NPC. GO enrichment analysis showed that these pathways are involved in the RNA-directed DNA Polymerase Activity associated with RNA-dependent DNA polymerase activity, Regulation of Cell Size, and cell proliferation. The identified pathways and biological processes are crucial to NPC pathogenesis. **Conclusion:** This study identified potential aberrantly methylated genes in NPC through integrated bioinformatics analysis, highlighting their potential as methylation-based biomarkers for more accurate NPC diagnosis. However, further experimental validation and complementary analyses, such as GSEA and PCR-based validation, are necessary to strengthen these findings and support their clinical translation.

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Corresponding Author:
Assoc. Prof. Ts. Dr. Rabiatul Basria S. M. N. Mydin, PhD
Email: rabiatulbasria@usm.my
Tel : +604-5622351

INTRODUCTION

Nasopharyngeal cancer (NPC) originates in the nasopharynx and exhibits significant geographical and demographic variations. The highest prevalence is in Southeast Asia, which accounts for over 68% of global NPC cases, primarily due to Epstein-Barr virus (EBV) infection as well as dietary and lifestyle factors(1) In Malaysia, NPC represents about 4.5% of all cancers, with the incidence being higher in males and particularly in the northern states of Penang and Kedah, where it can reach up to 20 cases per 100,000(2). The disease mainly affects individuals aged 30 to 50, and those with a family history of NPC are at greater risk(3). There are also ethnic differences in susceptibility, with Chinese populations being more prone to NPC compared to Malays and Indians(4). DNA methylation, especially the

hypermethylation of tumor suppressor genes, is crucial in NPC development, affecting gene expression and key signaling pathways involved in cancer metastasis(5). which can be detected in saliva(6), providing a non-invasive and cost-effective alternative to blood and nasopharyngeal swabs for NPC diagnosis. This study uses in silico analysis to identify key methylated genes in NPC, which may enhance understanding of its pathogenesis and support the development of methylation-based diagnostic biomarkers.

MATERIALS AND METHODS

Publicly the available mRNA expression and methylation data from GEO database with the accession GSE12452 and GSE52068 respectively, were analyzed(7–9)(10,11). GSE12452 included 31 NPC and 10 normal tissue samples for mRNA expression, while GSE52068 had 24 NPC and 24 normal tissue samples for methylation analysis. Significant gene methylation changes, both hypermethylated and hypomethylated, were analyzed with GEO2R and Galaxy, with differentially methylated genes (DMG) showing fold changes and adjusted P-values under 0.001 considered significant on the criteria established by (12) to minimize false positives, given the large number of genes tested. Overlapping DMEGs were analyzed using Venn diagrams and compared with salivary biomarkers from salivaDB (https://webs.iiitd.edu.in/raghava/salivadb/). Functional enrichment analyses were performed using GO and KEGG databases via Enrichr tool to explore gene roles in biological processes and pathways. Statistically significant methylation changes were defined by a p-value threshold of less than 0.05 to allow for broader inclusion of potential candidates for further validation.

RESULTS

Identification of differentially methylated genes in NPC

The analysis revealed several genes with changes in methylation status. The EGFR, PTEN, and SIK1 genes were hypermethylated and downregulated, which suggests they may act as tumor suppressors. In contrast, the RCN1 gene was hypomethylated and upregulated, indicating its potential role in promoting NPC development (Fig. 1).

GO and KEGG Enrichment Analysis of Methylation-Driven Expression Genes (MDEGs)

GO enrichment analysis revealed that the MDEGs are involved in several key biological processes, including 'Negative Regulation of Cell Differentiation,' 'Negative Regulation of Cell Size,' and 'Regulation of Striated Muscle Cell Differentiation.' Cellular components associated with these genes include the 'Intracellular Vesicle,' while their molecular functions involve 'RNA-directed DNA Polymerase Activity,' 'Kinase Binding,'

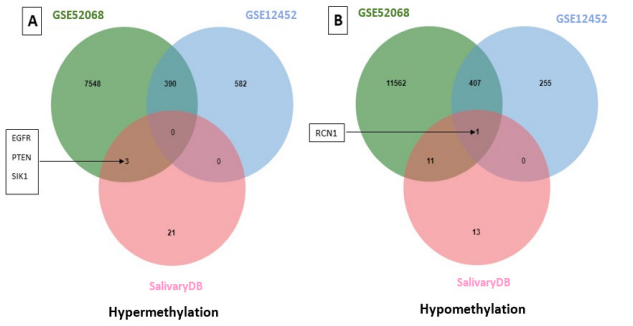

Figure 1: Identification of Hypermethylated-Downregulated Genes. (A) Overlap of hypermethylated and downregulated genes (EGFR, PTEN, SIK1) across the GSE52068, GSE12452, and SalivaryDB datasets. (B) Overlap of hypomethylated and upregulated gene (RCN1) across these datasets.

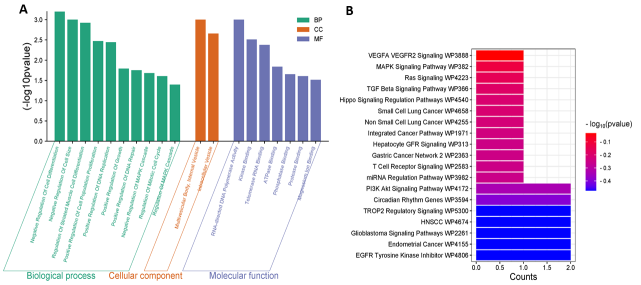

Figure 2: GO and KEGG Enrichment Analysis of MDEGs. (A) Bar graph displaying p-values for significant GO terms related to MDEGs. (B) KEGG pathway enrichment results, with adjusted p-values below 0.05 considered statistically significant.

and 'Telomerase RNA Binding' (Fig. 2a). KEGG pathway analysis indicated that MDEGs were primarily enriched in the ' PI3K/Akt signaling pathway,' 'VEGFA-VEGFR2 Signaling Pathway,' and 'MAPK Signaling Pathway' (Fig. 2b).

DISCUSSION

The results of this study emphasize the crucial role of DNA methylation in the pathogenesis of NPC. Through the identification of EGFR, PTEN, and SIK1 as significantly downregulated and hypermethylated genes, as well as RCN1 as an upregulated and hypomethylated gene, these genes are involved in essential pathways, including those related to the negative regulation of cell differentiation. The negative regulation of cell differentiation is crucial in NPC and other head and neck cancers, as disrupted differentiation can lead to cancerous changes and breakdowns in normal tissue function. In NPC, this disruption is evident from the overexpression of basal cell-specific proteins and the downregulation of luminal cell proteins, highlighting a disturbance in differentiation pathways(13). Similarly, the negative regulation of cell size in NPC cells is influenced by mechanisms affecting key signaling pathways like PI3K/Akt and focal adhesion kinase, which are essential for cell mitosis and interactions with the extracellular matrix(14). Additionally, the PI3K/Akt pathway plays a crucial role in NPC by promoting cell survival, proliferation, and metastasis, with its activation

being linked to the epithelial-to-mesenchymal transition (EMT), a process that enhances tumor cell motility and invasiveness(15).

RCN2 protein plays a significant role in disrupting calcium homeostasis, leading to mitochondrial apoptosis, a critical factor in NPC malignancy(16). RNA-directed DNA polymerase activity, which is often associated with reverse transcriptase, is crucial in NPC for integrating viral DNA into the host genome, thus influencing oncogenesis(17). This activity interacts with regulatory molecules like PTEN, whose methylation leads to the inactivation of its tumor suppressor functions(18), further emphasizing its role in NPC development and progression(19). There is no accurate information on how methylation changes in SIK1 influence NPC. However, methylation of tumor suppressor genes generally contributes to cancer progression by silencing these genes, whereas SIK1 facilitates apoptosis in cancer cells, contributing to tumor suppression(20). Nonetheless, low SIK1 expression in NPC is associated with aggressive tumor characteristics and poor outcomes, suggesting that SIK1 downregulation may promote cancer progression and metastasis(21). Moreover, Methylation of the EGFR promoter region typically downregulates EGFR expression, thereby reducing the activation of downstream pathways such as the MAPK pathway. Conversely, (22), EGFR activation triggers the PI3K/Akt /mTOR pathway, which is crucial for cell proliferation and survival, further enhancing the aggressive nature of NPC and supporting tumor growth(23).

KEGG analysis found that the VEGFA-VEGFR2 signaling pathway is vital for NPC progression, where VEGFA-VEGFR2 signaling is essential for angiogenesis, which is the formation of new blood vessels. This pathway supports tumor growth by ensuring a sufficient blood supply, promoting epithelial-mesenchymal transition, activating matrix metalloproteinases, and enhancing tumor invasion and metastasis promoting epithelial-mesenchymal transition, activating matrix metalloproteinases, enhancing tumor invasion and metastasis(24) (25). This pathway is closely linked to regulatory RCN1, EGFR, PTEN, and SIK1 genes, which impact cell differentiation and survival pathways(26). The MAPK signaling pathway, activated in over 50% of oral cancer cases, including NPC, is associated with the modulation of EGFR and PTEN genes, crucial for controlling cellular growth and preventing uncontrolled proliferation(27). Additionally, can inhibit pro-apoptotic signaling, thus preventing programmed cell death and supporting tumor growth(28). The dysregulation of these pathways underscores their significant roles in NPC tumorigenesis and malignancy, offering a deeper understanding of the molecular mechanisms driving the disease. It is worth mentioning here that, these genes were identified in the dataset obtained from the SalivaDB platform, which serves as a repository for saliva-derived genes, as shown in (Fig. 1). This finding, along with previous analyses, strongly supports the potential of these genes as biomarkers for early cancer detection, reinforcing the significance of saliva as a promising medium for non-invasive cancer diagnosis.

CONCLUSION

This study effectively identified critical methylated genes involved in NPC through comprehensive bioinformatics analysis. The genes EGFR, PTEN, and SIK1 were observed to be hypermethylated and downregulated, indicating their potential role as tumor suppressors. In contrast, RCN1 was hypomethylated and upregulated, suggesting its contribution to NPC progression. Pathway enrichment analyses underscored the significance of the MAPK and PI3K/Akt signaling pathways, particularly in regulating cell differentiation and size. These findings offer important insights into the molecular mechanisms of NPC and highlight methylation-based biomarkers that could enhance future diagnostic precision. Although this in-silico approach successfully identified key methylation biomarkers in NPC, additional analyses, including experimental validation and pathway enrichment through GSEA, are necessary to further substantiate these findings and explore their potential clinical applications.

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Ethical Clearance

This study uses only the publicly accessible and available datasets from NCBI Gene Expression Omnibus (GEO) database, with the accession GSE12452 and GSE52068, additional ethical clearance is therefore not applicable. These datasets are available in the GEO repository via http://www.ncbi.nlm.nih.gov/geo .

Conflict of interests

The authors declare no competing financial and non-financial interests.

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