ORIGINAL ARTICLE

Exploring Long Non-Coding RNAs As Potential Therapeutic Targets For Nasopharyngeal Cancer

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ABSTRACT

Introduction: Long non-coding RNAs (lncRNAs) play a key role in regulating cancer and other biological processes. Nasopharyngeal cancer (NPC), the fourth most common cancer in Malaysia, is often diagnosed at a late stage with a poor prognosis. Identifying reliable, non-invasive lncRNA biomarkers could enhance early detection and management of NPC. **Methods:** This study used an in-silico approach to analyze public datasets and identify significant lncRNA expression patterns associated with NPC. The significant lncRNAs are also subjected to pathway enrichment analysis which shows several significant pathways relating to NPC molecular landscape. **Results:** Through the analysis, the upregulation of lncRNA AATBC, MSC-AS1, and LHFPL3-AS1, as well as the downregulation NR2F2-AS1 and ZNF667-AS1 depicts a significant expression in NPC subjects that have either oncogenic and tumour suppressive properties that may be involved in NPC metastasis. These lncRNA were found to involve in specific pathway in NPC mechanism which previous study have not been investigated. **Conclusion:** As the dataset used were from the south eastern region, the results are constrained to that geographic area and are not generalizable to other areas. In any case, understanding these significant lncRNA biomarkers in NPC, as compared to healthy individuals, could provide valuable insights for improving NPC management, particularly in developing therapeutic strategies as they effect specific pathway in NPC.

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INTRODUCTION

Nasopharyngeal cancer (NPC) is a specific type of head and neck cancer that originates from the epithelial cells of the nasopharynx. It is notably prevalent in regions like Southeast Asia and is closely associated with Epstein-Barr virus (EBV) infection, genetic factors, and environmental influences (1,2). Despite progress in diagnostic techniques and treatment options, such as radiotherapy and chemotherapy, the prognosis for advanced NPC remains poor due to late diagnosis and high recurrence rates (3). Therefore, there is an urgent need to identify new therapeutic targets to improve outcomes for NPC patients. Recent research has emphasized the significant role of long non-coding RNAs (lncRNAs) in regulating essential cellular processes like gene expression, cell proliferation, apoptosis, and metastasis (4,5). Abnormal lncRNA expression has been linked to the development and progression of various cancers, including NPC, though the precise mechanisms involved are not fully understood (6). This study aims to explore the role of lncRNAs in NPC by comparing their expression in NPC tumours to normal nasopharyngeal tissues through in-silico analyses. The research will assess the functional impact of these lncRNAs on NPC cell behavior and determine whether they have oncogenic or tumor-suppressive properties. The results could lead to early detection and highlight potential new therapeutic targets by connecting lncRNA effects on tumor cell proliferation, apoptosis, migration, and invasion to existing findings, thereby potentially enhancing a more personalised treatment strategies for NPC patients.

MATERIALS AND METHODS

The analysis of differential expression of long non-coding RNAs (lncRNAs) in NPC begins by selecting appropriate datasets for identifying lncRNA biomarkers. RNA sequence data were sourced from the publicly available NCBI GEO database. The chosen dataset, accession number GSE118719 (7), includes six samples each of NPC and healthy tissue. As small sample size (only six NPC and six healthy tissues) creates a risk of bias in our results such as overfitting and limited generalizability. Hence, certain measures were taken to minimized the risk of biasing the results by comparing the results with studies which used larger datasets. Data were analyzed using the GEO2R tool (https://www.ncbi.nlm.nih.gov/geo/geo2r/), with standard parameters to obtain p-values, adjusted p-values (padj), log fold changes, and expression levels. LncRNAs were filtered based on their relevance from recent research, and criteria for p-values, padj-values, and log fold changes were set < 0.05 p-value and log fold change at >1.5. Significant lncRNAs were then visualized using heatmaps and bar graphs. The most notable upregulated and downregulated lncRNAs were further analyzed through pathway enrichment using KEGG and GO tools via the Enrich website platform (www.maayanlab.cloud/Enrich).

RESULTS

Differentially Expressed lncRNAs

The dataset chosen was obtained to find a neutral dataset that is not solely dependent on Epstein-Barr virus (EBV)-related cases and comes from the Southeast Asian region. From the chosen samples, the expression levels of 21 lncRNAs were obtained from the dataset and visualized using heatmaps and bar charts in Fig. 1. Among these, 18 lncRNAs were upregulated: AATBC, SNHG1, AFAP1-AS1, LHFPL3-AS1, LINC01503, LINC00173, MSC-AS1, SNHG15, PTPRG-AS1, LINC01376, SRRM2-AS1, MEG3, GNAS-AS1, LINC01515, SLC25A21-AS1, RGMB-AS1, FOXP4-AS1, and LINC00240. The remaining 3 lncRNAs were downregulated: NR2F2-AS1, LINC00324, and ZNF667-AS1. Additionally, some of these differentially expressed lncRNAs are known to be involved in other cancers. Among the lncRNAs stated, five lncRNAs were selected for detailed analysis due to their significant expression changes: three upregulated (AATBC, MSC-AS1, and LHFPL3-AS1) and two downregulated (NR2F2-AS1 and ZNF667-AS1). These selected lncRNAs were further investigated to understand their roles in the molecular mechanisms underlying NPC. In-silico studies provides useful insights, but they are restricted since they do not fully represent biological complexity or experimental settings. Future experimental validation is crucial for confirming these findings and ensuring their biological significan.

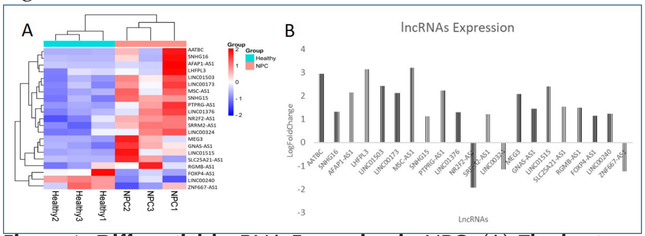

Figure 1: Differential lncRNA Expression in NPC. (A) The heatmap displays expression of lncRNAs. The heatmap showed significant differences in lncRNA expression profiles between NPC and healthy subjects, with NPC samples predominantly displaying red, indicating upregulation, while healthy samples predominantly showed blue, indicating downregulation. (B) The bar graph depicts lncRNA expression levels based on log fold change. The bar chart illustrated upregulated of lncRNAs having positive values ranging from 1 to 3, and downregulated lncRNAs having negative values between -1 and -2 Both of the heatmap and bargraph were obtain through GSE118719 dataset.

Pathway Enrichment

The pathway enrichment analysis included targeted genes associated with the chosen lncRNA, such as PNN, NR4A2, HOXA6, PTEN, and ABLIM1 as mentioned in the previous section based on the study carried out by recent articles. Key pathways related to NPC mechanisms identified in the analysis included the Regulation of Protein Kinase B signalling pathway, Actin Binding, and WNT signalling pathway in Fig.2(A). In KEGG enrichment analysis, significant pathways included are the TGF-beta, PI3K-Akt, and VEGFA-VEGFR2 signalling pathway in Fig. 2(B).

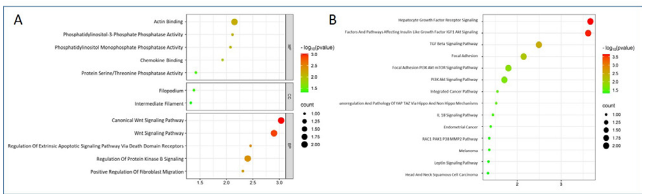

Figure 2: GO and KEGG Enrichment Pathways. (A) GO enrichment pathways, categorized into Molecular Function (MF), Cellular Component (CC), and Biological Process (BP). (B) KEGG enrichment pathways.

DISCUSSION

The study found 21 significant lncRNAs when compare the NPC subjects to healthy subjects, which five lncRNAs where deem to be the most significant which were three upregulated, AATBC, MSC-AS1, and LHFPL3-AS1 and two downregulated, NR2F2-AS1 and ZNF667-AS1. Through the extensive study, we carried out all five lncRNAs have a role in either tumour suppressive or oncogenic properties the mechanism of NPC, particularly focusing on AATBC. Similar outcomes to the study, a research found that AATBC promotes NPC cell migration and invasion by regulating PNN, which enhances the ZEB1-mediated epithelial-to-mesenchymal transition (EMT) process in NPC cells (8). Additionally, MSC-AS1 has been shown to induce NPC cell proliferation and inhibit apoptosis by sponging miR-524-5p, leading to increased expression of NR4A2, an oncogene implicated in various cancers, including NPC (9). For Lipoma HMGIC Fusion Partner-Like 3 Antisense RNA 1 (LHFPL3-AS1), it is highly expressed in NPC tissues and cells, where it is associated with radiosensitivity. LHFPL3-AS1 binds to miR-143-5p, resulting in increased expression of HOXA6, possibly contributing to radiation resistance in NPC cells (10). The elevated levels of LHFPL3 could potentially lead to radioresistance, affecting the efficacy of primary NPC treatments.

The lncRNAs with diminished expression include Nuclear Receptor Subfamily 2 Group F Member 2 Antisense RNA 1 (NR2F2-AS1) and ZNF667-AS1, each impacting NPC cells in distinct ways. NR2F2-AS1 acts as a tumor suppressor by promoting the expression of PTEN, a known tumor suppressor that inhibits the PI3K/Akt pathway, a crucial pathway for cancer cell survival. The reduced expression of NR2F2-AS1 leads to decreased PTEN levels, resulting in enhanced PI3K/Akt signalling and reduced apoptosis in cancer cells (11). In contrast, ZNF667-AS1 has the opposite effect on NPC cell proliferation through different mechanisms. Reduced ZNF667-AS1 expression increases miR-1290 levels, which binds to and decreases the expression of actin-binding LIM protein 1 (ABLIM1). This interaction promotes increased cell proliferation and migration in NPC cells (12). Thus, while NR2F2-AS1 functions as a tumor suppressor, ZNF667-AS1 exhibits oncogenic properties, highlighting the diverse roles of these lncRNAs in NPC.

As for the enrichment pathway the most significant GO pathway were Regulation of Protein Kinase B signalling and Actin Binding is involved in epithelial-to-mesenchymal transition (EMT), which is crucial in NPC progression, while the WNT signalling Pathway is also significantly affected in NPC (13). While for significant pathway in KEGG were the PI3K-Akt and VEGFA-VEGFR2 signalling Pathways are well-established in NPC, whereas the TGF-beta signalling Pathway plays a role in various cellular processes such as proliferation, migration, and EMT in NPC (14,15). Based on the results obtained, it could lead to a more robust and efficient diagnostic and tool which are feasible but issues such as establishing consistent expression patterns across varied populations, sensitivity in body fluids, and validation in larger cohorts must be addressed. With sufficient validation and technical improvements, these lncRNAs may become viable, non-invasive biomarkers for NPC detection. These challenges are also several limitations of the study. Nevertheless, there are reliable tools which can detect lncRNA precisely such as qRT-PCR, RNA sequencing and Microarray analysis, making lncRNAs to a more dependable biomarker. This study focuses on subset of lncRNAs expression which are distinct from other studies which depicts a preliminary information regarding possibilities of these lncRNAs based on their expression involving in specific pathway in NPC. There are also possibilities of the markers being much more significant in other cancers thrugh similar molecular pathways compared to NPC. Future studies need to carried out in confirming these discoveries.

CONCLUSION

This study identified five lncRNAs—AATBC, MSC-AS1, LHFPL3-AS1 (oncogenic), and NR2F2-AS1 (tumor suppressor), along with ZNF667-AS1 (oncogenic)—as significant in NPC. These lncRNAs exhibit critical roles in NPC progression and response to treatment. Pathway enrichment analyses highlighted crucial pathways such as TGF-beta, PI3K-Akt, and VEGFA-VEGFR2 signaling, relevant to NPC. These findings suggest that these lncRNAs could serve as potential biomarkers and therapeutic targets for improving NPC diagnosis and treatment strategies. Future studies are necessary to validate these findings such as performing RNA sequencing in clinical samples, larger sample size and functional assays.

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ETHICAL CLEARANCE

This study only use publically available dataset set from NCBI GEO coded under GSE118719, which does not require additional ethical approval.

CONFLICT OF INTEREST

The author declares no competing interests.

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