RESEARCH ARTICLE

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Circulating mRNA Expression of *VEGF, PTEN*, and *SOCS1* as Potential Prognostic Predictor for Nasopharyngeal Carcinoma Progression

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Abstract

Background: The molecular mechanisms underlying nasopharyngeal carcinoma (NPC) progression remain poorly understood. In particular, the roles of circulating mRNAs encoding key regulatory proteins have yet to be explored. This study aimed to identify NPC-associated expression signatures of circulating *VEGF*, *PTEN,* and *SOCS1* mRNAs and their potential as biomarkers. **Methods:** A case-control study was conducted comprising 160 nasopharyngeal carcinoma (NPC) patients and 80 controls, from whom peripheral blood samples. Total RNA was extracted and the levels of *VEGF*, *PTEN*, and *SOCS1* mRNAs were quantified using reverse transcription quantitative PCR (RT-qPCR). Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method. Bioinformatic analyses, including GeneMANIA, Gene Ontology (GO), and KEGG pathway analysis, were performed to predict the functional roles and interactions of these mRNAs. **Results:** We identified significantly increased circulating *VEGF* mRNA in lymph node metastases (1.66-fold, $p<0.05$) and elevated SOCS1 mRNA in late-stage NPC (20-fold, $p<0.05$). *PTEN* mRNA was reduced 4.26-fold in NPC patients. These data suggest that circulating *VEGF*, *PTEN*, and *SOCS1* mRNAs represent signatures of NPC progression and can potentially be biomarkers. Network analyses implicate these mRNAs in mechanisms enabling NPC pathogenesis. **Conclusions:** Our study reveals NPC-associated expression changes of circulating *VEGF*, *PTEN*, and *SOCS1* mRNAs. These molecular signatures may serve as biomarkers during NPC progression and provide insights into underlying mechanisms. Further validation of their utility as prognostic indicators of NPC is warranted.

Keywords: Nasopharyngeal Carcinoma- Circulation- mRNA Expression- Prognosis Biomarker- Clinical Outcome

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Introduction

Nasopharyngeal carcinoma (NPC) is an epithelial malignancy arising within the nasopharynx [1, 2]. In 2018, approximately 129,079 people worldwide, with 85% in Asia, were diagnosed with nasopharyngeal cancer, making it the 23rd most common cancer globally. Indigenous populations in East and Southeast Asia, the Arctic, North Africa, and the Middle East have been affected by NPC for many years. In Southeast Asia, NPC ranked 9th among incident cancers and 8th among cancer-related deaths [3]. In 2020, NPC caused the death of 80,008 cancer patients worldwide, with 85.5% in Asia of those deaths occurring in Asia [4]. Surprisingly, Indonesia ranked fifth globally regarding new NPC cases in 2020, with 19,943 new cases reported. NPC prevalence in Indonesia was approximately 28.35% (948 of 3344), followed by a prevalence of 14.35% for skin cancer and 12.3% for lymphoid malignancies, resulting in 13,399 deaths in the same year [4, 5]. Mortality and year-life rates for NPC showed an increase with age and were higher in males than females [6, 7]. Managing NPC poses significant challenges due to recognizing its signs and symptoms. Delays in seeking medical advice, misleading symptoms, and challenges in

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clinical examinations of the nasopharynx result in late-stage diagnoses [8]. Distinguishing early-stage NPC from nasopharyngeal lymphoma using imaging-based methods is challenging, as NPC tumors near the skull base exhibit irregular shapes. These challenges often rely on clinicians' subjective interpretations. Hence, developing alternative laboratory methods to assist with NPC diagnosis is necessary.

Early and effective diagnosis and treatment are crucial to prevent NPC progression, recurrence, or metastasis. The gold standard for diagnosing primary NPCs involves pathological assessment of postnatal space biopsies. However, tissue biopsy procedures are invasive and painful, leading to lower patient compliance and delayed diagnosis until the disease has advanced [9]. While tissue biopsy remains the preferred diagnostic method in a clinical setting, liquid biopsy of cancer-related materials in the bloodstream offers a promising alternative [10]. Liquid biopsy utilizes circulating tumor cells, tumor cell fragments, nucleic acids, and proteins to improve cancer diagnosis and enable early detection. One such molecule in tumor cells is messenger RNA (mRNA) [11].

mRNA (messenger RNA) is a large family of RNA molecules that carry genetic information of DNA and serve as templates for protein synthesis by ribosomes [12]. Circulating RNA, including tumor-associated cell-free mRNA, has been detected in the plasma/ serum of cancer patients and healthy individuals. Detecting tumor-associated cell-free mRNA opens new avenues for cancer screening and monitoring [13]. Accumulating evidence suggests that dysregulated mRNA expression, particularly abnormal expression of tumor-associated mRNA, is closely linked to the development of various diseases, including NPC [14]. Studies have shown that cell-free mRNA from cancer patients can be utilized as an early detection and diagnosis tool for various types of cancers [15]. Additionally, elevated mRNA expression has been associated with poor prognosis and clinical status in patients.

In this study, we aimed to quantify circulating *VEGF*, *PTEN*, and *SOCS1* mRNA expression in NPC patients compared to healthy controls. Correlations between mRNA levels and salient clinical and pathological features were evaluated. We further sought to assess the feasibility of plasma *VEGF*, *PTEN*, and *SOCS1* mRNA quantification for non-invasive diagnosis and prognostication in NPC. Our overarching goal was to evaluate these mRNA biomarkers to improve NPC detection and clinical management in high-risk groups such as Indonesian populations.

Materials and Methods

Ethical statement

This study was approved by the Ethical Committee Jenderal Soedirman University, Indonesia (Number: 016/KEPK/PE/IV/2022), and the research followed the Helsinki Declaration. All participants provided informed consent prior to enrollment.

Study Participants

The study comprised peripheral blood patients with NPC (N = 160) and non–tumor patients [80]. Peripheral blood was collected in the EDTA-contained tubes, and plasma isolation was conducted by centrifuge at 1500 rpm for 10 minutes at 4ºC. Long-term storage was saved at a temperature of -80 °C. NPC diagnosis was confirmed by histopathology and elevated EBV serological markers (EBV-EA, EBV-EBNA, EBV-VCA). Clinical data, including tumor stage, nodal status, and histological grade, were obtained from medical records. Demographic and clinical characteristics of the study cohort are summarized in Table 1.

RNA Extraction and cDNA Synthesis

The total plasma extraction volume was 200 µl utilizing miRCURY-Biofluids, Exiqon Denmark (Cat no. 30112). Plasma was combined with lysis buffer and protein precipitation solution before being centrifuged at 12,000 revolutions per minute (rpm). Total RNA was eluted with 25 µl of RNAse-free water, and internal control was conducted by adding sp6. 8–64 rxns cDNA synthesis using universal cDNA synthesis kit II (Cat No. 203301, Exiqon). The cDNA synthesis utilized a Bio-Rad C1000 thermal cycle with conditions of 42°C for 60 minutes, 95°C for 5 minutes, and 4°C. Every condition and technique followed the manufacturer's recommendations.

qPCR Analysis of mRNA Expression

The procedure of quantifying mRNA using SYBR Green master mix 2,5 mL (Catalog No. 203402, Exiqon) and sequencing primer is detailed in Table 2. cDNA was diluted with a ratio of 1:80 by adding nuclease-free water to 5 µl of cDNA. The primer sequence for this study is shown in Table 2. Quantification was performed using real-time PCR on Biorad CFX 96 with the following conditions: 95°C for 10 minutes, 95°C for 10 seconds, and 58°C, with a 1-minute ramp rate of 1.6°C/s. Every procedure followed the manufacturer's instructions.

Construction of PPI and Enrichment Analysis

An online server that explores the interconnection between proteins regarding physical interaction, co-expression, predicted, co-localized, common pathway, genetic interaction, and shared protein domain.

Functional enrichment and construction of a regulatory network

Functional enrichment analysis was conducted using the online software databases Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). Oncology-related mRNA *VEGF*, *PTEN*, and *SOCS1* were analyzed to determine their biological processes. In addition, the regulatory network was constructed using the internet application STRING (https://string-db.org/). Interaction score >0.7 (high confidence) was selected as the cutoff criterion; proteins with high scores were deemed hub factors in the network and may be significant candidate genes for disease development.

Data Analysis

Cycle quantification (cq) was accomplished via Genex

Pro with Exiqon qPCR wizard, qPCR analysis software, and a perpetual academic license (Cat No. 207006, Exiqon). Livak's methods were used to show the data [16]. The Software GenEx-6 Multid analysis and comparative expression data were used. Statistical analysis was also used to determine how the two groups' expressions and clinical status differed. All two-sided statistical tests with a p-value of 0.05 indicated statistical significance. GraphPad Prism 6 generated the graphical evaluation (La Jolla, CA, USA).

Results

Patient Characteristics

Participants' demographic and clinical characteristics in this research can be seen in Table 1. Peripheral blood was drawn from 240 tubes, including 80 non-control and 160 nasopharyngeal carcinoma (NPC) cases. More men were diagnosed than women. Between 14 to 68 years of age, 59% of males and 41% of women were diagnosed. Patients with NPC were grouped based on histology and pathology, a standard by the World Health Organization (WHO). Histologically, it has been known that >50% of NPC patients are WHO type IV, followed by types III and II. Histopathology related to EBV virus infection was found in the patients. The EA, EBNA, and VCA assays were used to measure and confirm the amount of EBV-encoding protein. Demographic and clinical characteristics of the study cohort are summarized in Table 1.

Protein Expression Status of EBV Infection

Validation of protein expression in 160 plasma samples from NPC patients was performed to assess the association between protein expression and the occurrence of NPC. The protein expression of EA, EBNA, and VCA has been assessed using an Elisa reader. EBV-EA was identified in the plasma of 43 (83%) of 132 patients, EBV-EBNA in 111 (69%) patients, and EBV-VCA in 145 (91%) patients. All subjects were verified to be infected with EBV using one or more protein EBV markers.

Circulating level expression mRNA PTEN, VEGF, and SOCS1 across all clinical data

To perform alterations in circulating expression, we utilized qRT-PCR methods, whose primer sequence can be seen in Table 3. A primer sequence detects and quantifies mRNA targets using SYBR fluorescence. With a fold change value, relative quantification analysis determines the expression's difference [16]. The results showed what kind of cycle quantification (CQ) was found in NPC and control samples. Analysis of the data showed that mRNA is linked to the growth of tumors.

Relative expression analysis was conducted based on clinical data using GenEx 6 MultiD. Statistical analysis used a T-test to compare differential groups. Table 3 and Figure 1 present the results of the differential expression of mRNA *VEGF*, *PTEN*, and *SOCS1* based on the clinical data of the participants. There are four groups, with the clinical status used as a basis for grouping: health and NPC, N-regional lymph nodes, T-primary tumor, and stage. Statistical analysis revealed significant expression of mRNA *VEGF* in N-regional lymph node status with a fold change of 1.66 [p-value <0.05]. Moreover, mRNA *SOCS1* indicated the alteration was associated with NPC status with a fold change of 20 [p-value <0.05] and stage status with a fold change of 4.26 (p-value <0.05).

Table 1. Clinical Pathology Data of the Subject Involved in This Study

Characteristic	N=240	Percent (%)
Nasopharyngeal Carcinoma	160	33
Non-tumor	80	67
Age		
Median	47	
Range	14-68	
Sex		
Male	106	59
Female	74	41
Histology		
WHO I	0	0
WHO II	24	15
WHO III	49	31
WHO IV	87	54
N Lymph Node		
N0	10	6
N1	43	27
N2	58	36
N3	49	31
T Classification		
T1	6	4
T2	71	44
T3	37	23
T4	46	29
Clinical Stages		
I	0	0
II	25	16
III	49	31
IV	86	54
Pathology Anatomy		
Undifferentiated	31	19
Non-Keratin, Undif Sub Type	117	73
Non-Keratin, Differentiated	9	6
Keratin	3	2
EBV - EA		
Positive	132	83
Negative	28	18
EBV - EBNA		
Positive	111	69
Negative	49	31
EBV - VCA		
Positive	145	91
Negative	15	9

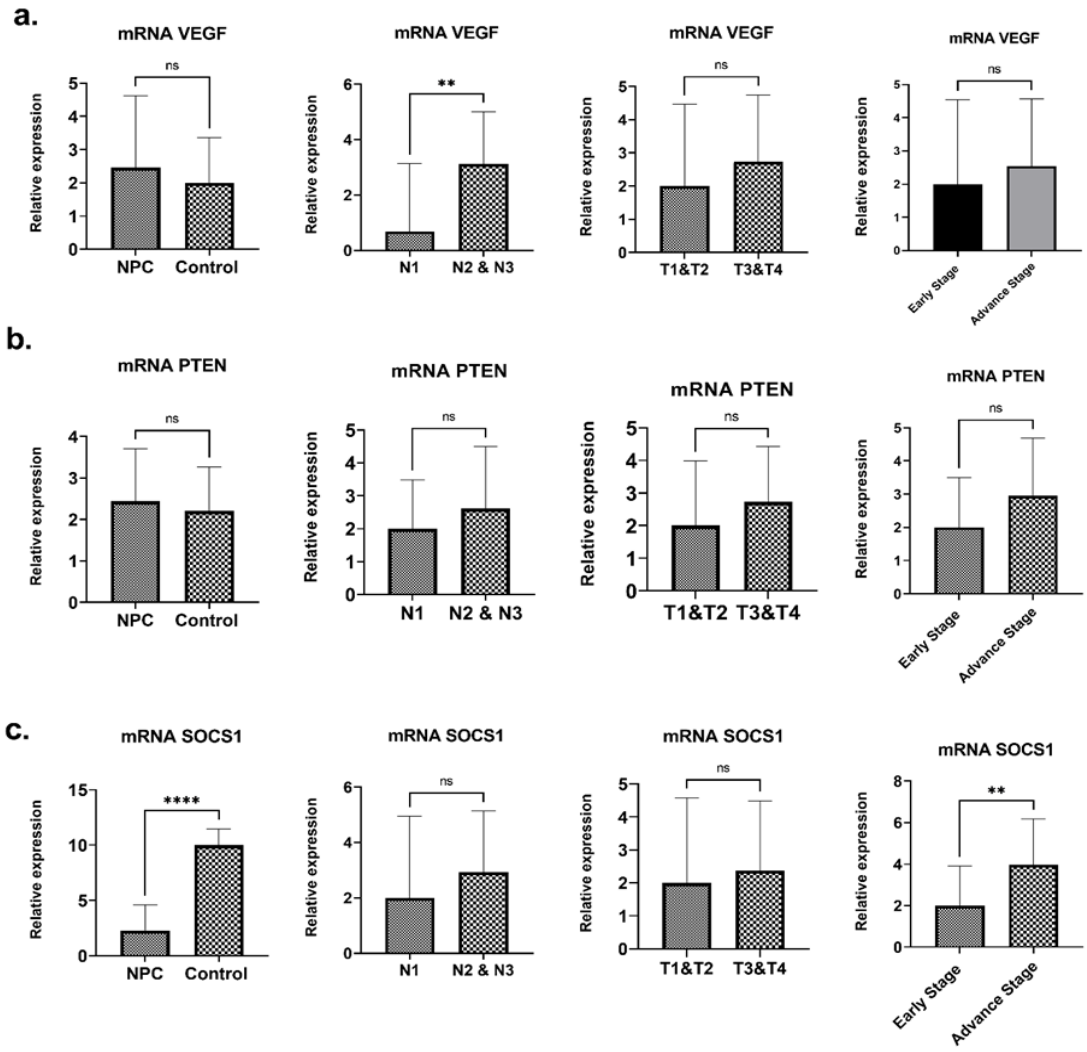

Figure 1. Shows the Correlation of mRNA *VEGF, PTEN*, and *SOCS1* Circulation with Clinical Status in Nasopharyngeal Carcinoma. a. mRNA *VEGF*; b. mRNA *PTEN*; c. mRNA *SOCS 1*, * indicated significant results based on statistical analysis.

Construction of PPI and Enrichment Analysis

To explore the potential PPI network, mechanism, function, and biological process of three genes, *VEGF*, *PTEN*, and *SOCS1*, we performed an analysis applying GeneMANIA. The relative findings are represented in Figure 2. Furthermore, we present the results of biological processes, interactions, and molecular mechanisms in Tables 2, 3. These indicated the potential mechanisms of three genes in NPC progression, which provide additional insights for further study.

Gene Ontology and Kyoto Encyclopedia of Genes and Genome Analysis

The annotation, visualization, and integrated discovery

Table 2. Relative Expression of Circulating mRNA *VEGF, PTEN*, and *SOCS1* Correlated with Clinical Status in Nasopharyngeal Carcinoma.

Variable	Fold Change		
	mRNA VEGF	mRNA PTEN	mRNA SOCS1
Cases			
NPC vs Healthy Control	1.02	1	20.0*
N- Regional Lymph Nodes			
N1 and N2 vs N3 and N4	1.66*	1.53	1.89
T- Primary Tumor			
T1 and T2 vs T3 and T4	1.5	1.51	1.11
Stage			
Early Stage vs Late Stage	1.28	1.8	4.26*

*, differences in significance P Value

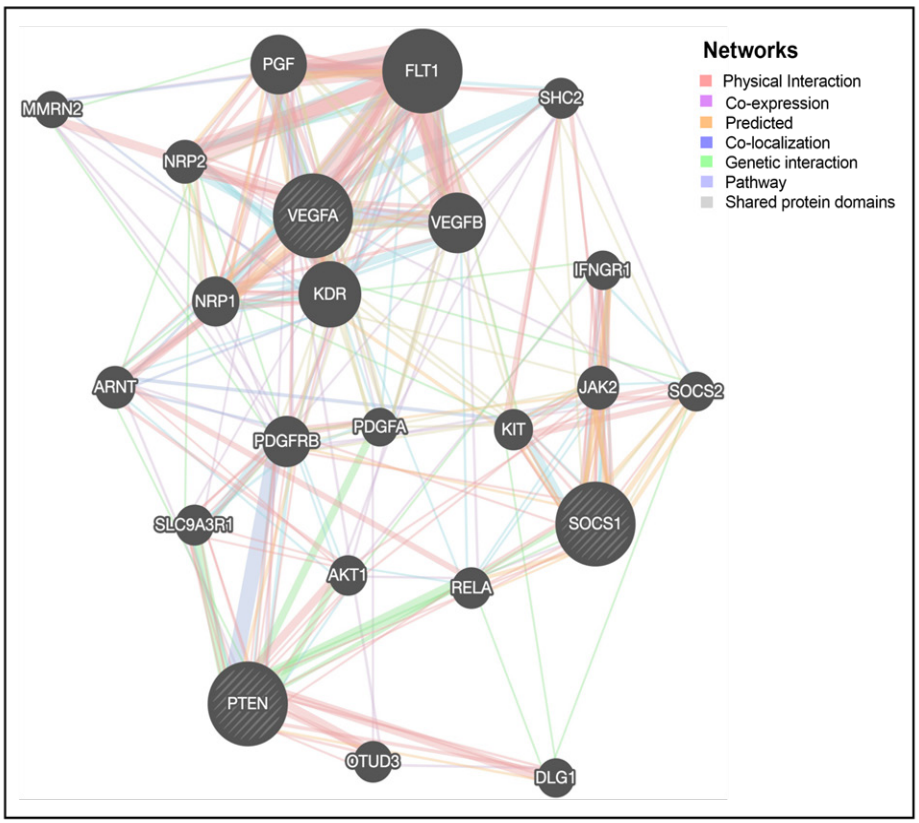

Figure 2. GeneMania Constructed the PPI Network of Three Genes. PPI networks are protein connections investigated in physical interactions, co-expressions, predicted co-localization, genetic interactions, pathways, and shared protein domains.

database have been analyzed using ShinyGo 0.77 (http://bioinformatics.sdstate.edu/go/). David KEGG Pathways (https://www.genome.jp/kegg/brite.html) were applied to perform genome ontology [GO] and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses to perform three hub genes and their most relevant conditions occurring in cancer. When the FDR was less than 0.05, enrichment terms were considered statistically significant, and the top 10 terms from each analysis were kept in a bubble chart (Figure 3).

Table 3. Sequence Primer for Quantification mRNA for This Study

Genes	Target sequence
VEGF A	
Forward	5'- GCACCCATGGCAGAAGG -3'
Reverse	5'- CTCGATGGATGGCAGTAGCT -3'
PTEN	
Forward	5'-GGGTCTGAGTCGCCTGTC-3'
Reverse	5'-CCGTGTAGGCAGTAGAAG-3'
SOCS1	
Forward	5'- GACGCCTGCGGATTCTACTG-3'
Reverse	5'- AGGCCATCTTCACGCTAAGG-3',
GAPDH	
Forward	5'- AAGACGGGCGGAGAGAAACC-3'
Reverse	5'- GTTGACTCCGACCTTCACCTT-3'

Discussion

NPC is a unique epithelial malignancy distributed in China, Southeast Asia, and Northern Africa. Its pathogenesis is correlated with EBV infection, genetics, diet (such as saltfish consumption), and environment. Chemotherapy and radiotherapy are often used to treat people with NPC, and it is hoped that this will increase the number of people who will live after being diagnosed [17,18]. In this study, we analyze the circulating differential expression of mRNA (*VEGF*, *PTEN*, and *SOCS*) from Nasopharyngeal Carcinoma (NPC) and examine its association with clinical outcomes. Moreover, co-expression analysis was conducted to recognize module and clinical traits.

On the other hand, individualized development, early diagnosis, and treatment at the molecular level required further development. Consequently, delays in diagnosis and treatment made improving the patient's condition challenging. Molecular biology could become a potential approach to eliminating the problem in the cancer field. Molecular biology has aggressively moved toward a simple phase that supports advanced technology, and bioinformatics is approaching a novel stage in data extraction and analysis. It became one of the best approaches for researchers to find out the problem and effectively find disease treatment through reading the nucleotide sequence, the exact location of all genes, genomic information, and alterations of genomes that have a role in diseases, including cancer. Identifying a

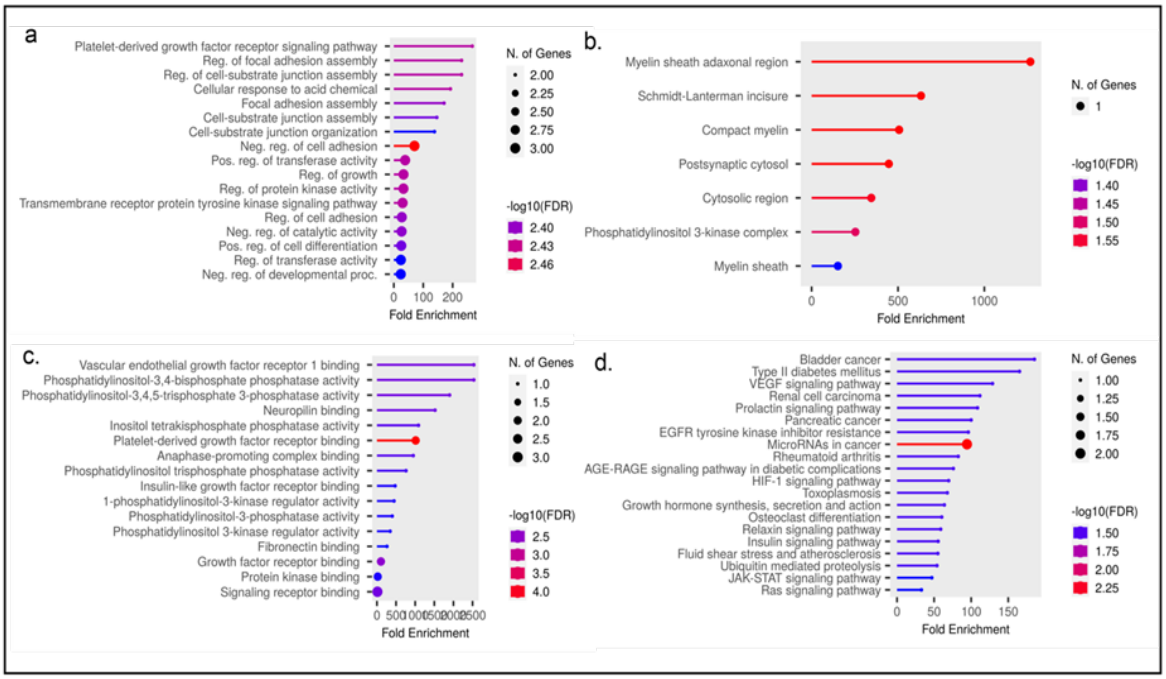

Figure 3. GO and KEGG Pathway Enrichment Analyses were Performed to Explain the Potential Mechanisms and Their Co-Operators: a. biological process, b. cellular component, c. molecular function, and d. KEGG pathway analysis. The size of each circle indicates the counting number on each part, while the color represents the FDR of the enrichment analysis.

gene, protein, cellular mechanisms, and profile expression could become more effective in finding biomarkers and therapeutic drug candidates for cancer.

Co-expression analysis is an efficient and effective strategy for gene or disease prediction. In this study, we looked at circulating messenger RNA [mRNA], which represents tumor cell condition and could be used as a support marker to detect and predict NPC prognosis. Moreover, an analysis of bioinformatics is needed to evaluate the correlation between gene expression and clinical outcome relating to the mechanism and high connecting activity for three genes (*VEGF*, *PTEN*, and *SOCS1*) related to cancer. We found that the expression of mRNA *VEGF*, *PTEN*, and *SOCS* was significantly correlated with the status of malignancy (fold change of 20; $p < 0.05$), lymph nodes (fold change of 1.66; $p < 0.05$), and stage (fold change of 4.26; $p < 0.05$).

VEGF is a platelet-derived growth factor family member contributing to vascular endothelial cells, mitogenesis, and angiogenesis. *VEGF* contributes to the suppression of maturing dendritic cells and the facilitation of the leakage of vascular matter, thus providing an extracellular matrix for the formation of vascularization [19]. Its expression was found in various circulating samples, indicating a prognostic indicator of lymph node, angiogenesis, metastases, recurrence, and decreased survival, including head and neck cancer [20, 9, 21]. Furthermore, alterations in the expression of *VEGF* were reported to be associated with advanced tumor stage and consistently with poor overall survival (OS) and disease-free survival (DFS) [22, 23]. Therefore, *VEGF* is a significant target for inhibiting tumor angiogenesis.

On the other hand, *PTEN* is located on chromosome 10q23, a tumor suppressor that correlates with initiation and progression in NPC. Alterations in the expression of *PTEN* would impact cellular processes, including senescence, angiogenesis, apoptosis, cell cycle progression, cell proliferation, chemotaxis, muscle contraction, and the DNA damage response [24]. Inactivated *PTEN* in somatic cancer is associated with the second most frequently mutated gene after p53 [25].

Cytokines in cancer cause *SOCS-1* dysregulation, which provides negative feedback on cytokine expression and inhibits macrophage response to IFN-. Negatively regulates JAK/STAT signaling through binding crucial phosphotyrosine residues in cytokine receptor cytoplasmic domains and the kinase inhibitory region pathway. It fits a previous study that showed that *SOCS1* mRNA expression was 200 times higher in people with NPC. These consistent findings revealed that *SOCS1* mRNA expression increased in NPC patients and more in early-stage NPC patients than in late-stage NPC patients. *SOCS1* can be used not only as a biomarker of NPC but *SOCS1* can also as an assessor of whether the NPC is in an early or advanced stage. Further research is needed to prove this.

PTEN is usually inactivated in somatic cancer and is the second most frequently mutated tumor suppressor gene after p53. The role of *PTEN* in cancer is known widely as a tumor suppressor [26]. It has a function for cell growth, proliferation, and differentiation [27]. Some previous studies show that loss of *PTEN* is associated with gastric, colorectal, and breast cancer [28–30]. Identifying *PTEN* in NPC is essential, whether it can be a marker for tracing the disease. We found a decreased amount of *PTEN* in nasopharyngeal carcinoma, which has the same result as the findings of Li et al. [7]. The average expression

levels of *PTEN* in a patient with NPC significantly declined compared to the control group. *PTEN* loss is also associated with poor prognostic tumors, but our study did not differentiate the clinical outcome of NPC between samples.

Our research further validated the role and function of the three mRNA for cancer. Furthermore, we employed GeneMANIA to construct a PPI network and GoShine to apply GO and KEGG analysis to identify the functional enrichment of three hub genes and the interaction that potential mechanism represents the condition in NPC progression. Analyses performed the alterations expressions significantly related to endothelial cell proliferation and cellular response to vascular endothelial growth factor stimulus. Our findings provided novel insight for hub three genes mediating endothelial cell proliferation and vascularization associated with NPC oncogenesis and development.

In conclusion, our study reveals significant alterations in *VEGF*, *PTEN*, and *SOCS1* mRNA expression in Nasopharyngeal Carcinoma [NPC], which are associated with poor prognosis. These findings suggest the potential of using these molecular signatures as biomarkers to track the progression of NPC and predict clinical outcomes. The comprehensive analysis of gene networks and pathways provides valuable insights into the underlying mechanisms of NPC progression. Further research and validation studies are needed to explore the clinical implications of these biomarkers and their potential for personalized treatment approaches in NPC.

Author Contribution Statement

TW, HS, SJSI, and ABD: Conceptualization, Supervision, writing, review, and editing; APN, MAPDP, FMLR, RR, TAP, ANA, RRA, MMH, TW, HS: Investigation; RRA, MMH, TW, HS, SJSI, and ABD: Data Curation; TW: Funding acquisition; APN, MAPDP, FMLR, RR, TAP, ANA, RRA, MMH, TW: writing original draft. .

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Availability of data

The data supporting this study's findings are available on request from the corresponding author.

Conflict of interest

All authors declared that there is no conflict of interest in this study.

References

1. Wu L, Li C, Pan L. Nasopharyngeal carcinoma: A review of current updates. Exp Ther Med. 2018;15(4):3687-92. https://doi.org/10.3892/etm.2018.5878.
2. Okekpa SI, RB SMNM, Mangantig E, Azmi NSA, Zahari SNS, Kaur G, et al. Nasopharyngeal carcinoma (npc) risk factors: A systematic review and meta-analysis of the association with lifestyle, diets, socioeconomic and sociodemographic in asian region. Asian Pac J Cancer Prev. 2019;20(11):3505-14. https://doi.org/10.31557/apjcp.2019.20.11.3505.
3. Chang ET, Ye W, Zeng YX, Adami HO. The evolving epidemiology of nasopharyngeal carcinoma. Cancer Epidemiol Biomarkers Prev. 2021;30(6):1035-47. https://doi.org/10.1158/1055-9965.Epi-20-1702.
4. Iarc. The global cancer observatory [globocan] 2020 database. Int agency res cancer (internet). 2020;441:1–2. Available from: Https://gco.Iarc.Fr/,.
5. Adham M, Kurniawan AN, Muhtadi AI, Roezin A, Hermani B, Gondhowiardjo S, et al. Nasopharyngeal carcinoma in indonesia: Epidemiology, incidence, signs, and symptoms at presentation. Chin J Cancer. 2012;31(4):185-96. https://doi.org/10.5732/cjc.011.10328.
6. Xie L, Shang Z. Burden of oral cancer in asia from 1990 to 2019: Estimates from the global burden of disease 2019 study. PLoS One. 2022;17(3):e0265950. https://doi.org/10.1371/journal.pone.0265950.
7. Li WZ, Lv SH, Liu GY, Liang H, Xia WX, Xiang YQ. Age-dependent changes of gender disparities in nasopharyngeal carcinoma survival. Biol Sex Differ. 2021;12(1):18. https://doi.org/10.1186/s13293-021-00361-8.
8. Abdullah B, Alias A, Hassan S. Challenges in the management of nasopharyngeal carcinoma: A review. Malays J Med Sci. 2009;16(4):50-4.
9. Zhang SQ, Pan SM, Liang SX, Han YS, Chen HB, Li JC. Research status and prospects of biomarkers for nasopharyngeal carcinoma in the era of high-throughput omics (review). Int J Oncol. 2021;58(4). https://doi.org/10.3892/ijo.2021.5188.
10. Ilié M, Hofman P. Pros: Can tissue biopsy be replaced by liquid biopsy? Transl Lung Cancer Res. 2016;5(4):420-3. https://doi.org/10.21037/tlcr.2016.08.06.
11. Heitzer E, Perakis S, Geigl JB, Speicher MR. The potential of liquid biopsies for the early detection of cancer. NPJ Precis Oncol. 2017;1(1):36. https://doi.org/10.1038/s41698-017-0039-5.
12. Goss dj, domashevskiy a v. Messenger rna (mrna): The link between DNA and protein. In: Encyclopedia of cell biology. 2016. P. 341–5. .
13. Tani N, Ichikawa D, Ikoma D, Tomita H, Sai S, Ikoma H, et al. Circulating cell-free mrna in plasma as a tumor marker for patients with primary and recurrent gastric cancer. Anticancer Res. 2007;27(2):1207-12.
14. Zeng ZY, Zhou YH, Zhang WL, Xiong W, Fan SQ, Li XL, et al. Gene expression profiling of nasopharyngeal carcinoma reveals the abnormally regulated wnt signaling pathway. Hum Pathol. 2007;38(1):120-33. https://doi.org/10.1016/j.humpath.2006.06.023.
15. Ng EK, Tsui NB, Lam NY, Chiu RW, Yu SC, Wong SC, et al. Presence of filterable and nonfilterable mrna in the plasma of cancer patients and healthy individuals. Clin Chem. 2002;48(8):1212-7.
16. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative pcr and the 2(-delta delta c(t)) method. Methods. 2001;25(4):402-8. https://doi.org/10.1006/meth.2001.1262.
17. Feng BJ, Jalbout M, Ayoub WB, Khyatti M, Dahmoul S, Ayad M, et al. Dietary risk factors for nasopharyngeal carcinoma in maghrebian countries. Int J Cancer. 2007;121(7):1550-5.

https://doi.org/10.1002/ijc.22813.
18. Tsao SW, Tsang CM, Lo KW. Epstein-barr virus infection and nasopharyngeal carcinoma. Philos Trans R Soc Lond B Biol Sci. 2017;372(1732). https://doi.org/10.1098/rstb.2016.0270.
19. Choi KS, Bae MK, Jeong JW, Moon HE, Kim KW. Hypoxia-induced angiogenesis during carcinogenesis. J Biochem Mol Biol. 2003;36(1):120-7. https://doi.org/10.5483/bmbrep.2003.36.1.120.
20. Kut C, Mac Gabhann F, Popel AS. Where is vegf in the body? A meta-analysis of vegf distribution in cancer. Br J Cancer. 2007;97(7):978-85. https://doi.org/10.1038/sj.bjc.6603923.
21. Krishna SM, James S, Balaram P. Expression of vegf as prognosticator in primary nasopharyngeal cancer and its relation to ebv status. Virus Res. 2006;115(1):85-90. https://doi.org/10.1016/j.virusres.2005.07.010.
22. Guang-Wu H, Sunagawa M, Jie-En L, Shimada S, Gang Z, Tokeshi Y, et al. The relationship between microvessel density, the expression of vascular endothelial growth factor (vegf), and the extension of nasopharyngeal carcinoma. Laryngoscope. 2000;110(12):2066-9. https://doi.org/10.1097/00005537-200012000-00017.
23. Chen X, Xu X, Pan B, Zeng K, Xu M, Liu X, et al. Mir-150-5p suppresses tumor progression by targeting vegfa in colorectal cancer. Aging (Albany NY). 2018;10(11):3421-37. https://doi.org/10.18632/aging.101656.
24. Govender D, Chetty R. Gene of the month: Pten. J Clin Pathol. 2012;65(7):601-3. https://doi.org/10.1136/jclinpath-2012-200711.
25. Di Cristofano A, Pandolfi PP. The multiple roles of pten in tumor suppression. Cell. 2000;100(4):387-90. https://doi.org/10.1016/s0092-8674(00)80674-1.
26. Lee YR, Chen M, Pandolfi PP. The functions and regulation of the pten tumour suppressor: New modes and prospects. Nat Rev Mol Cell Biol. 2018;19(9):547-62. https://doi.org/10.1038/s41580-018-0015-0.
27. Hopkins BD, Hodakoski C, Barrows D, Mense SM, Parsons RE. Pten function: The long and the short of it. Trends Biochem Sci. 2014;39(4):183-90. https://doi.org/10.1016/j.tibs.2014.02.006.
28. Lu YM, Cheng F, Teng LS. The association between phosphatase and tensin homolog hypermethylation and patients with breast cancer, a meta-analysis and literature review. Sci Rep. 2016;6:32723. https://doi.org/10.1038/srep32723.
29. Kang HJ, Lee IS, Park YS, Ho WJ, Sohn D, Ahn JY, et al. Biomarkers of ebv-positive gastric cancers: Loss of pten expression is associated with poor prognosis and nodal metastasis. Ann Surg Oncol. 2016;23(11):3684-92. https://doi.org/10.1245/s10434-016-5284-2.
30. Molinari F, Frattini M. Functions and regulation of the pten gene in colorectal cancer. Front Oncol. 2013;3:326. https://doi.org/10.3389/fonc.2013.00326.

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