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Correlation of P38 Mitogen-Activated Protein Kinase Expression to Clinical Stage in Nasopharyngeal Carcinoma

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Abstract

BACKGROUND: Nasopharyngeal carcinoma (NPC) is uncommon and usually diagnosed at the advanced stage. A subfamily of mitogen-activated protein kinase which is called p38 mitogen-activated protein kinase (MAPK) involved in response to stress, and plays an important role in cell regulation. There is a suggestion that p38 mitogen-activated protein kinase could be a potential biomarker to determine the clinical stage of nasopharyngeal carcinoma.

AIM: The aim of this study is for observing and analysing the correlation of p38 mitogen-activated protein kinase expression in regards to nasopharyngeal carcinoma patient's clinical stage.

METHODS: This study involved 126 nasopharyngeal carcinoma patients admitted to Haji Adam Malik General Hospital.

RESULTS: The result of this study indicates that nasopharyngeal carcinoma mostly found in the age group 41-60 years, male, non-keratinizing squamous cell carcinoma, and stage IV group. In immunohistochemistry evaluation, most of p38 mitogen-activated protein kinase overexpressed in non-keratinizing squamous cell carcinoma, T3-T4, N2-N3 and clinical stage III-IV. Spearman's test for categorical correlation yield p-value of < 0.001.

CONCLUSION: In conclusion, there is a significant correlation between p38 mitogen-activated protein kinase expression and the clinical stage of nasopharyngeal carcinoma.

Introduction

Nasopharyngeal carcinoma (NPC) is not a rare entity, which has a distinct distribution especially in Asia [1] [2] [3] [4] [5]. There are more than 13.000 new cases of NPC in Indonesia and associated with a high mortality rate [1] [6]. The aetiology of NPC is considered to be related to environmental and genetic factors as well as EBV infection [7] [8]. Because of the location of NPC is in the silent and painless area; therefore the disease is usually diagnosed at the advanced stages; hence early detection of NPC is difficult [9]. Regulation of signaling molecules in intracellular signal transduction, which regulate cell proliferation, apoptosis, and adhesion, underlines the basis of NPC pathogenesis [10] [11] [12] [13] [14] [15] [16] [17].

Mitogen-activated protein kinase (MAPK) is an important signal molecule that affects a variety of cellular process such as proliferation, differentiation, migration, and apoptosis [18] [22]. Aside from its

physiological functions, MAPK also plays a key role in many pathological conditions including cancer, cardiac hypertrophy, and diabetes [23] [24] [25] [26].

Two distinct classes of MAPKs have been identified so far: p42-p44 (ERK) MAPKs inducible by; and SAPKs (Stress-Activated Protein Kinases), which include p38 MAPKs, and p46-p54 JNKs inducible by cellular stress [24] [25]. Each MAPK class responds to distinct stimuli and induces a specific biological response, and they proofed to have a crucial role in cancer development [26].

The p38 MAPKs are a conserved subfamily of MAPKs involved in response to stress found in eukaryotic cells from yeast to mammals. p38 was found in 1994 as a MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells [27]. In the development of NPC, p38 MAPK activation plays an important role both in the ability to protect EBV-infected Raji cells from apoptosis and also for promoting EBV lytic gene expression [17].

The p38 MAPKs have been proposed as a novel biomarker for predicting the clinical stage of NPC [19] [20]. Considering the importance of a biomarker for NPC, the primary goal of the current study was to explore the prevalence and expression of p38 MAPK and their possible correlation to the clinical stage of NPC. This study might provide supportive evidence for the role of p38 MAPK in the clinical stage of NPC.

This study was analytic research, and we used paraffin samples of 126 NPC patients to analyse the correlation of p38 MAPK overexpression to the clinical stage of NPC.

Material and Methods

During July to October 2017, research with 126 samples of NPC patients was established in Adam Malik General Hospital, Medan. The samples were taken in 2015-2016 based on history taking, physical examination, and nasopharyngeal histopathological biopsy. The criteria include patients with NPC histopathologically examined and not yet received any radiotherapy, chemotherapy, or combination of both. To obtain an adequate result, non-probability consecutive sampling was used to receive a minimum of 68 patients in this study.

Paraffin-embedded pathological specimens of nasopharyngeal histopathological biopsy were obtained. All of these resection samples were treated with a standard fixation, dissection, and processing protocol. The blocks were then cut into 4 mm sections and processed for immunohistochemistry. After being washed with phosphate-buffered saline, the specimens were incubated with the primary antibody using GENETEX human p38 MAPK antibody.

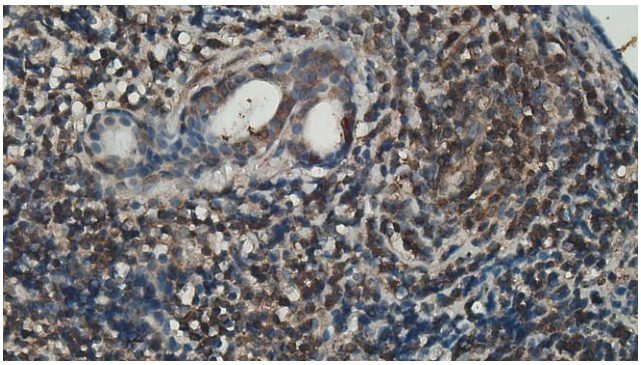

Figure 1: Cytoplasmic expression of p38 MAPK in non-keratinizing squamous cell nasopharyngeal carcinoma (x 400)

The tissues were examined using immunohistochemistry technique under fluorescent microscope evaluating the immunoreaction of p38 MAPK. Three pathologists who were blinded to the patients' clinicopathological data independently evaluated the p38 MAPK expressions. The results were evaluated using immunoreactivity score (score obtained by multiplying width score with intensity score) [21].

This study was approved by the Health Research Ethical Committee of the Medical Faculty of Universitas Sumatera Utara.

To define the correlation in this study, SPSS ver. 22 software was used to conduct all the statistical analysis. The association was identified by using Spearman's correlation, in which $p < 0.05$ was considered as statistically significant.

Results

There were 126 patients with NPC involved in this study that has fulfilled the study requirement. The presentation of demographic data was shown in table 1. Distributions of age-specific rates of NPC shows that NPC incidence increases simultaneously with age, but fells of after 60 years. Despite the same ratio of male: female over the year, the number of NPC based on gender are higher in men compared to women. After immunohistochemistry examination, the most frequent histological type was non-keratinizing squamous cell carcinoma. For the clinical stage, most of the samples in the study was stage IV. The main focus of this study showed most of p38 MAPK over-expressed was in non-keratinizing squamous cell carcinoma (63.5%), T3-T4 primary tumour size (53.1%), cervical nodes enlargement in the N2-N3 group (65.1%) and clinical stage in the III-IV group (71.8%).

The association uses Spearman's test to categorise the correlation which results in the p-value of < 0.001 indicating that the correlation of clinical staging and the expression of p38 MAPK is statistically significant.

Table 1: Demographic Data and Correlation of p38 MAPK and Clinical Stage in NPC Patients

Characteristic	p38 MAPK Expression				*P*
	Overexpression	%	Negative	%	
Age (y)					
≤ 20	3	2.4	4	3.2	
21-40	21	16.7	7	5.6	
41-60	59	46.8	13	10.3	
> 60	17	13.5	2	1.6	
Sex					
Male	69	54.8	21	16,7	
Female	31	24.6	5	4.0	
Histopathology					
Keratinizing SCC	8	6.3	0	0.0	
Non-keratinizing SCC	80	63.5	20	15.9	
Undifferentiated carcinoma	12	9.5	6	4.8	
Primary Tumor (T)					
T1	12	9.5	13	10.3	
T2	21	16.7	11	8.7	
T3 T4	40	31.7	1	0.8	
	27	21.4	1	0.8	
Nodes (N)					
N0	8	7.8	13	12.6	
N1	11	10.7	4	3.9	
N2 N3	18	17.5	0	0.0	
	49	47.6	0	0.0	
Clinical Staging					
I	4	3.9	9	8.7	<0.001
II	8	7.8	5	4.9	
III	16	15.5	2	1.9	
IV	58	56.3	1	1.0	

*p < 0.05 = statistically significant.

Discussion

In this study, the largest age group of a patient diagnosed with NPC is between 41-60 years old (57.1%). Similar to Adham et al., (2012), 40-49 years old is the highest age of the NPC patients found in their study [1]. This occurred because the DNA repair mechanism function and immune system have been lessened as mutation develops at the age of more than 40 years [5] [6] [13].

In our study, a trend was conducted, with 71.4% male and 28.6% female resulting in a 2.5:1 ratio. The male: female ratio was relatively stable over the years. In Cao (2011) study, the predominance was found as well with the incidence rate of NPC was less in women than in men, with a ratio of 2-3:1 [3]. The exposure to environmental pollution from occupation and lifestyle caused males got a higher ratio than females diagnosed with NPC [2] [5].

In our study, non-keratinizing squamous cell carcinoma (79.4%) was the most common form of NPC, similar to the studies done in other high-risk countries. In an endemic area, over 90% of NPC is non-keratinizing squamous cell carcinoma [2] [4] [5].

The p38 pathway has also been playing a role in the activation of p53 and p53-mediated apoptosis [18] [28] [29]. Many cancers are associated with decreased p38 activity because in the majority of cancers studied, reduced p38 activity implicated in the continuous cell proliferation, with downstream target activation such as ATF-2 and Elk-1 [30] [33].

Many factors support the role of p38 MAPK as a tumour suppressor, and the negative regulation of cell cycle and apoptotic induction mediate this p38α function, although terminal differentiation induction also supports tumour suppressor [24] [34] [35]. However, p38 MAPK as well as an oncogenic function that is mediated by the involvement of cancer progression processes, such as invasion, inflammation, and angiogenesis [36] [40]. The conclusion of this study is a statistically significant correlation between clinical staging and the expression of p38 MAPK. Further research with larger and multiple centres is required for assessing the role of p38 MAPK in the progression of nasopharyngeal cancer.

References

1. 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. Chinese journal of cancer. 2012; 31(4):185. https://doi.org/10.5732/cjc.011.10328 PMid:22313595 PMCid:PMC3777476

2. Tang L-L, Chen W-Q, Xue W-Q, He Y-Q, Zheng R-S, Zeng Y-X, et al. Global trends in incidence and mortality of nasopharyngeal carcinoma. Cancer letters. 2016;374(1):22-30.Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA: a cancer journal for clinicians. 2015; 65(2):87-108.

3. Cao S-M, Simons MJ, Qian C-N. The prevalence and prevention of nasopharyngeal carcinoma in China. Chinese journal of cancer. 2011; 30(2):114. https://doi.org/10.5732/cjc.010.10377 PMCid:PMC4013340

4. Zhang L-F, Li Y-H, Xie S-H, Ling W, Chen S-H, Liu Q, et al. Incidence trend of nasopharyngeal carcinoma from 1987 to 2011 in Sihui County, Guangdong Province, South China: an age-period-cohort analysis. Chinese journal of cancer. 2015; 34(3):15. https://doi.org/10.1186/s40880-015-0018-6 PMid:26058679 PMCid:PMC4593377

5. Jayalie VF, Paramitha MS, Jessica J, Liu CA, Ramadianto AS, Trimartani T, et al. Profile of Nasopharyngeal Carcinoma in Dr. Cipto Mangunkusumo National Hospital, 2010. eJournal Kedokteran Indonesia. 2017:156-62.

6. Nawaz I, Moumad K, Martorelli D, Ennaji MM, Zhou X, Zhang Z, et al. Detection of nasopharyngeal carcinoma in Morocco (North Africa) using a multiplex methylation-specific PCR biomarker assay. Clinical epigenetics. 2015; 7(1):89. https://doi.org/10.1186/s13148-015-0119-8 PMid:26300994 PMCid:PMC4546349

7. Tulalamba W, Janvilisri T. Nasopharyngeal carcinoma signaling pathway: an update on molecular biomarkers. International journal of cell biology. 2012; 2012.

8. Fles R, Bos A, Rachmawati D, Waliyanti E, Tan I, Haryana S, et al. The role of Indonesian patients' health behaviors in delaying the diagnosis of nasopharyngeal carcinoma. BMC public health. 2017; 17(1):510. https://doi.org/10.1186/s12889-017-4429-y PMid:28545416 PMCid:PMC5445307

9. Tsang CM, Tsao SW. The role of Epstein-Barr virus infection in the pathogenesis of nasopharyngeal carcinoma. Virologica Sinica.

2015; 30(2):107-21. https://doi.org/10.1007/s12250-015-3592-5 PMid:25910483

10. Zeng MS, Zeng YX. Pathogenesis and etiology of nasopharyngeal carcinoma. InNasopharyngeal Cancer 2010 (pp. 9-25). Springer, Berlin, Heidelberg.

11. Liu T-H, Zheng F, Cai M-Y, Guo L, Lin H-X, Chen J-W, et al. The putative tumor activator ARHGEF3 promotes nasopharyngeal carcinoma cell pathogenesis by inhibiting cellular apoptosis. Oncotarget. 2016; 7(18):25836. https://doi.org/10.18632/oncotarget.8283 PMid:27028992 PMCid:PMC5041948

12. Petersson F, Editor. Nasopharyngeal carcinoma: a review. Seminars in diagnostic pathology. Elsevier, 2015.

13. Young LS, Dawson CW. Epstein-Barr virus and nasopharyngeal carcinoma. Chinese journal of cancer. 2014; 33(12):581. https://doi.org/10.5732/cjc.014.10197

14. Burgos JS. Involvement of the Epstein-Barr virus in the nasopharyngeal carcinoma pathogenesis. Medical Oncology. 2005; 22(2):113-21. https://doi.org/10.1385/MO:22:2:113 PMid:28184182

15. Chan AT, Teo PM, Huang DP. Pathogenesis and treatment of nasopharyngeal carcinoma. InSeminars in oncology 2004 Dec 1 (Vol. 31, No. 6, pp. 794-801). WB Saunders.

16. Raab-Traub N, editor. Epstein–Barr virus in the pathogenesis of NPC. Seminars in cancer biology. Elsevier, 2002.

17. Yong H-Y, Koh M-S, Moon A. The p38 MAPK inhibitors for the treatment of inflammatory diseases and cancer. Expert opinion on investigational drugs. 2009; 18(12):1893-905. https://doi.org/10.1517/13543780903321490 PMid:19852565

18. Martín-Blanco E. p38 MAPK signalling cascades: ancient roles and new functions. Bioessays. 2000; 22(7):637-45. https://doi.org/10.1002/1521-1878(200007)22:7<637::AID-BIES6>3.0.CO;2-E

19. Cargnello M, Roux PP. Activation and function of the MAPKs and their substrates, the MAPK-activated protein kinases. Microbiology and molecular biology reviews. 2011; 75(1):50-83. https://doi.org/10.1128/MMBR.00031-10 PMid:21372320 PMCid:PMC3063353

20. Fedchenko N, Reifenrath J. Different approaches for interpretation and reporting of immunohistochemistry analysis results in the bone tissue–a review. Diagnostic pathology. 2014; 9(1):221. https://doi.org/10.1186/s13000-014-0221-9 PMid:25432701 PMCid:PMC4260254

21. Cuadrado A, Nebreda AR. Mechanisms and functions of p38 MAPK signalling. Biochemical Journal. 2010; 429(3):403-17. https://doi.org/10.1042/BJ20100323 PMid:20626350

22. Roux PP, Blenis J. ERK and p38 MAPK-activated protein kinases: a family of protein kinases with diverse biological functions. Microbiology and molecular biology reviews. 2004; 68(2):320-44. https://doi.org/10.1128/MMBR.68.2.320-344.2004 PMid:15187187 PMCid:PMC419926

23. Wagner EF, Nebreda ÁR. Signal integration by JNK and p38 MAPK pathways in cancer development. Nature Reviews Cancer. 2009; 9(8):537. https://doi.org/10.1038/nrc2694 PMid:19629069

24. Obata T, Brown GE, Yaffe MB. MAP kinase pathways activated by stress: the p38 MAPK pathway. Critical care medicine. 2000; 28(4):N67-N77. https://doi.org/10.1097/00003246-200004001-00008 PMid:10807318

25. Coulthard LR, White DE, Jones DL, McDermott MF, Burchill SA. p38MAPK: stress responses from molecular mechanisms to therapeutics. Trends in molecular medicine. 2009; 15(8):369-79. https://doi.org/10.1016/j.molmed.2009.06.005 PMid:19665431 PMCid:PMC3016890

26. Bradham C, McClay DR. p38 MAPK in development and cancer. Cell cycle. 2006; 5(8):824-8. https://doi.org/10.4161/cc.5.8.2685 PMid:16627995

27. Wang B, Jiang H, Ma N, Wang Y. Phosphorylated-p38 mitogen-activated protein kinase expression is associated with clinical factors in invasive breast cancer. Springer Plus. 2016; 5(1):934. https://doi.org/10.1186/s40064-016-2636-0 PMid:27386378 PMCid:PMC4929108

28. Olson JM, Hallahan AR. p38 MAP kinase: a convergence point in cancer therapy. Trends in molecular medicine. 2004; 10(3):125-9. https://doi.org/10.1016/j.molmed.2004.01.007 PMid:15102355

29. Leelahavanichkul K, Amornphimoltham P, Molinolo AA, Basile JR, Koontongkaew S, Gutkind JS. A role for p38 MAPK in head and neck cancer cell growth and tumor-induced angiogenesis and lymphangiogenesis. Molecular oncology. 2014; 8(1):105-18. https://doi.org/10.1016/j.molonc.2013.10.003 PMid:24216180 PMCid:PMC3946852

30. Kennedy NJ, Cellurale C, Davis RJ. A radical role for p38 MAPK in tumor initiation. Cancer Cell. 2007; 11(2):101-3. https://doi.org/10.1016/j.ccr.2007.01.009 PMid:17292820

31. Riebe C, Pries R, Schroeder KN, Wollenberg B. Phosphorylation of STAT3 in head and neck cancer requires p38 MAPKinase, whereas phosphorylation of STAT1 occurs via a different signaling pathway. Anticancer research. 2011; 31(11):3819-25. PMid:22110204

32. Beisswenger C, Coyne CB, Shchepetov M, Weiser JN. Role of p38 MAP kinase and transforming growth factor-β signaling in transepithelial migration of invasive bacterial pathogens. Journal of Biological Chemistry. 2007; 282(39):28700-8. https://doi.org/10.1074/jbc.M703576200 PMid:17650505

33. Lara HHR, Monroy A. Prevalence of nasopharyngeal carcinoma among patients with nasopharyngeal mass in a Philippine tertiary training hospital. Philippine Journal of Otolaryngology Head and Neck Surgery. 2016; 31(1):35-8.

34. Han J, Sun P. The pathways to tumor suppression via route p38. Trends in biochemical sciences. 2007; 32(8):364-71. https://doi.org/10.1016/j.tibs.2007.06.007 PMid:17624785

35. Lin ML, Lu YC, Chung JG, Wang SG, Lin HT, Kang SE, et al. Down-regulation of MMP-2 through the p38 MAPK-NF-κB-dependent pathway by aloe-emodin leads to inhibition of nasopharyngeal carcinoma cell invasion. Molecular carcinogenesis. 2010; 49(9):783-97. https://doi.org/10.1002/mc.20652

36. Zhong Y, Naito Y, Cope L, Naranjo-Suarez S, Saunders T, Hong S-M, et al. Functional p38 MAPK identified by biomarker profiling of pancreatic cancer restrains growth through JNK inhibition and correlates with improved survival. Clinical cancer research. 2014; 20(23):6200-11. https://doi.org/10.1158/1078-0432.CCR-13-2823 PMid:24963048 PMCid:PMC4866510

37. Zhu G-H, Dai H-P, Shen Q, Ji O, Zhang Q, Zhai Y-L. Curcumin induces apoptosis and suppresses invasion through MAPK and MMP signaling in human monocytic leukemia SHI-1 cells. Pharmaceutical biology. 2016; 54(8):1303-11. PMid:26134921

38. Bistrović A, Grbčić P, Harej A, Sedić M, Kraljević-Pavelić S, Koštrun S, et al. Small molecule purine and pseudopurine derivatives: synthesis, cytostatic evaluations and investigation of growth inhibitory effect in non-small cell lung cancer A549. Journal of enzyme inhibition and medicinal chemistry. 2018; 33(1):271-85. https://doi.org/10.1080/14756366.2017.1414807 PMid:29271659 PMCid:PMC6009932

39. Wakeman D, Schneider JE, Liu J, Wandu WS, Erwin CR, Guo J, et al. Deletion of p38- alpha mitogen-activated protein kinase within the intestinal epithelium promotes colon tumorigenesis. Surgery. 2012; 152(2):286-93. https://doi.org/10.1016/j.surg.2012.05.009 PMid:22828150 PMCid:PMC3408636