CONTRIBUTION OF CANCER STEM CELLS TO THE METASTATIC MECHANISM IN POSITIVE LMP I WHO TYPE III NASOPHARYNGEAL CANCER PATIENT BEFORE AND AFTER NEOADJUVANT CHEMOTHERAPY

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Abstract

Introduction: Nasopharyngeal cancer is the most common malignant tumour of the head and neck that caused high mortality. Most of patients came for treatment in late stage with enlargement of the neck lymphnode. Cancer stem cells are characteristic of stemness such as self-renewal and survival that caused tumour metastasis and recurrence. CD44, SOX2 and CCR7 are marker for NPC stem cells.

Methods: Nine new patients NPC, WHO type III, positive LMP1 were examined for volume of the neck lymphnode, CD44, SOX2 and CCR7 expression before and after treatment chemotherapy cisplatin based. Staging determined by AJCC/UICC 2010.

Results: The results of this study showed that all subjects have increased SOX2 and CCR7 expression after treatment with statistically negative correlation. CD44 did not altered before and after treatment and cannot be evaluate. Correlation between alteration of the increase SOX2 and CCR7 expression and reduction of the volume have shown statistically positive correlation.

Conclusion: Neoadjuvant Chemotherapy Cisplatin based eliminated progenitor cells but did not for cancer stem cells in NPC. This result indicates that NPC with higher cancer stem cells must be more attention forward potential resistance and recurrence of the tumour, although the have received all standard neoadjuvant chemotherapy

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1. INTRODUCTION

Nasopharyngeal Cancer (NPC) is the most common malignant tumour of head and neck that caused high mortality. Most of patients came for treatment with late stage of cancer. Neck tumour is very often as a first symptom or sign that stimulate patients to looked for hospital medication [1].

EBV-encoded latent membrane protein-1 (LMP-1) could induce development of CD44⁺ stem- like cell in NPC. The molecular modulation of CSC m NPC could shown that LMP-1 induced CSC through promotion of the expression of Epithelial-mesenchymal transition (EMT) [2-4].

LMP-1 provided and activated PI3-K/AKT pathway and subsequently important for induction and maintenance of LMP-1 induced CD44⁺ also activated PI3-K/AKT pathway. LMP-1 transformed cells led to phosphorylation to activated PI3-K/AKT pathway and induced CSC [5, 6].

Cancer stem cells are small population with characteristic of stemness such as self-renewal and survival that caused tumour metastasis and recurrences [7, 8]. NPC cancer stem cells are characterized by CD44⁺, SOX2 and CCR7 [9-12].

CD44⁺ as a receptor for hyaluronic acid (HA) high expression in transmembrane cells. CD44⁺ also receptor for extracellular matrix (ECM) and co-receptor for growth factors and cytokine. CD44v isoform are CSC markers and critical regulators of cancer stemness including self-renewal, tumour initiation and metastasis (Sex determining region Y)-box2 (SOX2) is a transcription factor that is important and essential for maintaining self-renewal, or pluripotency of embryonic stem cells. SOX2 also potential for somatic stem cells population maintenance, positive regulation of cell-cell adhesion, negative regulation of canonical Wnt signalling pathway and cell differentiation. SOX2 gene encodes transcription factor with function as an activator or suppressor of gene transcription. Recent finding SOX-2 also involved in cancer biology process especially in the fields of cellular signalling and cancer stem cells (5,6) In clinical settings, SOX-2 has shown

a highly influence on patient survival and prognosis, possible therapeutic intervention in the future [9, 10].

Chemokine (C-X-C motif) Receptor7 (CCR7) is a chemokine contain small peptide receptor binding which act as chemo-attractants for leucocyte lead to inflammation site. CCR7 involved in angiogenesis and malignant metastatic mechanism. Many researchers clarified that CCR7 functionally as a receptor for chemokine (C-X-C motif) ligand 19 and 21 in head and neck cancer patients. High expression of CXCR4 (SDF-1) and CCR7 have significantly correlated with regional and distant metastatic mechanism in head neck cancer [11, 12]

2. MATERIAL AND METHODS

Study design is observational cross sectional. We conducted to nine subjects who presented to ENT clinic at dr. Saiful Anwar Government Hospital, Malang by consecutive sampling.

2.1 Inclusion criteria

All subjects was diagnosed new NPC, WHO type III and positive LMP-1 after they agreed informed consent for this study. Stage of NPC determine according to AJCC/UICC 2010. All of subjects have late stage with module in the neck, varies in size. They never received standard treatment for cancer before this study. Karnofsky score more than 80.

2.2 Subject Measurement

Nine subjects measured lymphnode volume before and after treatment. All of biopsy tissues before and after treatment have been examined for immunohistochemistry (IHC) CD44⁺, SOX2, CCR7. CD44⁺ were evaluated by software immune membrane and for SOX2 and CCR7 by software Immunoratio.

3. RESULT

CD44, SOX2, CCR7 Before and after Neoadjuvant Chemotherapy. Measurement of CD44⁺ expression by immunomembrane shown all subject have some result 3⁺ before and after treatment. Statistic analysis for all CD44⁺ expression can not be evaluated as planned before study.

Table 1. Descriptive analysis SOX2 Expression before and after treatment

	SOX2 Expression		P
	Before	After	
Average	29.18	35.88	0.008
Standard deviation	11.19	10.21	
Mean	26.70	31.20	

Wilcoxon test shown significant difference SOX-2 expression before and after treatment.

Table 2. Analysis Descriptive CCR7 Expression before and after treatment

	CCR7 Expression		P
	Before	After	
Mean	30.21	34.34	0.008
Standard deviation	10.57	10.20	
Median	26.70	30.50	

Wilcoxon test shown significant difference CCR-7 expression before and after treatment.

Table 3. Analysis Descriptive lymphnode volume before and after treatment

	Lymphnode volume		P
	Before	After	
Mean	293.38	110.38	0.008
Standard deviation	310.63	186.01	
Median	159.51	8.184	

Wilcoxon test shown significant difference lymphnode volume before and after treatment. Correlation between SOX2 expression and alteration of lymphnode volume shown by Spearman correlation test resulted negative correlation before and after treatment with coefficient of 0.379 (moderate correlation). The increased of SOX2 expression correlated with decreased of lymphnode volume, also meant that smaller treatment response.

Picture 1. Correlation SOX2 expression before treatment with reduction of lymphnode volume

Picture 2. Correlation CCR7 expression before treatment with reduction of lymphnode volume

Correlation between CCR7 expression and alteration of lymphnode volume shown by Spearman correlation test resulted negative correlation before and after treatment with coefficient 0.134 (weak correlation). It is meant that increasing of CCR7 expression correlated with decreasing lymphnode volume, also shown smaller treatment response.

3.1 Analysis between alteration of CD44⁺, SOX2 and CCR7 expression and lymphnode volume before and after treatment

Analysis of CD44⁺ expression before and after treatment cannot be evaluated because same result before and after treatment. Also for analysis between CD44 expression and lymphnode volume cannot be evaluated because of same reason above.

Table 4. Spearman Test Correlation Alteration of SOX2 expression and Lymphnode volume after treatment

	Alteration Before and After Treatment			P
	SOX2	Lymphnode volume	Coeff. correlation	
Average	29.50	80.23	0.293	0.444
Standard deviation	36.81	21.62		
Mean	10.86	91.80		

Table 5. Spearman Test Correlation Alteration of CCR7 expression and Lymphnode volume after treatment

	Alteration Before and After Treatment			P
	CCR7	Lymphnode volume	Coeff. correlation	
Average	15.183	80.23	0.201	0.604
Standard deviation	11.282	21.62		
Mean	10.462	91.60		

All subject shown increase SOX2 and CCR7 expression after treatment. Spearman test result positive correlation between percentage alteration of lymphnode volume (decreased) and percentage alteration of SOX2 and CCR7 expression (increased) with coefficient correlation 0.293 for SOX2 and 0.201 for CCR7. This result meant higher increased SOX2 and CCR7 expression would be more decreased lymphnode volume. The correlation above statistical not significant (P=0.444; P=0.604)

4. DISCUSSION

All subject of this study are NPC WHO type III with positive LMP-1 and all in late stage according to American Joint Committee Cancer/International Union Against Cancer (AJCC/UICC) 2010. 9 subjects contain of six males and three females with age 40-56 years. All of patients have many size enlargement of neck lymphnode.

Treatment in this study are neoadjuvant chemotherapy cisplatin-based give for three times with three weeks interval. Measurement of all variable have done before and after treatment. CD44⁺ which did not alter expression shown that LMP-1 modulated NPC stem cells through promotion of the EMT [2-4]. LMP-1 also provided and activated PI3-K/AKT pathway that very important for induction and maintain of CSC properties in NPC [5, 6]. CD44 is once of cell surface markers which expressed in the membrane cells. CD44 as a receptor for ECM, growth factors and cytokine, have critical role as regulators of cancer stemness including self-renewal, tumour initiation and metastasis [5, 6].

Expression SOX2 after treatment increased significantly. SOX2 regulate EMT and involved at the metastasis, invasion and migration process. SOX2 also preserved CSC phenotype by modulation of histone acetyltransferase (HAT) [9, 10]. NPC stem cells contain of high level of SOX2, OCT4 and Nanog. High expression of SOX2 can be founded in cancerous tissue but only 5% expressed in normal tissue [9]. High expression of SOX2 are correlated with ability to pluripotency, progressivity, aggressiveness and invasion. Once study clarified that NPC stem cells which expression of SOX2 are correlated with metastatic ability to lymphnode and predicted poor prognosis [9, 10].

Expression of CCR7 increased after treatment. It is shown that CCR7 has been high expressed in head and neck cancer. Secondary lymphnode as main sites for metastatic of many cancers. CCR7 mediated expansion of the cancer cells to lymphnode by PI3-K/AKT pathway [11, 12].

CCR7 and CXCR4 have correlated significantly to the patients with lymphnode metastasis compared to patient without lymphnode metastasis. CCR7 more linked with late stage cancer, but CXCR4 mostly founded significantly high in patient with distant metastasis [13-15]. CCR7 also upregulated MMP9 which subsequently lead the dendritic cell to move to

the lymphnode and increased resistance to the treatment [16, 17]. NPC as an epithelial malignancy have close related to EBV and shown highly ability to invasion and metastasis, subsequently predicted to poor prognosis because of resistance to therapy [16-19].

CCR7 mostly expressed in cells highly built spheroid in NPC and correlated with metastatic to the lymphnode. CCR7 expression also contributed to recurrence and distant metastatic process.

Nine subjects was given neoadjuvant chemotherapy according to the standard procedure, three times with three weeks time interval. We used chemotherapy cisplatin based for NPC patient was killed eliminated all cancer cells, altered cancer cells to be more sensitive to radiation and can be eliminate systemic micro metastatic molecules as early as possible. Also all patients must have been waited for radiation because of lack radiotherapy facilities for long time 6-12 months.

All subjects shown decreases of the lymphnode volume significantly. It was compared to the result of increased SOX2 and CCR7 expression and level of CD44⁺ which did not altered after treatment. There was explained that NPC progenitor cells still sensitive to the chemotherapeutic agent. We founded negative correlation between CD44⁺, SOX2, CCR7 expression with reduction lymphnode volume before treatment and positive cumulation after treatment.

5. CONCLUSION

Neoadjuvant chemotherapy cisplatin based can be eliminated progenitor cells, but did not for cancer stem cells (CD44⁺) SOX2 and CCR7 in NPC. Positive correlation between alteration of SOX2 and CCR7 expression after treatment indicate that higher increase of cancer stem cells expression will following with high reduction lymphnode volume. We must pay more attention or more focus toward resistance of the cancer stem cells in NPC after treatment because of the potential tumour recurrence in the future although they have been received all standard therapy for NPC.

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