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Correlation between HIF-1α and CD44 with Radiotherapy Response in Stage IIB-IIIB Cervical Squamous Cell Carcinoma

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Abstract: ***Cervical cancer is the fourth most commonly malignancy in women worldwide and one of the most common malignancies in Indonesian women. Radiotherapy is one of the therapeutic modalities of cervical carcinoma. On the other hand, radioresistance can cause failure in this treatment. Under certain circumstances, reoxygenation can ensure the successfulness of radiotherapy. HIF-1α and CD44 are proteins markers that play role in hypoxia condition and stemness of cancer cells which has correlation with radiotherapy resistance. The aim of this study is to find the correlation between HIF-1α and CD44 immunoexpression with radiotherapy response in stage IIB-IIIB cervical squamous cell carcinoma. A retrospective case control analysis design has been used in this study, using secondary data of patientsstage IIB-IIIB cervical squamous cell carcinoma at the Department of Anatomical Pathology of Dr Hasan Sadikin Hospital, Bandung. There are 64 samples consist of 32 radiotherapy sensitive cases and 32 radiotherapy resistant cases. Immunohistochemical staining of HIF-1α and CD44 were performed to all samples. The result of this study shows a statistically significant correlation between HIF-1α (p=0.0001) and CD44 (p=0.011) immunoexpression with radiotherapy response respectively, with Odd Ratio (OR) 27.35 and 4.78 respectively. In conclusion, radiotherapy response in Cervical squamous cell carcinoma is influenced by hypoxia condition and stem cell status. Immunoexpression of HIF-1α and CD44 can be used to predict radiotherapy response. Increase of HIF-1 α and CD44 immunoexpression has a positive correlation with the possibility of radiotherapy resistance.***

Keywords: CD44, HIF-1α, Cervical Squamous cell carcinoma, Radiotherapy

1. Introduction

Cervical cancer in general according to Globocan (2012) is the fourth leading cancer in women, and the seventh of overall cancer worldwide.[1] In developing countries, including Indonesia, cervical cancer is reported as the second leading cause of death in women with high mortality rate (59%).[2] Moreover in 2013, according to Pusdatin (Center for Data and Information) Ministry of Health Republic of Indonesia, cervical cancer is claimed as the highest incidence of cancer occupied by women.[3]Squamous cell carcinoma is one of the types of cervical cancer which composed of squamous cells with various degree of differentiation.[4]

The five-years survival rate of cervical cancer patients is generally varies depending on the stage of the disease, i.e, 97-100% in stage IA, 84% in stage IB, 65-73% in stage II, then continue to decrease to 36% in stage III, and lastly < 15% in stage IV.[5]

Cervical cancer treatment at early stage is done by surgery, whereas radiation therapy is applied at all stages with the mass of the tumor still localized in the pelvis. Especially in patients with advanced stages of cancer, radiation therapy is recommended as the primary therapy, while in high-risk patients such as comorbid or obese patients, radiation therapy may be given as adjuvant therapy to reduce the risk of recurrence after surgery. Radiation therapy is a very effective therapy in stage IB1 of cervical cancer, with systemic and local controls reaching 98% and 95% respectively, and the disease-free survival rate is reaching 90%. However, maintaining high percentage of local control is still become a major constraint in locally advanced stage IB2-IIIB cervical cancer, due to high rate of local recurrence (60% to 70%). The rate of patient's 5-year survival ranged from 40% to 50%, and it continue to decrease along with the cancer progression, 5-15% in stage III and stage IV cervical cancer.[6]In Indonesia, cervical cancer management with radiotherapy or chemoradiation is considered as the main therapy for stage IIB-IIIB cervical cancer, according to cervical cancer management guidelines issued by The Ministry of Health, Republic of Indonesia. Cervical cancer stage is stipulated based on clinical staging, which is staging corresponding to pre-treatment primary tumor clinical examination. Currently, cervical cancer stage is determined by International Federation of Obstetricians and Gynecologist (FIGO) 2000. Hence, this research was conducted to early-detect the radiotherapy response on cervical squamous cell carcinoma patients that had underwent biopsy procedure.

There are several factors that can alter the radiosensitivity in cervical cancer such as apoptotic protein mutation (bcl-2, bax, and p53); tumor DNA damage that caused by free radical formed by COX by product in inflammation reaction; angiogenesis which can be detected by VEGF and EFGR expression; hypoxia conditions which can be assessed by examination of oxygen levels by using electrodes and with hypoxic markers HIF-1α; and temperature.[7]

Hypoxia inducible factor-1α (HIF-1α) is a transcription factor that regulates cell biology in hypoxia condition, and tumor cells are known to activate this condition other than physiological hypoxia.[8] Cancer stem cells (CSC) are also contribute to cancer invasiveness. Biomarker stem cell CD44 is a transmembrane receptor protein which also categorized as adhesion molecule group is involved in cell-cell and cell-matrix interaction. Several studies have mentioned that CD44 and HA interaction can elicit various

intracellular pathways, i.e PI3K/Akt pathway which can lead to continuous tumor cell proliferation of and eventually result in radioresistence.[9]

2. Method

This study uses analytic observational method with case control study design and retrospective data retrieval / collection. Ethical clearance has been approved / assessed by Health Research Ethic Commission, Padjajaran University, with assessment number 14/UN6.KEP/EC/2018.

Samples preparation: The samples were obtained from patients registered at Hasan Sadikin Hospital, histopathologically diagnosed with cervical squamous cell carcinoma during January 2016 to September 2017, and had received radiotherapy 30 times, then classified as radiosensitive or radio resistant group. The tumor cells were then collected through biopsy and fixated in paraffin block.

Analysis of immuno expression: Samples from paraffin blocks were prepared for the immunohistochemistry analysis; immunohistochemistry analysis was performed based on the protocol provided by anatomical pathology laboratory. The slides were then visualized under the microscope.

Histoscore calculation: Positive result was shown/visualized as brown staining on the tumor cell. Analysis on HIF-1α and CD44 immunoexpression was evaluated by brown staining assessed on the tumor cell nucleus and the cell membrane respectively. The stain intensity measured under the microscope was then converted into score. Scores obtained from the samples were represented on a scale of 0-4. Score 0 represent blank samples or no colored tumor cells; score 1+ for positive weak, or less than 25% colored/stained tumor cells; score 2+ for moderate positive or 25%-50% stained tumor cells; and score 3+ for strong positive or 51-80% stained tumor cells; and lastly score 4+ represent >80% of colored tumor cells. Histoscore was obtained from score attained by the software multiplied to distribution of tumor cell.

Statistical analysis: All the data collected from IHC staining was evaluated for significant difference through non-parametric test Chi-Square.

3. Results

During January 2016 to September 2017 there were 140 cervical squamous cell carcinoma cases registered, however only 64 samples that was matched the inclusive criteria.

Table 1: Squamous cell carcinoma characteristic

Variable	Radiotherapy Response		P Value
	Sensitive	Resistant	
	N=32	N=32	
Histopatology			**1.000**
Keratinizing Squamous cell carcinoma	16(50.0%)	16(50.0%)	
Non Keratinizing Squamous cell carcinoma	16(50.0%)	16(50.0%)	
Staging			**0.999**
II B	17(53.1%)	20(62.5%)	
III A	3(9.4%)	1(3.1%)	
III B	12(37.5%)	11(34.4%)	
Grading			**1.000**
Poor	5(15.6%)	4(12.5%)	
Moderate	21(65.6%)	21(65.6%)	
Well	6(18.8%)	7(21.9%)	

Table 1 shows several characteristics of the subjects such as histopathology profile, cancer stages, and grades. The samples are distributed equally between radiosensitive and radioresistant, with most cases classified as IIB cancer stage (17 cases or 53.1% in radiosensitive group; and 20 cases or 62.5% in radioresistant group), as well as moderate grading in both group (21 cases or 65.6%).

Table 2: HIF-1α and CD44 histoscore data on radiotherapy responses group.

Variable	Radiotherapy Response		OR CI (95%)	P Value
	Sensitive	Resistant		
	N=32	N=32		
Histoscore HIF-1α			27.35 (3.320-225.36)	0.0001
Low (0-4)	31(96.9%)	17(53.1%)		
High (6-12)	1(3.1%)	15(46.9%)		
Histoscore CD44			4.78 (1.354-16.936)	0.011
Low (0-4)	13(40.6%)	4(12.5%)		
High (6-12)	19(59.4%)	28(87.5%)		

Table 2 shows the association between HIF-1α and CD44 immunoexpression with radiotherapy response. There is a significant association between HIF-1α immunoexpression with radiotherapy response (p=0.0001) with odds ratio 27.35 which means patients with weak expression of HIF-1α are 27.35 times more likely to be radiosensitive compared to patients with strong expression of HIF-1α. In addition, CD44 immunoexpression is also significantly correlated with radiotherapy response (p=0.011) with odds ratio 4.78 which represent the patients with weak expression of CD44 are 4.78 times more likely to be radiosensitive compared to patients with strong CD44 expression.

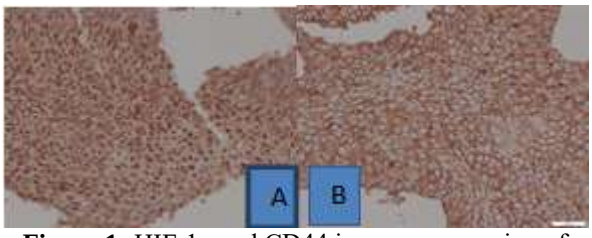

Figure 1: HIF-1α and CD44 immunoexpression of squamous cell carcinoma cervix (A) HIF-1α strong expression, tumor cells stained with HIF-1α antibody in the nuclear and cytoplasm (B) CD44 strong expression, tumor cells stained with CD44 antibody in the membrane. (400X Magnification)

4. Discussion

Histopathology data on table 1 was statistically analyzed with Chi-Square analysis. Statistical analysis shown that sample's characteristics such as histopathology, cancer stages, and grades, obtain the p value that is higher than 0.05 which represent no significant difference between each

characteristic on radiosensitive and radioresistant group. Statistical analysis was also performed on both groups. Result shown that the both groups are homogenous and the p value is higher than 0.05 which mean no significant difference in characteristic between both group. Hence the data is qualified for further statistical analysis.

HIF-1α is a transcription factor that is activated when the oxygen tension is depleted. Previous studies have shown the association between HIF-1α and radioresistence through several mechanisms such as apoptosis suppression which also lead to continuous cell proliferation. This hypoxia condition also causes angiogenesis and ineffective DNA strand break, which both will lead to radioresistence.[8]

This research performed immunoexpression analysis on HIF-1α on stages IIB-IIIB cervical squamous cell carcinoma which already attained full radiotherapy with complete response. Result from immunohistochemistry staining discover that 31 samples (96.9% of the population) shown weak expression, and 1 sample (3.1% of the population) shown strong expression. Statistical analysis Chi-Square reveal that there is a significant difference (p=0.0001) which explains that there is a correlation between the expression of HIF-1α and radiotherapy response. Strong expression of HIF-1α results in high possibility of resistance in radiotherapy.

Previous studies have similar results compare to this research. Zhu et. al showed significant difference between strong HIF-1α expression and partial radiotherapy response group as well as complete radiotherapy response group in locally advanced cervical cancer. Patients with weak HIF-1α expression tend to be more sensitive to radiation.[10]Dellaset. al discovered a strong correlation between HIF-1α expression and high metastasis incidence as well as local tumor progression in invasive cervical carcinoma.[11]

There are various HIF-1α activity pathways, lead to many researches about HIF-1α inhibitor. This study has found that the increased HIF-1α immunoexpression parallel with radioresistence occurrence. Thus, HIF-1α immunohistochemistry staining can be advised to cervical squamous cell carcinoma patients before radiotherapy treatment.

Radiotherapy is one of many therapies that can kill and support cancer cell shrinkage. However, radioresistence and local tumor recurrence can be a major problem for radiotherapy patient's survival. The mechanism behind radioresistence occurrence is yet unknown and can be altered by many factors. One factor that has been under a lot of studies and suspected to cause radioresistence is cancer stem cell. Cancer therapy management is still under continuous development while concurrently local recurrence and metastasis is still taking place after radiotherapy or other combined treatment. Poor effectiveness in CSC eradication is suspected as one factor that cause radioresistence. Thus, in this research we examine the CD44 expression as CSC marker.[9, 12]

CD44 is a membrane glycoprotein on mammal cell, including endothelial cells, epithelial cells, fibroblasts, keratinocytes, and leukocytes. Physiologically CD44 involved in cell-cell and cell-matrix interaction, such as cell proliferation, cell adhesion, cell migration, hematopoiesis, and lymphocytes activation. Hyaluronic acid (HA) is the main ligand for CD44, and CD44 signaling plays an important role in tumor growth and cancer metastasis. Binding of HA to CD44 on the cell surface can elicit various pathways such as PI3K, Akt, PP2A, ERK, and Ras/Raf/Rac. These events will then leads to tumor progression and growth. CD44 activation will inhibit the transforming growth factor β (TGF-β) signal which will increase the cancer's self-renewal activity, as well as promote antiapoptotic effect through Akt pathway.[9]

This study also performed CD44 immunoexpression analysis on stages IIB-IIIB squamous cell carcinoma which already received full radiotherapy treatment with a complete radiotherapy response. Result from immunohistochemistry staining confirm that there are 13 samples (40.6% of the population) shown weak CD44 immunoexpression and 19 samples (59.4% of the population) shown strong CD44 immunoexpression. According to Chi-Square analysis, there is a significant difference (p=0.011) between CD44 expression and radiotherapy response. Strong CD44 immunoexpression results in high possibility of resistance in radiotherapy response. Study performed by Liu et. al confirmed this result, that radiotherapy related-CD44 expression could inhibit cervical cancer cell apoptosis.[13]

5. Conclusion

There was a significant association between HIF-1α and CD44 with radiotherapy response in stage IIB-IIIB cervical squamous cell carcinoma. Strong HIF-1α and CD44 expression positively correlated to the likelihood of radio resistance.

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Author Profile

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