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Research Article

Chemokine C-X-C Receptor Type 4 (CXCR4) and Autocrine Motility Factor Receptor (AMFR) expression in Renal Cell Carcinoma and Their Correlation with Metastatic in Dr Hasan Sadikin Hospital, Bandung, Indonesia

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Abstract:

Background: Renal cell carcinoma (RCC) is the most aggressive urinary tract cancer. More than one-third of patients experience metastatic. Metastatic is responsible for 90% of cancer deaths compared to the primary tumor itself. Factors that play a role in the occurrence of metastatic are chemokine CXC receptors type 4 (CXCR4) and autocrine motility factor receptor (AMFR). This study aims to determine the relationship between CXCR4 and AMFR expression with metastatic in RCC.

Methods: Case control research design was done to 44 Fixed Formalin Paraffin Embedded (FFPE) of RCC cases at the Department of Anatomical Pathology of Dr. Hasan Sadikin Hospital, Bandung, Indonesia. FFPE samples consist of 22 cases of metastatic RCC and 22 cases of non-metastatic RCC. Immunohistochemical staining of CXCR4 and AMFR was performed to all samples. All data were analyzed using Chi-Square test with p-value < 0.05 of significant level and then processed using SPSS 24.0 for Windows.

Results: The result of this study showed a significantly different statistics of CXCR4 (p = 0.015) and AMFR (p = 0.014) expression between metastatic RCC and non-metastatic RCC. AMFR expression is the stronger factor that affects metastatic compared to CXCR4 expression ((Odds Ratio AMFR: OR CXCR4 = 4.911: 4.667).

Conclusions: Increased CXCR4 and AMFR expression were associated with higher possibility of metastatic in RCC. These findings suggest that high expression of CXCR4 (histoscore ≥8) and AMFR (histoscore ≥5) can be considered to predict metastatic in RCC.

Keywords: AMFR, CXCR4, metastatic, renal cell carcinoma

Introduction

Renal Cell Carcinoma (RCC) is a malignant tumor originated from tubular epithelium. RCC is the third most common urogenital cancer in men. RCC is the most aggressive urinary tract with high mortality rates.[1, 2] The incidence of renal cell carcinoma in the world is 2-3% of all cancer cases with an increased incidence per year.[2] The incidence of renal carcinoma in Indonesia, based on Globocan in 2018, is 0.84% of all cancer cases or an estimate of 2112 cases, with a mortality rate of 1225 cases.[3] In Dr. Hasan Sadikin General Hospital Bandung, the incidence of RCC increased by 2.44 times between periode 2010-2014 and 2015-2018.[4]

Patients with metastatic renal cell carcinoma (mRCC) are very resistant to chemoradioresitance with a low survival rate (8%). Once metastatic occurs, most patients will relapse and eventually die after receiving targeted tyrosine-kinase and mTOR-inhibitor therapy. This target therapy is not effective for all patients and requires substantial costs. Prognostic and predictive markers are needed for more effective systemic therapy for each patient.[1]

Clinical studies show growth factors and their receptors involved in the development of cancer metastatic.[5] Chemokine C-X-C receptor type 4 (CXCR4) is a chemokine factor involved in the transition/ chemotaxis process,[1] whereas AMFR is an autocrine motility factor in tumor cells involved in invasive cells and tumor angiogenesis.[6] CXCR4 and AMFR can be studied for new treatment strategies for cancer metastatic.

CXCR4 is a chemokine receptor that is specific to CXCL12. CXCR4 is the most participating chemokine receptor and has been shown to play an important role in metastatic.[7] At present, CXCR4 has proven overexpression of more than 23 malignancies, such as thyroid, breast, pancreas, liver, prostate, and others. The results of several studies show that published overexpression of CXCR4 is proven by metastatic and poor prognosis.[1, 6, 8, 9]

The locomotor activity of tumor cells plays an important role in invasion and metastatic. Some researches show that autocrine motility factor receptor (AMFR) shows a regulator of tumor cell motility. AMFR overexpression has been seen in colon, gastric, lungs, skin, breast, esophageal, liver carcinomas and soft tissue sarcomas. Furthermore, increasing AMFR levels can be used as tumor markers, which correlate with the development of metastatic and poor prognosis.[10, 11] In the Endo et al. Study, AMFR significantly correlated with tumor recurrence, local metastatic and advanced metastatic in squamous cell carcinoma of the tongue.[12]

Thus, an accurate value of CXCR4 and AMFR expression

from a tumor specimen will provide valuable predictive information on metastatic for the prognosis of the disease and require therapeutic intervention.

To the best of our knowledge, no data has been reported until now about the role of the immunohistochemically assessed expression of CXCR4 and AMFR of RCC in a large, single-institution series in Indonesia.

Materials and Methods

Patients

This study was started after had been approved by the Universitas Padjadjaran Research Ethical Committee (1420/UN6.KEP/EC/2018). RCC tissue samples were collected from Dr. Hasan Sadikin Hospital, Bandung, Indonesia from January 2014 to September 2018. We use analytic observational method with case-control study design and retrospective data collection There were 72 cases of RCC during that period, only 44 cases could be included. On the basis of the metastatic status, the patient samples were categorized into two groups: non metastatic RCC (n = 22), and metastatic RCC (n = 22).

Immunohistochemistry (IHC)

Immunohistochemistry was done by using labeled streptavidin-biotin immunoperoxidase complex method with *Starr Trek Universal Horseradish peroxidase Detection system* (Biocare Medical, California, USA). Primary antibodies used were rabbit anti-CXCR4 polyclonal antibody with dilution 1:100 (cat No. PA1237, BosterBio, USA) and rabbit anti-AMFR polyclonal antibody with dilution 1:100 (cat No. GTX112393, Genetex, USA). Ovarium and breast carcinoma tissue were used as external positive controls for CXCR4 and AMFR. IHC staining was performed on 4 µm-thick, FFPE tissue sections manually. Counterstaining was done using Mayer's hematoxylin.

Evaluation of Immunostaining

The expression of CXCR4 and AMFR were assessed using a semiquantitative scoring system (histoscore) in all of the immunohistochemical-stained sections according to the method used by Zhao et al. and Grewal et al.[13, 14] CXCR4 and AMFR positivity were defined by membrane and/or cytoplasmic staining in tumor cells. The staining intensity was scored as 0 (negative), 1+ (weak), 2+ (moderate), and 3+ (strong). The distribution percentage of positively stained cells were scored as 0 (none), 1 (<10%), 2 (10-50%), 3 (50-80%), or 4 (>80%) for CXCR4 and were scored as 0 (none), 1 (<10%), 2 (10-50%), 3 (>50%) for AMFR. The final score was calculated by multiplying the staining intensity and percentage score, ranging from 0 to 12 for CXCR4 and 0 to 9 for AMFR. The patients were divided to high or low expression using the median score of each marker as cut off points (median score of 6 for CXCR4 and 4 for AMFR). Histoscores CXCR4 were classified as low (≤ 6) and high (8-12). Histoscores AMFR were classified as low (≤ 4) and high (5-9). All immunohistochemical stainings were evaluated blindly by two independent investigators.

Statistical Analysis

Numeric data of patients' characteristics were recorded as mean, median, range, and standard deviation. Comparisons of clinical data between groups were carried out using the Chi-Square, Mann-Withney, and Kolmogorov-Smirnov test. P-values were calculated and $p<0.05$ was considered statistically significant. The analysis was performed using Statistical Package for Social Science (SPSS version 24.0, IBM Corp., Armonk, NY, USA).

Result

Patient Characteristics

Forty-four RCC samples that were included in the present study, more common in male and in 6th decade of life and most common histopathology type was clear cell RCC (table 1) . The overall distribution of metastatic site is in table 2. The most common sites were lung (38.71%), lymph node (32.26%) ,and liver (22.58%). The rate of single site metastatic was 63.64% versus 36.36% for metastatic at two or more sites.

Table 1. Characteristics of Patients from each group

Variable	Group		P value
	Non-metastatic RCC N=22	Metastatic RCC N=22	
Age (year old)			0.347*
Mean±Std	51.68±12.755	56.95±14.244	
Median	52.50	53.50	
Range(min-max)	22.00-74.00	40.00-89.00	
Sex			0.472**
Male	18 (81.8%)	16 (72.7%)	
Female	4 (18.2%)	6 (27.3%)	
Type of histophatology			0.860***
Clear Cell	20 (90.9%)	16 (72.7%)	
Papillary	2 (9.1%)	4 (18.2%)	
Chromophobe	0 (0.0%)	2 (9.1%)	

Note: $p < 0.05$ means statistically significant

*Mann-whitney test **Chi-square test ***Kolmogorov-smirnov test

From the analysis of the characteristics of the two groups above with p value > 0.05 in table 1, it shown that the two groups are homogen. The differences in age, sex and type of histopathology in each group were not confounding factors in this study.

Table 2. Characteristics of metastatic group

Variable	N=44
Site of metastatic	
Lungs	12 (38.71%)
Lymph node	10 (32.26%)
Liver	7 (22.58%)
Bone	1 (3.23%)
Brain	1 (3.23%)

Number of metastatic sites	
1	14 (63.64%)
2	7 (31.82%)
3	1 (4.55%)

CXCR4 and AMFR expression in RCC tissue

The correlations between CXCR4 and AMFR expression in RCC are summarized in table 3. In CXCR4 expression, fourteen patients of metastatic group (63.6%) displayed high histoscore ≥8 (Fig. 1A, 1B and 1C). There was correlation between CXCR4 expression and metastatic status in RCC, with P value 0.015 (P value <0.05), with Odd Ratio 4.667 and confidence interval 1.299-16.761. Patients with high CXCR4 expression (histoscore ≥8) will get higher possibility of metastatic 4.667 times compared to patients with low CXCR4 expression (histoscore ≤ 6). In AMFR expression, thirteen patients of metastatic group (59.1%) displayed high histoscore ≥5 (Fig. 2A, 2B, 2C and 2D). There was correlation between AMFR expression and metastatic status in RCC, with P value 0.014 (P value <0.05), with Odd Ratio 4.911 and confidence interval 1.325-18.205. Patients with high AMFR expression (histoscore ≥5) will get higher possibility of metastatic 4.911 times compared to patients with low AMFR expression (histoscore ≤ 4).

Table 3. CXCR4 and AMFR data on non-metastatic and metastatic RCC

Variable	RCC		OR CI (95%)	p value
	Non-metastatic	Metastatic		
CXCR4 Histoscore				0.015 *
Low	16 (72.7%)	8 (36.4%)	4.667 (1.299-16.761)	
High	6 (27.3%)	14 (63.6%)		
AMFR Histoscore				0.014 *
Low	17 (77.3%)	9 (40.9%)	4.911 (1.325-18.205	
High	5 (22.7%)	13 (59.1%)		

Note: p < 0.05 means statistically significant. *Chi-square test

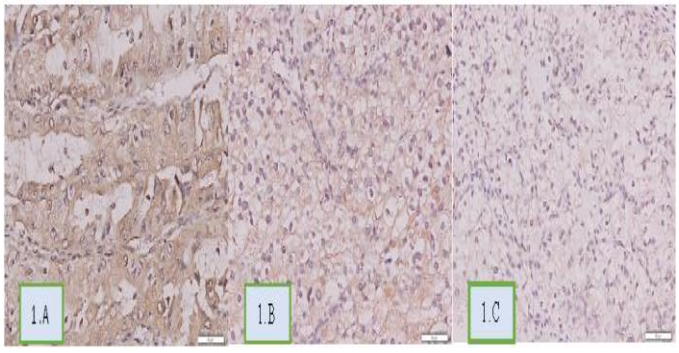

Figure 1: CXCR4 expression of clear cell RCC. Tumour cell stained with CXCR4 antibody in the cytoplasmic and/or membrane cells. (A) strong expression, (B) medium expression, (C) low expression. (200x Magnification).

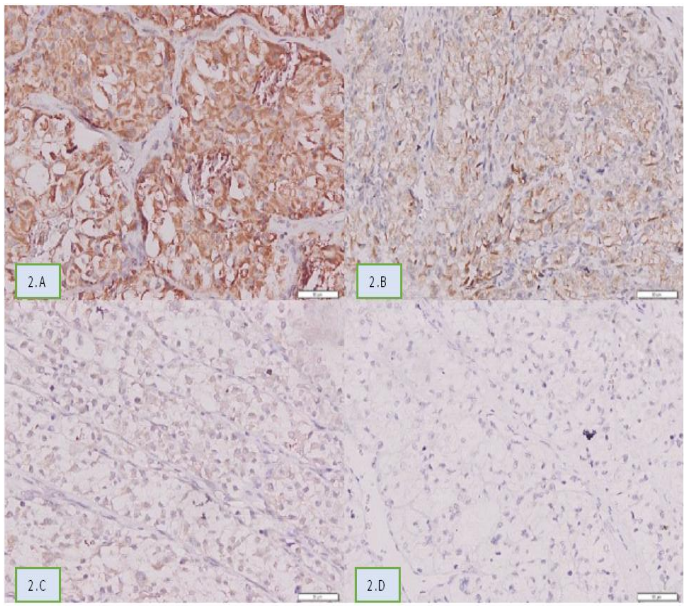

Figure 2: AMFR expression of clear cell RCC. Tumour cell stained with CXCR4 antibody in the cytoplasmic and/or membrane cells. (A) strong expression, (B) medium expression, (C) low expression, (D) negative expression. (200x Magnification).

Average histoscore of CXCR4 and AMFR in RCC was shown in table 4. The highest expression of CXCR4 was found in chromophobe subtype, lung metastatic, and multiple site of metastatic. The highest expression of AMFR was found in chromophobe subtype, lung and liver metastatic, and single site of metastatic.

Table 4. Average Histoscore CXCR4 and AMFR in RCC

Variable	CXCR4	AMFR
Type of histopathology		
Clear cell	6.4	4.4
Papillary	5.3	3.5
Chromophobe	8	7.5
Site of metastatic		
Lung	8.5	6.25
Liver	7.25	6.25
Lymph Node	5.67	6
Number of metastatic site		
Single	6.9	5.3
Multiple	7.5	5

Discussion

The prognosis of RCC is influenced by many factors, which are broadly divided into anatomical, histopathological, clinical, and molecular factors. Invasion of lymph nodes and distant metastatic are anatomical factors, which are generally used in the classification of stages according to the Tumor

Node Metastatic (TNM). Existing prognostic factors have limited accuracy, for example, in micrometastatic formation, it cannot be detected radiologically or clinically, so molecular markers are needed to improve the accuracy of the prognostic.[7, 15, 16]

CXCR4 is a pair of G-protein receptors with seven-transmembrane helices.[17] CXCR4 expression has been shown to play an important role in migration, metastatic, and poor prognosis in malignancy.[18] In clear cell RCC, the pVHL protein encoded by the VHL gene plays an important role in the mechanism of metastatic. The pVHL protein has the ability to degrade hypoxia-inducable factor (HIF) under normal conditions, but at the time of mutation and the presence of hypoxic conditions, it will result in accumulation of HIF. This accumulation of HIF will cause activation of CXCR4.[19]

Although the exact mechanism of CXCR4 activity has not been fully explained, CXCR4 can induce or support carcinogenesis through the interaction of CXCR4 with CXCL12 ligand, which mediates the activation of phosphatidylinositol 3-kinase (PI3K) and Akt, resulting in cell proliferation and survival. The activated CXCR4-CXCL12 axis also activates signals from MAPK promotes chemotaxis and proliferation. CXCR4-CXCL12 axis also activates phospholipase C (PLC)/ protein kinase C (PKC)-Ca^{2+} signals, promotes cell migration.[6, 9, 20]

CXCR4 target therapy is being developed, which in preclinical studies has the ability to prevent tumor growth and metastatic in animal breast, head, and neck carcinoma models.[21] In small cell lung carcinoma, antagonist CXCR4 (TN14003) interferes with CXCR4-CXCL12 interactions and inhibits cell adhesion and chemoresistance.[22]

In this study, the percentage of lungs metastatic (38.71%) was lower than incidence in WHO (75%). This is because in this study, more lymph node metastatic was found more than in other studies, reaching 32.3%. Low expression of CXCR4 was found in the metastatic group in 8 cases (5 cases in lymph node metastatic and the rest metastatic are in lung, liver, and bone), while low expression of AMFR was found in the metastatic group in 9 cases (7 cases in lymph node metastatic, and the rest metastatic are in lung and liver).

AMFR is protein that is encoded by the AMFR gene on chromosome 16q12.2, an increase in AMFR can function as a tumor marker, which correlates with the development of metastatic and poor prognosis.[11] AMFR is bind to AMF. The AMF-AMFR bond then stimulates various signaling pathways, including those regulated by protein kinase C (PKC) and tyrosine kinase, small GTPases Rac1 and RhoA.[23, 24] Binding receptors also lead to activation of the phosphatidylinositol 3-kinase pathway (PI3K) and the pathway of mitogen-activated protein kinase (MAPK), thereby increasing the proliferative, migration and angiogenic activity of tumor cells.[25]

At present, there are no drugs that selectively target AMFR, but anti-AMFR antibodies have been developed and used to analyze AMFR expression. The molecular and physiological properties of AMFR are very attractive as target therapy compared to other cell surface tumor markers, and their role in metastatic and tumorigenicity makes it a promising functional target.

In conclusion, there was a significant correlation between CXCR4 and AMFR expression with metastatic in RCC. Increased CXCR4 and AMFR expression were associated with higher possibility of metastatic in RCC. These findings suggest that high expression of CXCR4 (histoscore ≥8) and AMFR (histoscore ≥5) can be considered to predict metastatic in RCC.

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Conflict of Interest

The authors declare that there are no conflicts of interests in this study.

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