

The Role of EMMPRIN in Perirenal Fat Invasion Clear Cell Renal Cell Carcinoma

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Abstract

Renal Cell Carcinoma (RCC) is a common malignancy of the kidney, and Clear Cell Renal Cell Carcinoma (CCRCC) is the most common type. Accurate prediction of prognosis is valuable for therapy and follow up. EMMPRIN is a transmembrane protein of the immunoglobulin family and is associated with tumour proliferation, invasion and metastasis. This study aims to prove the role of EMMPRIN in perirenal fat invasion CCRCC. This analytic observational study with a cross-sectional approach conducted in Anatomical Pathology Institute of Dr. Soetomo Hospital, Surabaya that used 44 samples of paraffin blocks from radical nephrectomy preparations for CCRCC patients at the period of January 2013-December 2018, which were divided based on perirenal fat invasion status. The analyzed was using Spearman test. EMMPRIN expression is positively correlated with perirenal fat invasiveness ($p = 0.019$) in clear cell renal cell carcinoma. EMMPRIN expression is related with perirenal fat invasiveness, in clear cell renal cell carcinoma.

Keywords: Clear cell renal cell carcinoma, EMMPRIN, perirenal fat invasion

Introduction

The Renal Cell Carcinoma (RCC) is the sixth most common malignancy diagnosed in men and tenth in women and accounts for approximately 85% of malignant kidney tumors and 2% of all malignant tumors. In Europe and North America, the lifetime risk for developing renal cell carcinoma ranges between 1.3% and 1.8%. According to the latest data from the World Health Organization (WHO), there are more than 140,000 deaths related to renal cell carcinoma each year, with renal cell carcinoma ranking as the 13th most common cause of cancer death worldwide.^{1,2}

Tumor staging (TNM), defined by the disease's anatomical involvement, is recognized as one of the

strongest prognostic factors in the clinical outcome of patients with RCC, as described in the eighth edition of the American Joint Commission on Cancer (AJCC) Cancer Staging Manual. The system currently used is according to the 2016 WHO classification system. This system concerns tumor size, tumor growth through the renal capsule, tumor invasion of the renal veins, lymph nodes, and metastases to the adrenals, and distant metastases. These factors indicate a poor prognosis compared to tumors confined to the kidney.^{3,4}

EMMPRIN/CD147, also known as Basigin (BSG) or Extracellular Matrix Metalloproteinase Inducer (EMMPRIN), is a transmembrane glycoprotein belonging to the immunoglobulin superfamily that is highly expressed on the cell surface of various types of tumors, including breast, lung, mouth, esophageal cancer, larynx and kidney.⁵ EMMPRIN expression was positively expressed by 88.7% in advanced RCC.⁶ EMMPRIN is known to induce the production of various Matrix Metalloproteinases (MMPs) in cancer cells and

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fibroblasts after epithelial-stromal interactions. MMP is a major protease in degrading the extracellular matrix, which leads to cancer cell invasion and metastasis.⁷

EMMPRIN binds to Cyclophilin A (CypA). A previous study showed that the CypA-EMMPRIN interaction-initiated growth was signaling via a variety of pathways, including the MAPK, ERK1/2, and p38 signaling pathways that induce G1 to S transitions via cyclin D1 and p-RB in cholangiocarcinoma.⁸ Correlation between EMMPRIN expression in cell carcinoma kidney has not been reported. Therefore, this study was conducted to analyze EMMPRIN expression with perirenal fat invasion status in renal cell carcinoma. This study aims to prove the role of EMMPRIN on the status of perirenal fat invasion in Clear Cell Renal Cell Carcinoma (CCRCC).

Materials and Methods

Preparation and Sample of the Study

This study's research design was an analytic observational study with a cross-sectional approach, which was carried out in the Anatomic Pathology Installation of Dr. Soetomo General Academic Hospital Surabaya, Indonesia. The study sample used 44 blocks of paraffin radical nephrectomy preparations for CCRCC patients at the Anatomical Pathology Institute of Dr. Soetomo General Academic Hospital, Surabaya, for the period January 2013-December 2018. The samples were divided into two groups based on perirenal fat invasion status. The parameter of assessment was the expression of EMMPRIN, which streaked positively on tumor cells. This study was approved by the Health Research Ethics Committee of Dr. Soetomo General Academic Hospital, Surabaya, Indonesia (Ethical Clearance No.1705 / KEPK / XII / 2019).

Immunohistochemical Procedures

EMMPRIN expression in samples was observed using immunohistochemical staining. Paraffin blocks were cut 4 μ m, deparaffinized, and rehydrated with graded alcohol, then warmed with citrate buffer pH 6 for 20 minutes in the microwave. The primary

antibody, namely EMMPRIN (sc-71038, Santa Cruz Biotechnology, Inc.), was dripped by diluting 1: 250 at 40°C overnight. The secondary antibody is then dropped and incubated for 20 minutes. The final step, diaminobenzidine (DAB), was dripped, and counterstain was carried out with Meyer Hematoxylin.

Immunohistochemical Staining Analysis

EMMPRIN expression was assessed using an Immunoreactive Score (IRS), which is the multiplication of the percentage of tumor cells stained (A) and the intensity of staining (B). The percentage is divided into a score of 0 = no positive tumor cells, score 1 = positive tumor cells < 10%, score 2 = positive tumor cells 10 - 50%, score 3 = positive tumor cells 51 - 80%, and score 4 = cells positive tumors > 80%. Intensity was divided into a score of 0 = colorless, score 1 = weak intensity, score 2 = moderate intensity, and score 3 = strong intensity. The IRS (AXB) was divided into four groups, namely negative (score 0), weak (score 1 - 3), moderate (score 4 - 8), and strong (score 9 - 12). EMMPRIN expression was observed in the membrane and cytoplasm of tumor cells.⁹ EMMPRIN expression was observed using a binocular light microscope and evaluated by two pathologists.

Statistical Analysis

The correlation between perirenal fat invasion status and EMMPRIN expression was tested by the Spearman correlation test. The test results are said to have a significant correlation if the *p*-value is <0.05.

Results and Discussion

The patients' average age was 53.89 years with a male to female ratio of 2:1. In this study, clear cell, non-perirenal fat invasive (non-PFI) renal cell carcinoma was found in 59.1% (26/44) of cases, whereas clear cell, perirenal fat invasive (PFI) type renal cell carcinoma was only 40, 9% (18/44) of cases. The highest grade in this study was grade 3 (54.5%) cases (24/44). The clinicopathological characteristics of the patients are shown in Table 1.

Table 1. The clinicopathological characteristics of the patient.

Characteristics	n (%)
Age (years) #	
£ 40	2 (4.6)
41-50	13 (29.5)
51-60	17 (38.6)
61-70	11 (25.0)
>70	1 (2.3)
Gender	
Male	32 (72.7)
Female	12 (27.3)
Tumor Grade	
Grade 1	5 (11.4)
Grade 2	12 (27.3)
Grade 3	24 (54.5)
Grade 4	3 (6.8)
Perirenal Fat Invasion	
(pT1-2) / Non PFI	26 (59.1)
(pT2-4) / PFI	18 (40.9)

EMMPRIN expression in this study was stained on the membrane and cytoplasm of tumor cells (Figure 1). The results of this study indicated that EMMPRIN expression with a strong IRS score was more common in clear cell renal cell carcinoma with perirenal fat invasion,

namely 66.7% (Table 2). The Spearman correlation test results showed a significant correlation between perirenal fat invasion status and EMMPRIN expression ($p < 0.05$) with a value of $r = 0.352$ (Table 3). These results indicate that the higher the EMMPRIN expression is in line with the perirenal fat invasion status.

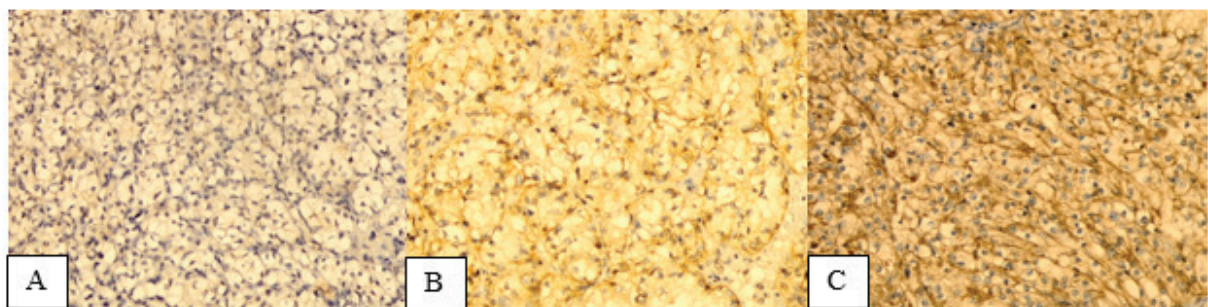


Figure 1. EMMPRIN expression by immunohistochemical staining on clear cell renal cell carcinoma, magnification: 400×. A: EMMPRIN expression with weak intensity; B: Medium intensity EMMPRIN expression; C: EMMPRIN expression with strong intensity.

Table 2. EMMPRIN expression on the status of perirenal fat invasion of Clear Cell renal cell carcinoma.

EMMPRIN Expression	Category	Non-invasive perirenal fat	Invasive perirenal fat	P
Percentage	<10%	0 (0%)	0 (0%)	
	11-50%	1 (3.8%)	0 (0%)	
	51-80%	21 (80.8%)	11 (61.1%)	
	>80%	4 (15.4%)	7 (38.9%)	
Intensity	Weak	3 (11.5%)	0 (0%)	
	Moderate	14 (53.9%)	7 (38.9%)	
	Strong	9 (34.6%)	11 (61.1%)	
IRS Score	Weak	4 (15.4%)	0 (0%)	0.019
	Moderate	13 (50%)	6 (33.3%)	
	Strong	9 (34.6%)	12 (66.7%)	

Table 3. The Spearman correlation test results of EMMPRIN expression with perirenal fat invasion status.

		EMMPRIN Expression
Perirenal fat invasion status	r	0.352
	p	0.019
	n	44

Most of the patients in this study were in the age range 51 - 60 years with 17 cases (38.6%), with a male to female ratio of 2:1 where the number of male cases was 32 cases (72.7%). This is in accordance with previous research which states that the highest incidence is found in the sixth and seventh decades of life and about 80% are between the ages of 40 - 69 years with the distribution of men more than women.^{10,11}

The results of this study indicate that EMMPRIN expression is stronger in Clear Cell Renal Cell Carcinoma (CCRCC) with perirenal fat invasion, and there is a significant correlation which indicates that the higher EMMPRIN expression is in line with the occurrence of perirenal fat invasion in Clear Cell Renal Cell Carcinoma (CCRCC).

The results of this study are in line with the research conducted by Zheng et al., which stated that the increased expression of EMMPRIN was significantly correlated with tumor size, depth of invasion, lymph vessel invasion, MMP 2, MMP 9, and tumor VEGF ($p < 0.05$) in gastric carcinoma. This study showed that the abnormal expression of EMMPRIN can increase tumor cell invasion and angiogenesis by increasing the expression of MMP and VEGF in stromal fibroblasts and gastric carcinoma cells so that increased EMMPRIN expression could be used as an effective and objective marker in predicting invasion and prognosis in gastric carcinoma.¹² A study by Nakamura et al. also found that high EMMPRIN expression is a significant marker of poor prognosis in endometrial cancer. EMMPRIN affects the proliferation, migration, and invasion of tumor cells

through the expression of TGF- β , EGF, VEGF, MMP-2, MMP-9. The binding between growth factors such as TGF beta, EGF, IGF, and TNF alpha with their receptors can activate cadherin E inhibiting factors such as Snail. The decrease in E-cadherin expression resulting in the loss of bonds between cells is an early stage of EMT.¹³

Various studies have shown that EMMPRIN plays an important role in the invasion and metastasis of various tumors, such as hepatocellular carcinoma, astrocytic glioma, retinoblastoma, and oral squamous cell carcinoma through increased MMP production. EMMPRIN has also been found to play a role in urothelial carcinoma invasion through the secretion of MMP2, MMP9, MMP14, and VEGF.¹⁴

The multifunctional role of EMMPRIN in advanced RCC is not only as an adhesion molecule involved in Cell-Matrix-Extracellular interactions (ECM) but also as a mediator for tumor invasion and angiogenesis through stimulation of VEGF production. Multivariate analysis showed a strong association between EMMPRIN and VEGF expression and poor prognosis in advanced RCC.⁶ EMMPRIN expression was found to be significantly associated with increased tumor invasion. These observations strongly suggest that EMMPRIN may be actively involved in the growth, invasion, and metastasis of OSCC. In addition, measurement of EMMPRIN levels can help predict a patient's prognosis.¹⁵

EMMPRIN is a transmembrane glycoprotein belonging to the immunoglobulin superfamily that is highly expressed on the cell surface of various types of tumors, including kidney cancer.⁵ EMMPRIN acts as a cellular adhesion molecule and induces the secretion of matrix metalloproteinases (MMPs) and the release of cytokines.¹⁶ EMMPRIN stimulates cancer cells and fibroblasts peritumoral to secrete matrix metalloproteinases (MMPs), which are capable of lowering extracellular matrix protein (ECM), and EMMPRIN directly promotes tumor proliferation, invasion, and metastasis.¹⁷

EMMPRIN has been shown to be involved in the regulation of tumor cell invasion and metastasis. First, EMMPRIN combines with the alpha6beta1 integrin into the FAK P13K-Ca (2+) pathway and the MARK signal, which then produces interstitial collagenase (MMP-1), forming a CD147-MMP-1 complex on the surface

of tumor cells, thus modifying the pericellular cell matrix tumor to promote invasion. Second, EMMPRIN is a receptor for platelet GPVI and mediates platelet movement through the GPVI-EMMPRIN Combination, thereby increasing the potential for metastasis. High EMMPRIN expression can be used to determine the TNM stage, histopathological stage, metastases, and worse survival in patients with kidney cancer.¹⁸

Another study investigated the effects of EMMPRIN on prostate cancer proliferation. EMMPRIN is expressed on the cell surface of most tumor cells, which results in proliferation, invasion, metastasis, and angiogenesis of cancer cells. Previous studies have shown that EMMPRIN can increase prostate cancer invasion and metastasis. The study showed that the inhibition of the EMMPRIN gene had a significant effect on the prostate cancer cell cycle, where a decrease in EMMPRIN expression resulted in an increase in the G0/G1 phase and a significant decrease in the S and G2 phases, indicating the cessation of the G1 phase. The G1 phase, the cell cycle phase in which cells grow and synthesize mRNA and protein for DNA synthesis, is very important because it determines whether the cell is committed to division or escape the cell cycle. The study states that EMMPRIN suppresses the progression of cancer cells by resting the cell cycle in the G0/G1 phase of cancer by suppressing cyclin D1 expression, there by inhibiting cell proliferation.^{19,20}

Conclusion

In conclusion, EMMPRIN expression was significantly correlated to the perirenal fat invasion. EMMPRIN expression has an important role in the Clear Cell Renal Cell Carcinoma (CCRCC).

Conflict of Interest: The authors declare that they have no conflict of interest.

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References

411. Capitanio, U., Bensalah, K., Bex, A., Boorjian, S. A., Bray, F., Coleman, J., Gore, J. L., Sun, M., Wood, C., & Russo, P. Epidemiology of renal cell carcinoma. *European Urology*. 2019; 75(1): 74–84.
2. Rabjerg M. Identification and validation of novel prognostic markers in Renal Cell Carcinoma. *Danish Medical Journal*. 2017; 64(10): B5339.
3. Sims, J. N., Yedjou, C. G., Abugri, D., Payton, M., Turner, T., Miele, L., and Tchounwou, P. B. Racial disparities and preventive measures to renal cell carcinoma. *International Journal of Environmental Research and Public Health*. 2018; 15(6): 1089.
4. Ljungberg, B. Prognostic significance of the heidelberg classification of renal cell carcinoma. *European Urology*. 2004; 36: 565-569.
5. Barata, P.C and Rini, B. I. Treatment of renal cell carcinoma: Current status and future directions. *CA: A Cancer Journal for Clinicians*. 2017; 67(6): 507-524.
6. Li, Hui, Wu, D., Shi, S., Xu, Y., Wei, L., Liu, J., and Liu, Y. Expression and clinical significance of CD147 in renal cell carcinoma: A meta-analysis. *Oncotarget*. 2017; 8(31): 51331–51344.
7. Liang, Y. X., He, H. C., Han, Z. D., Bi, X. C., Dai, Q. S., Ye, Y. K., Zhong, and W. De. CD147 and VEGF expression in advanced renal cell carcinoma and their prognostic value. *Cancer Investigation*. 2009; 27(7): 788–793.
8. Xu, J., Lu, Y., Qiu, S., Chen, Z.N., Fan, Z. A novel role of EMMPRIN/CD147 in transformation of quiescent fibroblasts to cancer-associated fibroblasts by breast cancer cells. *Cancer Letter*. 2013; 335: 380–6.
9. Obchoei, S., Sawanyawisuth, K., Wongkham, C., Kasinrerker, W., Yao, Q., Chen, C., & Wongkham, S. Secreted cyclophilin A mediates G1/S phase transition of cholangiocarcinoma cells via CD147/ERK1/2 pathway. *Tumor Biology*. 2015; 36(2): 849–859.
10. Dewi, H., Hassan, A. H., & Hernowo, B. S. Analisa imunoekspressi extracellular matrix metalloproteinase inducer (EMMPRIN) pada karsinoma sel ginjal subtype sel jernih yang telah metastasis dan tidak bermetastasis. *The Indonesian Journal of Pathology*. 2018; 27(3).
11. Petejova, N., and Martinek, A. Renal cell carcinoma: Review of etiology, pathophysiology and risk factors. *Biomedical papers of the Medical Faculty of the University Palacky, Olomouc, Czechoslovakia*. 2016; 160(2): 183–194.
12. Padala, S. A., Barsouk, A., Thandra, K. C., Saginala, K., Mohammed, A., Vakiti, A., Rawla, P., and Barsouk, A. Epidemiology of renal cell carcinoma. *World Journal of Oncology*. 2020; 11(3): 79–87.
13. Zheng, H. C., Takahashi, H., Murai, Y., Cui, Z. G., Nomoto, K., Miwa, S., and Takano, Y. Upregulated EMMPRIN/CD147 might contribute to growth and angiogenesis of gastric carcinoma: A good marker for local invasion and prognosis. *British Journal of Cancer*. 2016; 95(10): 1371–1378.
14. Nakamura, K., Kodama, J., Hongo, A., and Hiramatsu, Y. Role of emmprin in endometrial cancer. *BMC Cancer*. 2012; 12(1).
15. Xiong, L., Edwards, C. K., and Zhou, L. The biological function and clinical utilization of CD147 in human diseases: A review of the current scientific literature. *International Journal of Molecular Sciences*. 2014; 15(10): 17411–17441.
16. Ahmed, E.M. and Farag, A.S. Expression of EMMPRIN/CD147 and Ki-67 in oral squamous cell carcinoma: An immunohistochemical study. *Journal of American Science*. 2014; 10(12): 241-249.
17. Calvisi, D.F. CD147/Basigin: A warburg oncogene in hepatocellular carcinoma. *Chinese Journal of Cancer Research*. 2016; 28(3): 377–379.
18. Sato, M., Nakai, Y., Nakata, W., Yoshida, T., Hatano, K., Kawashima, A., Nonomura, N. EMMPRIN promotes angiogenesis, proliferation, invasion and resistance to sunitinib in renal cell carcinoma, and its level predicts patient outcome. *PLoS ONE*. 2013; 8(9): 1–10.
19. Li, H., Wu, D., Shi, S., Xu, Y., Wei, L., Liu, J., and Liu, Y. Expression and clinical significance of CD147 in renal cell carcinoma: A meta-analysis. *Oncotarget*. 2017; 8(31): 51331–51344.

20. Sweeny, L., Liu, Z., Bush, B. D., Hartman, Y., Zhou, T., and Rosenthal, E.L. CD147 and AGR2 expression promote cellular proliferation and metastasis of head and neck squamous cell carcinoma. *Experimental Cell Research*. 2012; 318(14): 1788–1798.