Expression and prognostic significance of EPAS-1 in renal clear cell carcinoma

Ann Ital Chir, 2021 92, 6: 671-675
pii: S0003469X21036630
Online ahead of print 2021 - Sept. 6
free reading: www.annitalchir.com

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OBJECTIVE: *predict the relationship between EPAS-1 in the occurrence and development of renal cell carcinoma and its effect on prognosis.*
METHODS: *The immunohistochemical EnVision two-step method was used to detect the expression of EPAS-1 protein in 145 cases of renal cell carcinoma and adjacent tissues to verify the correlation between EPAS-1 expression and clinicopathological characteristics and its effect on survival.*
RESULTS: *The expression of EPAS-1 protein in renal cell carcinoma and adjacent tissues was 75.9% and 15.9%, respectively. Univariate analysis showed that positive expression of EPAS-1, PT staging, lymphatic metastasis, distant metastasis, and ISUP grade were associated with poor prognosis of renal cell carcinoma (P <0.05); Multivariate analysis results showed that EPAS-1 protein and ISUP classification are independent prognostic factors for renal cell carcinoma. Kaplan-Mier survival analysis showed that the survival rate of renal cell carcinoma patients increased with the expression of EPAS-1 protein decreased significantly (P <0.05).*
CONCLUSION: *The expression of EPAS-1 protein is related to the PT stage, lymphatic metastasis, distant metastasis, and ISUP classification of renal cell carcinoma. EPAS-1 can be used as a potential prognostic marker or Treatment target.*

KEY WORDS: EPAS-1 prognosis, Immunohistochemistry, Molecular markers, Renal neoplasms, Renal clear cell carcinoma

Introduction

ccRCC is one of the most malignant and most common tumors of the urinary system, also known as renal adenocarcinoma, accounting for about 80% to 90% of renal malignancies[1]. The early clinical manifestations of ccRCC are often atypical, such as intermittent hematuria, abdominal masses and low back pain, the incidence is less than 15% [2]. Most of the ccRCC diagnosed by imaging and pathological examinations are advanced, and most of the tumors have spread and metastasized to a large area, and the prognosis is poor. In terms of treatment, curative surgery is currently the main treatment in China, but nearly 30% of ccRCC patients will have distant metastases after surgery. Therefore, the discovery of biomarkers that can effectively predict the prognosis of ccRCC and the development of targeted drugs for ccRCC are very important. Endothelial PAS domain protein epas-1, also known as hypoxia inducible factor (hzf-2 α) [3], is the main transcription factor of tissues and cells during hypoxia, and plays an important role in the changes of enzymes or factors under hypoxia [4]. EPAS-1 mainly induces expression in hypoxic regions and can regulate genes necessary for tumors to adapt to hypoxic conditions, such as the expression of genes encoding EPO, VEGF, Glue glycolysis, etc. Thus, EPAS-1 can play an important role in energy metabolism, vascular growth, bone marrow hematopoiesis, tumorigenesis and development [5]. hypoxia-inducible factor-1α (HIF-1α) correlate with prognosis in high stage ccRCC [6], At present, the bioinformatics analysis of gene chip data shows that [7] EPAS-1 is differentially expressed in ccRCC, but the relationship between the differential expression of EPAS-1 in RCC and the pathological parameters of ccRCC and the prognosis of ccRCC patients is not clear.

Pervenuto in Redazione Maggio 2021. Accettato per la pubblicazione Luglio 2021
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The purpose of this study is to analyze the differential expression of EPAS-1 in ccRCC and adjacent tissues, and verify the correlation and prognostic value of EPAS-1 expression with RCC clinicopathological characteristics.

Materials and Methods

General Information

A total of 145 wax specimens from our hospital from February 2007 to February 2010, were pathologically confirmed to be ccRCC patients. At the same time, 69 adjacent tissues were selected as the control group. All patients gave informed consent. The protocol was approved by the medical ethics committee of our hospital. The specimens of the cases were fixed with 10% neutral formalin, embedded in paraffin, continuous 4 m thick section, and stained by HE. All cases were confirmed by two senior pathologists in our hospital. Tumor TNM staging was performed according to the 8th edition of 2017 AJCC standard [8]. The patients were followed up at home or by telephone after discharge. The follow-up period was 10 years, and the follow-up was terminated in February 2020. The follow-up data was 126 cases, the follow-up rate was 86.89%, and the number of survival and death was counted.

Inclusion and Exclusion Criteria

Inclusion criteria: 1) Patients who were diagnosed with ccRCC; 2) The clinical pathological data of the patients were completely preserved; 3) All patients were newly ill(The patient was diagnosed for the first time and had not received relevant treatment before); 4) Patients who had not received chemotherapy and radiotherapy before being included in the study; 5) Patients themselves agreed and willing to cooperate with the study, and signed an informed consent form. Exclusion criteria: 1) Patients with other kinds of malignant tumors; 2) Patients with autoimmune diseases; 3) Patients with infectious diseases or diseases of vital organs; 4) Patients with missing pathological data.

Methods

The immunohistochemical staining method uses EnVision two-step method, the staining steps are as follows: section deparaffinization, hydration, 3% H_2O_2 block endogenous peroxidase activity, autoclave method for antigen retrieval (EPAS-1 antibody uses pH 9.0, EDTA antigen retrieval solution), and then the pressure cooker was used to heat the retrieval solution until gassing for 2 minutes, then rinses the pot with cold water to room temperature, and removes the section. Incubate with normal serum, add anti-human antibody EPAS-1 (1: 200) dropwise, overnight at 4°C. Add EnVision secondary antibody dropwise. DAB color development. Hematoxylin counterstaining, dehydration, transparency and sealing. Use PBS, mouse IgG of the same type instead of primary antibody as negative control.

Result Judgment

Under the microscope, the positive staining sites of EPAS-1 were both nucleus and plasm.
Which were dyed yellow. Randomly select 10 high-power fields, and comprehensively consider the proportion of positive cells in the slice and the staining intensity of positive cells to determine the result semi-quantitatively. Score based on staining intensity: Light yellow 1 point, brown yellow 2 points, tan 3 points, low expression 1 point (The color is too light), no expression 0 points, among which low expression or even no expression group is regarded as protein table deletion, otherwise it is regarded as positive expression.

Statistical Analysis

The SPSS 25.0 software was used for statistical analysis. The χ^2 test was used to compare the expression of EPAS-1 in ccRCC and adjacent tissues, and analyze the correlation between EPAS-1 and clinicopathological factors. Cox regression model was used for univariate and multivariate analysis. The Kaplan-Meier method and Log-rank test were used to compare survival rates between groups. P <0.05 means the difference is statistically significant.

Results

The Expression of EPAS-1 in Ccrcc and Adjacent Tissues

The expression of EPAS-1 in the adjacent cancer and ccRCC was 15.9% (11/69) and 75.9% (110/145),

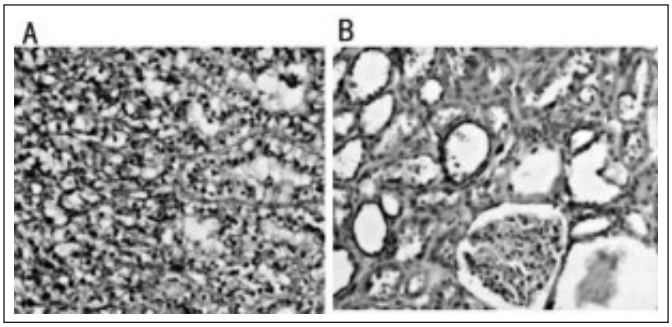

Fig. 1: Expression of EPAS-1 in ccRCC (×200): Expression of EPAS-1 in adjacent tissues (×200).

TABLE I - *The expression of EPAS-1 in ccRCC and adjacent tissues*

Tissues	No. of patients	EPAS-1protein positive	P
ccRCC	145	110(75.9%)	0.05
adjacent tissues	69	11(15.9%)	

respectively. The expression of EPAS-1 protein in ccRCC was significantly increased. χ^2 test showed that EPAS-1 expression difference between the two groups was significant (P<0.05). (Table I, Fig. 1)

The Relationship Between Epas-1 Expression and Ccrcc Clinicopathological Characteristics

EPAS-1 expression is not related to the age and gender of ccRCC patients (P>0.05), and is related to PT staging, lymphatic vascular metastasis, distant metastasis, and ISUP classification (P < 0.05, Table II).

The Relationship Between the Expression of Epas-1 in Ccrcc and the Prognosis

The overall average survival time of 145 ccRCCs with follow-up data was (42.62±1.53) months. The average survival time of patients in the EPAS-1 positive expression group was (44.32±1.58) months, and the average survival time of patients in the EPAS-1 loss-of-expression group was (60.78 ± 2.64) months. The survival time of ccRCC patients varies significantly with the increase and decrease of EPAS-1 expression (P<0.01, Fig. 2).

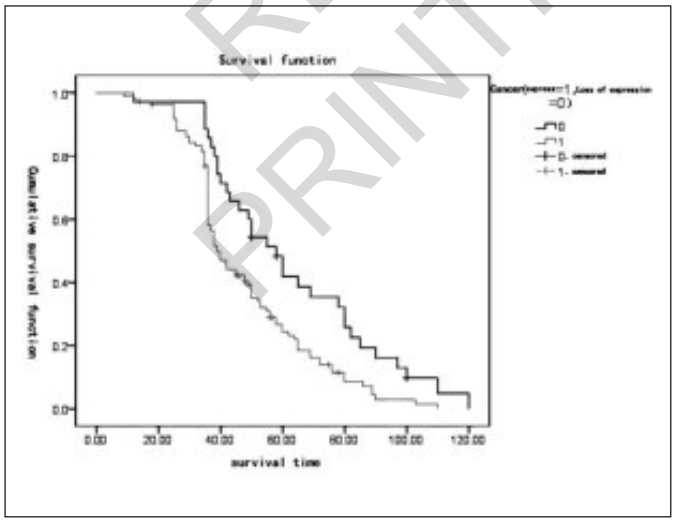

Fig. 2: The relationship between EPAS-1 expression in ccRCC and prognosis.

TABLE II - *The relationship between EPAS-1 expression and ccRCC clinicopathological characteristics*

Clinical pathological parameters	n	EPAS-1 Loss of expression	positive	χ^2	P
Age				0.037	>0.05
≤60y	56	14(25.0%)	42(37.5%)		
>60y	89	21(23.6%)	68(76.4%)		
Sex				1.982	0.05
male	97	20(20.6%)	77(79.4%)		
Female	48	15(31.2%)	33(68.8%)		
PTStaging				8.640	>0.05
T1	95	30(31.58%)	65(68.4)		
T2	26	3(11.5%)	23(88.5%)		
T3	19	2(10.5%)	17(89.5%)		
T4	5	0(0)	5(100%)		
Lymphatic invasion				4.818	<0.05
None	108	31(28.7%)	77(71.3%)		
Have	37	4 (10.8%)	33 (89.2%)		
Distant metastases				6.411	<0.01
None	113	36(31.9%)	3(2.6%)		
Have	32	3(10.3%)	29(90.6%)		
ISUP classification				15.654	<0.01
I	50	20(40.0%)	30(60.0%)		
II	42	7(16.7%)	35(83.3%)		
III	40	3(7.5%)	37(92.5)		
IV	13	5(38.5%)	8(61.5%		

ISUP classification. International Association of Urological Pathology; T. Tumor; N. Lymph nodes; M. Distant shift; PTNM staging. TNM stage of pathology

TABLE III - *Univariate analysis of the prognosis of ccRCC patients*

Factor	Univariate analysis P	RR(Relative risk)	95%CI
Sex	0.304	0.833	0.588 ~ 1.180
Age	0.148	0.768	0.536 ~ 1.099
pTStaging	0.821	0.973	0.767 ~ 1.234
EPAS-1expression	<0.01	0.554	0.366 ~ 0.838
Lymphatic invasion	<0.05	1.484	1.049 ~ 2.1
Distant metastases	<0.05	0.704	0.496 ~ 0.998
ISUPclassification	<0.05	0.489	0.250 ~ 0.955

Univariate Analysis of the Prognosis of Ccrcc Patients

Univariate analysis of the prognosis of ccRCC patients: The results of univariate analysis showed that the positive expression of EPAS-1, lymphatic and vascular metastasis, distant metastasis, ISUP grade are related to the prognosis of ccRCC patients (P<0.05, Table III).

Multivariate Analysis of the Prognosis of Ccrcc Patients

The significantly correlated risk factors in the univariate analysis were included in the Cox regression model for

TABLE IV - *Multivariate analysis of the prognosis of ccRCC patients*

Factor	Univariate analysis		
	P	RR(Relative risk)	95%CI
EPAS-1expression	<0.05	0.619	0.398 ~ 0.964
Lymphatic invasion	0.160	1.816	0.791 ~ 4.171
Distant metastases	0.402	0.684	0.281 ~ 1.664
ISUPclassification	<0.05	0.494	0.248 ~ 0.984

analysis. The results showed that the positive expression of EPAS-1 and the ISUP grade were independent factors affecting the prognosis of ccRCC (P<0.05, Table IV).

Discussion

Renal clear cell carcinoma (ccRCC) currently accounts for about 3% of all new adult malignancies every year [9], and its incidence has been on the rise in the past two decades [10]. Renal cell carcinomas of the renal transplant affect approximately 0.2% of recipients, Predominant pathology is clear cell carcinomas [11]. ccRCC has the characteristics of atypical early clinical manifestations, and the prognosis is poor if distant metastasis occurs in the late stage [12]. This requires researchers to do more in-depth research on the pathogenesis of ccRCC. In recent years, with the rapid development of molecular biomedicine, a large number of tumor markers have been discovered and applied to the clinic, providing powerful help for the clinic. Therefore, it is particularly important to find effective ccRCC tumor markers and discover new targeted therapeutic drugs.

When malignant tumor grows, local tissues and cells are often in a state of hypoxia. In order to adapt to hypoxia and enhance the ability to invade the body, the angiogenesis of tumor cells increases [13]. The expression of EPAS-1 is widespread in the human body under hypoxia, which accelerates the formation of tumor new blood vessels, resulting in the enhancement of malignant behavior of tumor cells, and resistance to radiotherapy and chemotherapy [14]. There is little or no expression of EPAS-1 in normal tissues [15], however, EPAS-1 expression exists in a variety of tumor tissue cells and participates in regulating biological behavior [16]. There are two main reasons for the increased expression of hypoxia factor EPAS-1 in tumor tissue cells [17]: On the one hand, the excessive proliferation of tumor tissue cells will cause local hypoxia; on the other hand, some genetic changes, such as EPO , VEGF, Glue will be transcribed and translated under the action of hypoxia-inducible factors. Currently, there are few studies on the relationship between EPAS-1 and tumors, but it has attracted the attention of scholars at home and abroad.

This study shows that EPAS-1 expression in ccRCC tissues is significantly higher than that in normal cancer tissues. This may be due to the strong metabolism of ccRCC and the local hypoxic environment in ccRCC leads to increased expression of EPAS-1, which in turn promotes tumor neovascularization and accelerates the growth of tumor cells [18]. In this study, EPAS-1 protein expression is related to PT staging, lymphatic vascular metastasis, distant metastasis, ISUP grade but not to age and gender. EPAS-1 plays a role in accelerating tumor progression during the development of ccRCC and participates in tumor deterioration. Studies on the prognosis of EPAS-1 expression in ccRCC show that the survival time of ccRCC patients with EPAS-1 positive expression is significantly reduced. The results of multivariate analysis showed that the positive expression of EPAS-1 in ISUP is also an independent prognostic factor for good prognosis of ccRCC. This result suggests that EPAS-1 may play an important role in the early stages of ccRCC. However, the specific mechanism of EPAS-1's participation in the occurrence and development of ccRCC needs further study.

In summary, EPAS-1 has important research value in the diagnosis and prognosis of ccRCC. Detecting the expression level of EPAS-1, and exploring its relationship with ccRCC pathogenesis, clinical features and prognosis may provide a new direction for ccRCC targeted therapy. This study needs to further explore the molecular mechanism of EPAS-1 in tumor progression in order to serve the clinic.

Conclusion

The expression of EPAS-1 protein is related to the PT stage, lymphatic metastasis, distant metastasis, and ISUP classification of renal cell carcinoma. The low expression group of EPAS-1 protein benefits from survival. EPAS-1 can be used as a potential prognostic marker or Treatment target.

Riassunto

La proteina EPAS-1 (Endothelial PAS domain-containing protein 1) è un gene espresso in modo differenziato nel carcinoma renale a cellule chiare (ccRCC). Ipotizziamo che EPAS-1 possa influenzare la prognosi del ccRCC, quindi abbiamo studiato il livello di espressione della proteina EPAS-1 nel carcinoma a cellule renali e per prevedere la relazione tra EPAS-1 l'insorgenza e lo sviluppo del carcinoma a cellule renali e il suo effetto sulla prognosi.

Metodi: Il metodo immunoistochimico in due fasi EnVision è stato utilizzato per rilevare l'espressione della proteina EPAS-1 in 145 casi di carcinoma a cellule renali e nei tessuti adiacenti per verificare la correlazione tra l'espressione di EPAS-1 e le caratteristiche clinicopatologiche e il suo effetto sulla sopravvivenza.

Risultati: L'espressione della proteina EPAS-1 nel carcinoma a cellule renali e nei tessuti adiacenti era rispettivamente del 75,9% e del 15,9%. L'analisi univariata ha mostrato che l'espressione positiva di EPAS-1, stadiazione dei tumori primitivi (PT), metastasi linfatiche, metastasi a distanza e grado ISUP International Society of Urologic Pathology) erano associate a prognosi infausta nel carcinoma a cellule renali (P <0,05); I risultati dell'analisi multivariata hanno mostrato che la proteina EPAS-1 e la classificazione ISUP sono fattori prognostici indipendenti per il carcinoma a cellule renali. L'analisi di sopravvivenza di Kaplan-Mier ha mostrato che il tasso di sopravvivenza dei pazienti con carcinoma a cellule renali è aumentato con l'espressione della proteina EPAS-1, diminuito significativamente (P <0,05).
Conclusione L'espressione della proteina EPAS-1 è correlata allo stadio PT, metastasi linfatiche, metastasi a distanza e classificazione ISUP del carcinoma a cellule renali. EPAS-1 può essere utilizzato come potenziale marker prognostico o target del trattamento.

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