Expression and Significance of Coxsackie and Adenovirus Receptor in Renal-Cell Carcinoma

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OBJECTIVE To investigate the expression of Coxsackie and Adenovirus receptor (CAR) in renal-cell carcinoma and the relationship of the CAR to the biological behavior of the carcinomas.

METHODS The immunohistochemical SP method was used to detect the expression of Coxsackie and Adenovirus receptor in 48 cases of renal-cell carcinoma and in 12 cases of normal renal tissue 2 cm away from the tumor tissue.

RESULTS The positive rates of CAR were 100% in 12 cases of para-tumor normal renal tissue and 35.4% in 48 cases of renal-cell carcinoma respectively. The difference of CAR expression between them was significant ($P<0.05$). The grades of the tumor were as follows: 22 in Grade I, 17 in Grade II and 9 in Grade III with the CAR positive rate being 54.5%, 23.5% and 11.1%, respectively. There was a negative correlation between CAR expression and tumor grading ($P<0.05$). In addition, the number of the cases in stages I to IV were 19, 13, 11 and 5 respectively, with the respective positive rates being 57.9%, 30.8%, 18.2% and 0.0%, i.e. there also was a negative relationship between CAR expression and the stage ($P<0.05$).

CONCLUSION CAR expression is down-regulated in renal-cell carcinoma compared with normal tissue. The level of CAR may be a sensitive predictor of differentiation, invasion and metastasis. Loss of CAR expression correlates with the invasive phenotype in our analysis of renal-cell carcinoma.

KEYWORDS: renal-cell carcinoma, Coxsackie and Adenovirus receptor, immunohistochemistry.

The Coxsackie virus and Adenovirus receptor (CAR) is a 46-kDa transmembrane glycoprotein that contains intracellular, transmembrane and extracellular domains with 2 immunoglobulin-like motifs that interact with the knob protein from the Adenovirus type 5.[1] The (CAR) protein has been demonstrated to be a high-affinity receptor for Adenovirus type 5,[1] and the presence of this protein significantly enhances the efficiency of Adenoviral vector-mediated gene transfer.[2] The function of CAR is not fully understood, but its localization in the tight junctions suggests a role in cell adhesion.[3] Recent evidence has shown that CAR expression is often down regulated in various types of advanced clinical tumors.[2,4] Especially, CAR expression may be correlated inversely with the aggressiveness of tumors.

Renal-cell carcinoma is the most lethal urological carcinoma in China. Despite intensive treatments including surgery, radiation and chemotherapy, current therapy for renal-cell carcinoma is often unsatisfactory. So, intensive studies on the mechanism of renal-cell carcinoma metastasis may provide some new therapeutic methods to reverse the malignant disease.

In this study, we examined the expression of CAR in 48 renal-cell carcinomas by a immunohistochemical S-P method to explore whether it is related to malignant progression.

MATERIALS AND METHODS

Specimens

The tissues tested in the present study were retrieved from the surgical pathology archives of the First Hospital of Xi'an Jiao Tong University during 1997~2003. These tissues included 12 para-tumor normal renal tissues (about 2 cm away from the tumor tissue) and 48 renal-cell carcinomas. In the renal-cell carcinoma group, 37 were males and 11 females with a mean age of 56.7 years (ranging from 32 to 74 years). The histopathological results were obtained for all 48 renal-cell carcinomas. The stages were determined according to the Robson classification. Nineteen patients were in stage I, 13 in stage II, 11 in stage III and 5 in stage IV. The Grades were determined according to the Fuhrman classification.[5] The distribution of tumor Grades was as follows: I, 22 patients; II, 17 patients; III, 9 patients. Each paraffin embedded tissue section was cut into 2 slices in succession of 4 mm thick, one was stained by haematoxylin-eosin (H&E) to confirm the diagnosis, another was used for immunohistochemical staining.

Reagents

Rabbit anti-human polyclonal antibody against hCAR (a gift from Professor Jer-Tsong Hsieh, the University of Texas Southwestern Medical Center, USA) was used at a working concentration of 1:350. A SP reagent kit (KIT-9710) and DAB reagent kit (DAB-0030) were purchased from Fujian Maixin Biological Technology Ltd. (Fujian China).

Immunohistochemical staining

Four-micron sections of renal cell carcinomas were deparaffined in xylene and rehydrated with a gradient alcohol series. Endogenous peroxidase activity was blocked by treatment with 3% H_2O_2. These sections were heated in citrate buffer (pH 6.0) in a microwave oven for 10 min, blocked with goat serum for 10 min, incubated with anti-hCAR antibodies at 4℃ overnight, then treated with biotinylated second antibody and streptavidin HRP conjugate for 15 min each. The DAB-substrate solution was added to the slide at room temperature. Between each step in the staining procedures, the slides were rinsed 3 times in PBS. PBS was used to substitute for the first anti-hCAR antibody as a negative control.

Immunohistochemistry assay for CAR

The CAR expression is localized in the membrane of renal cells. Staining was measured as the percentage of positive staining in 1,000 to 1,500 tumor cells in a consecutive field. Counting was conducted on most intensely stained areas. We scored the CAR expression as follows: negative (-), < 5% of the tumor cells were stained; cases exhibiting definitive cytomembrane staining in ≥ 5% of the cells were considered positive[6].

Statistical analysis

The relationship between CAR expression and various clinical and pathological parameters were determined using the Chi-squared test (χ^2 test) All statistical analyses were performed using SPSS10.0 software. *P* values less than 0.05 were considered to be statistically significant.

RESULTS

The expression of CAR in renal-cell carcinoma tissues and normal renal tissues

Immunohistochemistry demonstrated that the CAR proteins mainly existed in the cytomembrane. The results of immunohistochemistry staining showed that the degree of positive expression of the CAR protein in 48 cases of renal-cell carcinomas was 35.4% (17/48), and 100% (12/12) in the group of normal renal tissues. The positive expression rate of CAR in renal-cell carcinoma group was significantly lower than that in normal renal tissues ($P<0.05$)(Table 1).

Table 1. CAR expression in renal-cell carcinoma tissue and normal renal tissue groups.

Groups	n	CAR +	CAR -	Positive rate (%)	*P*
Normal renal tissue	12	12	0	100	<0.05
Renal cell carcinoma tissue	48	17	31	35.4	-

Correlation between CAR protein expression and the clinical histopathological characteristics

Correlation between CAR protein expression and the pathological grade

Cases with CAR (+) or CAR (-) were considered with

regard to histopathological parameters. The expression rate of CAR in renal-cell carcinoma with grade I, II and III respectively was 54.5%, 23.5% and 11.1% (Table 2). There was a significant difference between different Grade cases ($P<0.05$), there being an inverse correlation between CAR expression and the renal-cell carcinoma Grade.

Table 2. CAR expression and clinical histopathologi cal Grade of renal-cell carcinoma.

		CAR		Positive	
Clinical pathology	n	+	-	rate (%)	*P*
Histological Grade					<0.05
I	22	12	10	54.5	---
II	17	4	13	23.5	---
III	9	1	8	11.1	---

Correlation between CAR protein expression and differencial pathological stage

The positive expression rate of CAR in renal-cell car cinoma in stage I and II was significantly higher than that in renal-cell carcinomas in stage III and IV ($P<0.05$) (Table 3). No statistical differences were found regarding histopathological type (no data shown). So, CAR expression was correlated with the renal-cell carcinoma stage.

Table 3. CAR expression and clinical histopathological stage of renal-cell carcinoma.

		CAR		Positive	
Clinical pathology	n	+	-	rate (%)	*P*
Histological Grade					<0.05
I	19	11	8	57.9	-
II	13	4	9	30.8	-
III	11	2	9	18.2	-
IV	5	0	5	0	-

DISCUSSION

The Coxsackie virus and Adenovirus receptor (CAR) protein was purified from HeLa cell lysates by immunoaffinity chromatography using mAb RmcB. The sequences of 4 tryptic peptides were determined, and a cDNA clone was isolated from a HeLa cell library.[1] CAR, first identified as the high affinity receptor for both Coxsackie and Adenovirus (type 2 and 5),[1,7] is a typical Ig-like molecule with two Ig domains that may have adhesion activity, which mediate homophilic interaction.[8] Structurally, CAR is a transmembrane protein containing an extracellular Ig loop (2U), trans membrane domain, and intracellular domain. The CAR was widely distributed in organisms. There was some differences in the expression pattern of CAR between mice and humans (and other animals). CAR expression is species specific and tissue specific. Mouse CAR was found in the heart, liver, brain, kidney, and lung and greatest in liver.[9] And variable levels of human CAR mRNA expression have been found in human liver, kidney, lung, brain, heart, colon, small intestine, testis, prostate and pancreas, whereas no expression was found in skeletal muscle, spleen, ovary, thymus, or placenta.[10]

Few reports on the expression of CAR in primary tumors have appeared to date. Based on real-time PCR analysis, Jonas et al.[11] found a great variability in hCAR expression in primary human astrocytic tumors. Grade IV tumors expressed significantly lower levels of hCAR than Grade II/III tumors. Interestingly, the level of hCAR expression has been found to inversely correlate with tumorigenicity [12]. These studies suggest that CAR may function as a tumor suppressor. Loskog et al.[13] found that 27 out of 27 biopsies from human urinary bladder cancer were positive for hCAR, with expression levels varying between tumors. Bladder cancer samples had only been analyzed semiquantitatively by mRNA expression, suggesting an apparent down-regulation in the invasive tumors. Sachs et al.[14] found that the more invasive cancers tended to have a decreased CAR pattern of expression. And the result by immunohistochemistry showed that clinical specimens of invasive bladder cancers reveal a marked stage and Grade-dependent down-regulation of CAR.

Because CAR seems to play a role in cell-cell adhesion and migration, such a reduction might cause cells to lose cohesiveness and therefore may be a major contributing factor in the process of developing the invasive phenotype.

Haviv et al.[15] found there were different expression decreases or losses of CAR in human renal carcinoma cell lines ACHN, A498, CaKi and SW157 by immunofluorescence and flow cytometry. However, there are no published papers about the relation between CAR expression and stage or Grade of renal carcinoma.

Our study showed that there were different degrees of the expression losses of CAR in the renal-cell carcinoma progression. With an increase in the stage and Grade of renal-cell carcinoma, CAR expression de-

creased progressively. This result is similar to CAR expression in other tumors. Bruning and Runnebaum [16] found that high expression of CAR in tumor cells can result in significantly depressed cell invasion and migration, but enhanced cell-cell adhesion. Our results suggested CAR expression was gradually decreasing with an increasing renal-cell carcinoma pathological Grade. Low expression or expression losses of CAR contribute to developing the invasive phenotype of renal cell carcinoma.

For this reason, CAR maybe is a potential metastatic inhibitor in renal carcinoma, the decrease or loss of its expression was correlated with renal carcinoma progression. Furthermore, we also confirmed the results from renal-carcinoma cell lines of Haviv et al.[15]. However, further studies concerning the precise mechanisms of CAR expression in renal-carcinoma are needed.

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