RESEARCH ARTICLE

COX-2 Expression in Renal Cell Carcinoma and Correlations with Tumor Grade, Stage and Patient Prognosis

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Abstract

Background: Cyclooxygenase 2 (COX-2) is important as an enzyme in the pathway leading to the production of prostaglandin E2 (PGE2) and arachidonic acid. This pathway is known to play a role in inflammation, tumor growth, invasiveness and metastasis, inhibition of apoptosis and angiogenesis. Inhibition of COX-2 has been shown to be a promising antitumor and antiangiogenic strategy in several tumor types, including renal cell carcinoma (RCC). Therefore, we decided to evaluate the immunohistochemical expression of this marker and its association with several clinicopathological characteristics in a series of cases. Materials and Methods: COX-2 expression was examined immunohistochemically in tumor tissues obtained from 96 patients who underwent radical (94 cases) or partial (2 cases) nephrectomy. Correlations between COX-2 expression and clinicopathologic findings including pathologic stage, nuclear grade and other indicator of prognosis were examined. Results: Of 96 tumors, 20.9% were positive for COX-2 expression. A correlation was found between COX-2 expression and tumor histological subtype (P=0.03).The papillary subtype showed maximum expression of this marker (43.8%) and the clear subtype minimum (14.7%). There were also possible links between COX-2 expression and pathologic stage, nuclear grade and nodal involvement but the results were not statistically significant (P=0.8, P= 0.14 and P=0.06, respectively). No correlation was found between COX2 expression and patient age, gender, tumor size, metastasis or survival. Conclusions: In our study, COX-2 expression was correlated with the histological subtype of RCC. Additional research is required to determine the link between COX-2 expression and prognosis and also evaluation of probable effectiveness of COX-2 inhibitor drugs in treatment of RCC patients.

Keywords: Renal cell carcinoma - COX-2 expression - IHC - prognosis

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Introduction

Renal cell cancer (RCC) accounts approximately for 2% of all cancers and 85% of kidney cancers in adults (Kumar et al., 2010). The incidence has increased substantially over the last two decades, accompanied by an improved 5 year survival. Undoubtedly, both trends are at least in part a result of improved diagnostic techniques and early detection. Despite the facts, up to 30% of patients of RCC present with metastatic disease. The median overall survival in patients with metastatic disease is 12 months (Carter et al., 2010). Patients prognosis depends on several clinicopathologic parameters including tumor stage, size, microscopic grade, distant metastasis, clear versus granular cytoplasm, presence of cystic changes, DNA ploidy, cell proliferation, P53 expression, Renal vein invasion, CD44, etc. Rosai (2011).

However, in many cases, these parameters were insufficient to predict the clinical behavior of RCC tumors. Therefore, it is important to identify additional indicators of biological aggressiveness of RCC (cho et al., 2005). Nephrectomy is the treatment of choice but partial nephrectomy to preserve renal function is being done with increasing frequency and similar outcome. RCC is resistant to chemotherapy and radiation therapy. Various immune-based therapeutic approaches (such as interlukin-2 and α-interferon) are currently being tried in metastatic RCCs Rosai (2011). Molecular targeted therapy in this tumor has received more attention in recent years. One of these attractive targets is Cyclooxygenase 2 (COX-2), an enzyme in the pathway leading to production of Prostaglandin E2 (PGE2) and Arachidonic acid. This pathway is known to play a role in inflammation, tumor growth, invasiveness and metastasis, inhibition of apoptosis and angiogenesis. Inhibition of COX-2 has been shown to be a promising antitumor and antiangiogenic strategy in several tumor types including renal cell carcinoma (RCC) (Wang et al., 2013).

COX-2 expression in RCC is reported between 16-100% by different studies (Miyata et al., 2003; Hashimoto

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et al., 2004; Tuna et al., 2004; Mungan et al., 2006; Sozen et al., 2007; Tawfik et al., 2007; Drim et al., 2008; Hasan et al., 2009; Sun et al., 2009; Schwandt et al., 2011; Tian et al., 2011) and some studies have mentioned the probable role of COX-2 in tumor progression (Mungan et al., 2006), inflammation and RCC progression (Tuna et al., 2004), relation to tumor size and nuclear grade (Sun et al., 2009).

There are no precise statistics on the incidence rate of this type of marker on RCC and so far there has been no study concerning the importance of the expression of COX-2 marker, its recurrence and even its treatment. Therefore, the present study tries to determine the IHC expression of this marker in RCC and its relationship with some clinicopathologic characteristics, especially tumor grade and stage so that through its help, we can provide the probable effect of some drugs to control this pathway in the treatment of these patients in future studies.

Materials and Methods

Patient selection

Cases were obtained from the surgical pathology archive at Sina Hospital Department of Pathology, Tehran University of Medical Sciences. After reviewing the pathology reports from April 2005 to April 2011, 96 patients with bladder RCC including 94 radical and 2 partial nephrectomy cases were selected. Then, all the Hematoxylin and Eosin – stained slides were re-examined by blinded review of two pathologists to achieve assurance on diagnosis, grade (Fuhrman nuclear grading), stage (pathologic stage, PT) and other histopathological characteristics. Finally, the appropriate formalin – fixed paraffin – embedded blocks were selected for immunohistochemical (IHC) study, for about two years patients were followed up ,in crude estimation there was a good correlation between patient low long survival and cox2 expression but this finding was not statistically remarkable. The most cox2 expression was seen in pT2.

Immunohistochemistry

Paraffin - embedded blocks were cut into 3μm sections and then mounted on Poly-L Lysine coated slides. After overnight incubation at 370 C, the specimens were deparaffinized in xylene and rehydrated in a graded series of alcohol. They were washed with phosphate buffered saline (PBS) and then endogenous peroxidase was inactivated by hydrogen peroxide 3 % for 20-30 min. Also, antigen retrieval was done by immersing the slides in 0.01 M. Tris buffer (PH 9.0) and performing autoclaving for 20 min in a 120c temperature.

After washing with PBS, the slides were incubated with protein Block serum - free (Code X0909, Dako, Denmark) for 10 minutes at room temperature to decrease nonspecific antibody binding and the samples were incubated for 1 hour with Monoclonal mouse anti-human antibody for COX-2 (Clone: CX- 294, Dako, Denmark). After rewashing with PBS, the reagent kit was used including secondary antibody bond to marked polymer with peroxidase Hoseradish for 30 min. After the rewashing with buffer, the samples were exposed to substrate diaminobenzidine for 10min. For coloring the background, hematoxylin was used.

Specimens showing at least 10% staining of tumor cells were assumed as positive (Kankuri- Tammilehto et al., 2010)

Statistical analysis

For statistical analysis, SPSS (version 19.0) was used and the expression of COX2 marker and its relation with each of the variables was studied with the use of Chi-square, Fisher's exact, T-test and non-parametric tests. We considered $P<0.05$ as a statistically significant value.

Results

Ninety-six patients with definite diagnosis of RCC were evaluated. The patients mean age was 56.5 (14.4±SD) years ranging from 21 to85. The patients were monitored for 33 months. There were 54 males (56.2%) and 42 females (43.8%). Of all 96 patients, 94 (98%) underwent radical nephrectomy and 2 (2%) underwent partial nephrectomy. Overall 20 patients (20.9%) expressed the COX-2 marker and 79.1% had negative COX-2.

COX-2 marker was expressed by 18.5% of the males (10) and 23.8% of the females (10) but the relation between the incidence of this marker and gender was not statistically significant through the use of Chi-Square test (P=0.52).

The mean age of the patients with COX-2 marker was 53.2 with the standard deviation of 16.7 while this was 57.4 for the negative COX-2 group with the standard deviation of 13.7. It seems that people who expressed COX-2 were younger than the group with negative COX-2, but this difference was not significant through T-Test (p=0.25).

We considered the relation between COX-2 expression and the histological subtype of RCC. The highest expression belonged to papillary subtype (43.8%) and the lowest expression was in clear subtype (14.7%) and statistical tests showed a significant relation between the above parameters (P=0.03).

Tumor size in the group with COX-2 expression was 9cm with the standard deviation of 13.7. The size in the group who did not express COX-2 was 7.6cm with the standard deviation of 4, but this difference was not statistically significant through the use of T-Test (P=0.17).

The relation between microscopic grade and the result of COX-2 expression shows that the highest amount of COX-2 expression in the grade was related to tumors that were not categorized, i.e. papillary and chromophobe groups (47.3%) and the lowest expression was observed in grade 4 (0.00 %) and based on Chi-Square, P=0.015 that shows a relation between these two parameters, but in order to have a more accurate interpretation of the results, based on the low number of samples in each group, tumors were divided into two groups of high grade (grades 3 and 4) and low grade (grades 1 and 2 and groups without nuclear grade), for which COX-2 expression in low grade group was 24.6% and 11.1%in the high grade group , but the statistical analysis showed no significant relation (P=0.14).

For the incidence of COX-2 marker at different stages

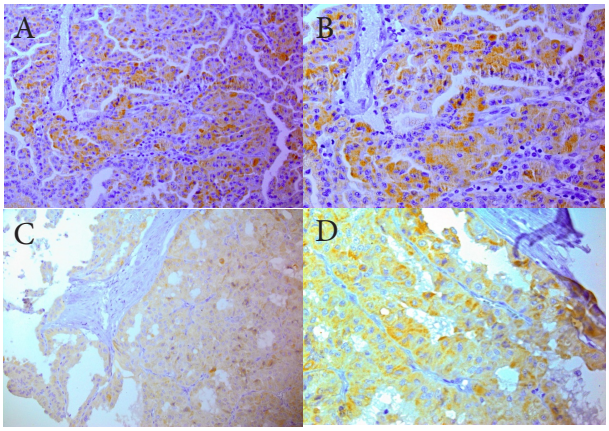

Figure 1.-A) Renal papillary Carcinoma (magnification 200x), B) Renal papillary Carcinoma (magnification 400x), C),) Renal Chromophobe Carcinoma (magnification 200x) and D) Renal Chromophobe Carcinoma (magnification 400x)

of tumor was 40% in PT2, 8.8% in PT1, 12.2% in PT3and 22.8% in PT4, which highest was in PT2.

From the two cases of partial nephrectomy patients that were undividable regarding the tumor stage, one case expressed COX-2 (50%).

Through T-test, P=0.04 shows a relation between the two above parameters, but because of the limitation of low expression of COX-2 and low number of subjects in each subgroup, tumors were divided into two groups of low grade (PT1, PT2) and high grade (PT3, PT4) for generalizing the results. The amount of marker expression in low grade tumors was 20.3% and in high grade tumors, it was 23.5 %, but the results were not statistically significant (P=0.89).

The other parameter in our study was the relation between COX-2 expression and the lymph node condition of our patients. The amount of the expression of this marker in patients without tumoral lymph node was less than the group with tumoral involvements (10.5% vs. 19%), but the statistical relation between them was not significant (P=0.06).

Also, in our study, no significant relation was observed between COX-2 expression and tumor metastasis or the survivability of the patients. After two years of follow up, COX-2 expression was not statistically relevant with prognosis.

Discussion

The increase of COX-2 expression has been observed in various human tumors including kidney and this increase in expression is related to carcinogens and weak prognosis of patients (Cho et al., 2005). In recent years, COX-2 marker, as one of the important target molecules in tumor treatment, has gained much attention and is seen in many human tumors including kidney (Miyata et al., 2003; Hasan et al., 2009; Tian et al., 2011).

Many studies have dealt with the relation between the incidence of this marker and clinical-pathologic indexes in renal tumors (Yoshimura et al., 2004; Mungan et al., 2006; Tawfik et al., 2007; Dirim et al., 2008; Sozen et al., 2008; Hasan et al., 2009; Sun et al., 2009; Schwandt et al., 2011; Tian et al., 2011).

In our study, the expression of COX-2 marker was seen in 20.9% of the patients without considering the grade and stage of tumor. These results were similar to the results of Dirim et al. (2008) study (Dirim et al., 2008). Nevertheless, the incidence of this marker in kidney tumor has been various in different studies, in a way that in Hashimoto et al study, COX-2 expression was seen in all of the patients (Hashimoto et al., 2004).

Also, in our study, the association of COX-2 marker was related to the histologic type of tumor. It was more than others in papillary subtype and it was expressed in 43.8% of the cases and has the minimum incidence rate in clear subtype with 14.7% expression. Our findings regarding the significant relation between COX-2 marker and histologic tumor were similar to the results of Sun's study (Sun et al., 2009). But, Hashimoto showed the relation between COX-2 expression and the histologic type and in their study, similar to ours, COX-2 in granular subtype was more than the clear (Hashimoto et al., 2004). In Dirim et al. study, the expression of COX-2 marker in clear carcinoma subtype of renal cell was extremely lower than the papillary type (Dirim et al., 2008).

In our study, no relationship was seen between the COX-2 expression and the age of the patients; this was similar to the results of the studies done by Hasan and Tuna (Tuna et al., 2004; Hasan et al., 2009).

According to our study, the relation between COX-2 expression and the gender of the patients was investigated but no statistically significant relation that was similar to the results of Tuna's (Tuna et al., 2004) study and different from the results of Lee et al study in which COX-2 expression was related to the male gender was seen (Lee et al., 2012). But in other similar studies, this parameter was not investigated.

We found no relation between COX-2 expression and the size of the tumor, although in the group that expressed COX-2, is was bigger and related to the results gained by Hasan (Hasan et al., 2009); however, it was different from the Tian et al investigation (Tian et al., 2011). They reached the conclusion that there is a relation between the COX-2 expression and the size of tumor.

In the present study, the relationship between the increase of COX-2 expression and the microscopic grade of the tumor was found similar to the study of Hasan Gucer, i.e. its expression was more in lower nuclear grades with no statistically significant relationship (Hasan et al., 2009), but Sun (Sun et al., 2009) was able to find a relationship between these two parameters.

We also studied the relationship between the pathologic stage of tumor and the highest expression in PT2 as well as the lowest expression in PT4. But no statistically significant association was identified similar to the results of Hasan et al. (2009) study (Hasan et al., 2009) and different from Sun et al. (2009) study (Sun et al., 2009) which reveals the relationship between these two parameters. We also studied its relation with the involvement of tumoral lymph node and the highest amount of its expression was in tumors with tumoral lymph node involvement, but the statistical relation was

not significant and this was different from Miyata's study (Miyata et al., 2003).

We were not able to find a relation between COX-2 expression with the occurrence of metastasis or the survivability of the patient, whereas Kankuri-Tammilehto, in his studies found that the share of COX-2 positive tumors in renal cell carcinoma undergoing metastasis in future is more and survivability without metastasis in COX-2 positive patients is higher (Kankuri-Tammilehto et al., 2010).

In our study, there were some limitations such as the low number of patients in subgroups under study for each parameter and the unavailability of some patients. In general, the difference between the results of different studies can be related to the sample size, use of cut-offs and different interpretations for the expression of COX-2, use of different anti-bodies and different IHC techniques.

In this study, the COX-2 marker was expressed in20.9% of patients with maximal positivity in papillary subtype, other than histology mentioned, other surveyed variables did not show significant statistical correlation with cox2 expression, and of coarse some of these parameters like tumor stage, metastasis, lymph node involvement and prognosis are of great importance in ultimate outcome.

Another important parameter is prognosis which most published studies have shown cox2 expression with poor prognosis (Miyata et al., 2003; Sozen et al., 2008) ,however predicted for a longer median overall survival (Kankuri-Tammilehto et al., 2010), remains unclear how to reconcile these seeming disparate findings. It is possible that the discrepancies could relate to clinical context in which the tissue was obtained (Wang et al., 2013).

According to previous experiments and our study we do suggest a more comprehensive study with a larger scale with and even designing an analytical study confirming with more suitable technique like more sensitive IHC and molecular studies, sampling of different parts of viable tumors and lowering the threshold can be also helpful (Wang et al., 2013).

Figures. Positive expression of COX-2 marker, A) Renal papillary Carcinoma (magnification 200x), B) Renal papillary Carcinoma (magnification 400x)C),) Renal Chromophobe Carcinoma (magnification 200x) and D) Renal Chromophobe Carcinoma (magnification 400x).

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