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Research Article

RELATIONSHIP OF STAT-3 EXPRESSION WITH RENAL CELL CARCINOMA SUBTYPES AND FUHRMAN PROGNOSTIC GRADE

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ABSTRACT

Aim: The STAT 3 (Signal Transducer and Activator of Transcription) gene is a member of a family known as the Stat genes. These genes enable the production of proteins that are part of the basic chemical signaling pathways within the cell. This protein acts in many cellular functions by regulating genes including cell growth, cell division and cell motions. In addition to these functions, it also regulates self-destruction of the cell, called apoptosis. The effects of nuclear grading (Fuhrman) and its subtypes on survival in RCC (Renal Cell Carcinoma) are known. In this study, the relationship between STAT 3 and RCC will be evaluated.

Material and Methods: 41 RCC cases were included in this study. The cases were 26 clear cell RCC (CCRCC), 7 chromophobe cell RCC (ChRCC), 8 papillary RCC (PRCC). Twelve of the CCRCC lesions were Fuhrman grade 1. Five of the CCRCC lesions were Fuhrman grade 2. Nine of the CCRCC lesions were Fuhrman grade 3. STAT 3 was administered immunohistochemically to paraffin blocks of these cases.

Results: When the distribution of staining intensity was evaluated with respect to the groups, the frequency of those with staining intensity "3" was found as 19.2% in the CCRCC group, 28.6% in the chromophobe group and 50% in the papillary group. However, these differences are not statistically significant. When the relationship between the Fuhrman grade and the degree of staining was examined in the CCRCC group (n = 26), it was determined that the degree of staining increased significantly as the Fuhrman grade increased. The correlation was r = 0.456 and there was a positive correlation (p = 0.019)

Conclusion: In this study, STAT 3 expression was found to be in positive corelation with Fuhrman grading.

INTRODUCTION

Renal cell carcinomas (RCC) account for 2-3% of all cancer cases (1). Of all cancer deaths, 2% are RCC and worldwide, 95,000 deaths occur annually (2,3). There are 208,000 new cases of RCC in the world every year (4). The most common age group of RCC cases is 60's and 70's, and male/female ratio is 1.6/1.0 (5). The highest rates are in North America and the lowest rates are in Asia and Africa (6). With the introduction of advanced imaging techniques, the rate of RCC detected at early stage was increased (7). These early detected tumors are usually small in size and low- stage. Prognostic models are being developed for this purpose. In these models, Fuhrman nuclear grading has an important place besides prognostic parameters such as TNM stage and performance score. Fuhrman nuclear grading system, which is described by Fuhrman *et al*. and is the most widely used histopathological grading system since 1982, has been shown to be an independent prognostic factor in the survival of RCC (8-10). The most distinct difference of Fuhrman nuclear grading

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from other grading systems is that nucleus appearance and the size of the nuclei are evaluated under light microscopy. Accordingly, the nuclear grade is composed of 4 groups (Table 1). Significant differences were found between grade 1 and grade 2-4 tumors in terms of metastasis. In the study conducted by Fuhrman *et al*., 4 different nuclear grades were defined through survival analysis, but when analyzing the results by grouping grade 2 and 3, it was reported that there was a statistically significant difference between grade 1, grade 2+3 and grade 4 in terms of survival. (8,10)

Epidemiological studies have shown an approximate increase of 2% in the annual worldwide incidence of RCC in Europe (11). Due to deficiencies of chemo and radiation treatment efficiency in stage IV RCC, 5-year survival rates in RCC range from 5% to 10% (12.13).

RCC is a heterogeneous disease with different types, having specific histopathological and genetic characteristic (14). RCC as defined by the Heidelberg classification system has four main subtypes, respectively; clear cell RCC (CCRCC), papillary RCC (PRCC), chromophobe RCC (ChRCC) and collecting duct carcinoma (14).

CCRCC is the most common type of adult RCC and represents 70% of all RCC cases. Among the remaining RCCs, papillary RCC accounts for 10-15%, chromophobe RCC 4-6%, renal oncocytoma 5%, and 4-5% for unclassified lesions (15).

Average survival is even weaker in all cases in which the tumor is resected, the 5- year survival rate is 50 %. The prediction for RCC survival rate is still arguable. Despite the prognostic significance of a lot of parameters are tested, very few of them have seen widespread acceptance in clinical practice (12-14). Today, TNM stage and tumor grade is regarded as the most commonly used decisive tool in the survival prediction (16).

Fuhrman grading system is the most widely used one in RCC studies showing that nuclear grading systems are directly related to survival. The Fuhrman grading system is evaluated according to nuclear size, nuclear shape and nucleolar prominence (17).

STATs (Signal Transducer and Activator of Transcription) were first described in 1996 as members of the interferon signal complex. STAT gene takes part in many cellular activities such as development, differentiation, proliferation (8, 18, 19). STATs are found in the cytoplasm in latent form and activated in response to stimulation generated by cytokines, growth factors, hormones and peptides (20).

STAT 3 is activated structurally in many type of cancers and causes a cross reaction between tumors and immune cells. Cytokines produced by the tumor are activated in immune cells that are infiltrated with STAT 3, and they suppress tumor cell activity. Inhibitor STAT 3 is a potential treatment approach to be used in a variety of RCC types (21). When phosphorylated, STAT 3 molecules activate to create dimers and transport into the nucleus to edit the protein transcription, and they control the cell survival and proliferation (22, 23). Also activated STAT 3 organizes the expression of antiptotic, proliferative and immune response genes (22, 24).

In our study, the relationship between RCC subtypes and Fuhrman grade and STAT 3 is assessed.

MATERIAL AND METHODS

41 RCC cases were included in our study. There were 26 CCRCC, 7 ChRCC, and 8 PRCC cases. Twelve of the CCRCC lesions were grade 1. Five of the CCRCC lesions were Fuhrman grade 2. Nine of the CCRCC lesions were Fuhrman grade 3.STAT 3 was administered immunohistochemically to the paraffin blocks of these cases.

Immunohistochemistry

Immunohistochemical sample was stained with Leica Bond-Max IHC Staining Device (Vision Biosystems, Melbourne, Australia).

The Leica Bond-Max IHC Staning Device protocol was applied this way. 5µ- thickness tissues that were cut and put on slide were placed in the machine.Waited for 30 minutes at 60 degrees Celsius. For deparaffinization, they were put in Bond Devax solution at 72 degrees Celsius. After rinsing 3 times with alcohol they were rinsed 3 times with Bond Wash solution. With previously identified antibody pretreatment solution they were held on for 10 mins in 100 degrees. They were rinsed again with Bond Wash solution 3 times more.10 mins peroxide blockage was done. They were rinsed 3 times more again with Bond Wash solution. They were incubated with identified Primary Antibodies for 15 minutes. They were rinsed 3 times more again with Bond Wash solution. They were treated with Post Primer for 7 mins. They were rinsed with Bond Wash rinse solution 3 times again and treated with the polymer for 7 mins. They were rinsed 2 times more again with Bond Wash solution and then rinsed with distilled water. Incubated with DAB for 7 minutes and rinsed 3 times with distilled water. After these processes, Stat -3 (cytoplasmic or nuclear) positivity was evaluated by light microscopy. Positive cells were evaluated by counting at least 1000 cells on 10x magnification view (400 x) per each tissue section. The number of Stat3 positive neoplastic cells was estimated semi-quantitatively. Evaluation was graded as; (-) the absence of immuno-reaction; (+) <10% positive cells; (++) 10-50% positive cells and (+++) >50% positive cells (Figure 1).

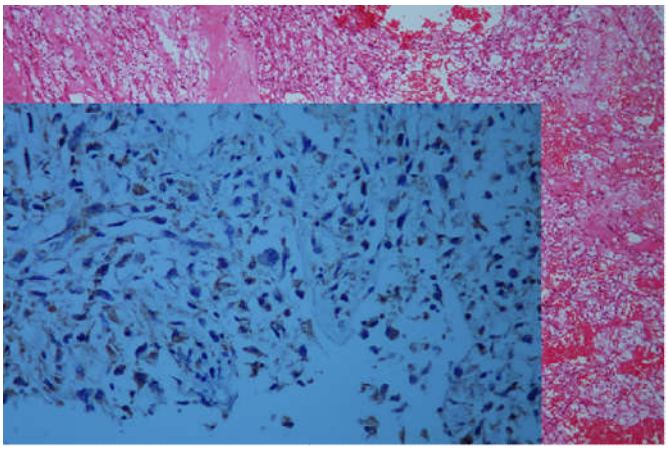

Figure1 Fuhrman grade 2 CCRCC (H&EX100), small picture: grade 2 staning with Stat 3 (x200)

Statistical analysis

In statistical analysis of the data, SPSS 20.0 (for Windows) software package was used. Whether the intensiy of the staining between groups shows a significant difference or not was evaluated using the Kruskal-Wallis H test. In addition, the relationship between the degree of staining and Fuhrman grade in CCRCC Group (n = 26) was assessed with Spearman rank correlation analysis. Data were expressed as mean ± SD. Probability values under $p<0.05$ were accepted as statistically meaningful.

RESULTS

Distribution of the staining intensity according to the groups is given in Table 1. The frequency of the ones with staining intensity "3" was found to be 19.2% in CCRCC group, 28.6% in ChRCC group and 50% in PRCC group. However, these differences are not statistically significant.

Table 1 Distribution of Stat 3 staining intensity according to subtypes

STAT 3 grade		RCC Subtypes						
		CCRCC		ChRCC		PRCC		
		n	%	n	%	n	%	n
cytoplasmic	0	1	3,8	1	14,3	0	0,0	2
	1	9	34,6	3	42,9	3	37,5	15
	2	11	42,3	1	14,3	1	12,5	13
	3	5	19,2	2	28,6	4	50,0	11
Total		26		7		8		41

Besides when the relationship between the Fuhrman grade and the degree of staining was examined in the CCRCC group (n = 26), it was determined that the degree of staining increased significantly as the Fuhrman grade increased. The correlation was r = 0.456 and there was a positive correlation (p = 0.019) (Table 2)

Interestingly, each STAT 3 and p53 mRNA expression level was correlated with tumor tissues while STAT 3 was associated with tumor-free tissues with p53 (31).

Crotty *et al*. presented that 86% of the CHRCCs in their serial were Robson stage I (32). Beck *et al*. stated that chromophobe and papillary histology were better in 5- year survival compared to clear cell RCC. However when the size and stage of the tumor were considered, not papillary but chromophobe RCC was significantly associated with a good survival rate (33). When compared to PRCC and CCRCC, in ChRCC patients, time passed from nephrectomy to metastasis and from metastasis to the time of death was 2 times longer (34).

The ability of Fuhrman grading system to predict specific survival rates was demonstrated in a lot of studies, regardless of the pathological stage (35). The metastasis rates of grade I tumors in Fuhrman grading system were found significantly low. However when grade 1 through 4 metastasis rates were compared, 3 categories have been identified; classified as grade 1, grade 2 and 3 and grade 4 (36).

In our study it is seen that there are differences between STAT 3 staining among RCC subtypes. However, in our study there was no statistically significant difference between the intensity of STAT 3 staining and subtypes (p=0.516), so there was no significant difference between the groups and the intensity of the staining. The reason of the intensity of staining STAT 3 being low in CCRCC (19.2%) was thought to be related with that 46% of cases constituted Fuhrman grade I.

It is not a surprise that STAT 3 expression in PRCC (50%) was higher compared to ChRCC (28.6%). Besides the similar survival rates, there are publications showing that, especially in metastatic RCCs, prognosis of ChRCC is better than that of PRCC (37, 38).

Table 2 Fuhrman grade STAT 3 staining intensity relation in CCRCC

		Fuhrman grade						Total	
		1,00		2,00		3,00			
		Count	%	Count	%	Count	%	Count	%
STAT 3	,00	0	0,0	0	0,0	1	11,1	1	3,8
	1,00	8	66,7	1	20,0	0	0,0	9	34,6
	2,00	3	25,0	3	60,0	5	55,6	11	42,3
	3,00	1	8,3	1	20,0	3	33,3	5	19,2
Total		12	100,0	5	100,0	9	100,0	26	100,0

DISCUSSION

STAT 3 which is commonly activated in cancer cells is an important intersection point for many signaling pathways (25). Among the seven members of the STAT family (STAT 1, 2, 3, 4, 5a, 5b and 6) Stat 3 and Stat 4 is activated frequently in human cancers (26, 27). All positive samples showed cytoplasmic and/or nuclear positivity. In non-lactating normal breast tissue and also in hyperplastic tissues low numbers of positive cells are shown. In neoplastic tissues, a great number of positive cells were observed having variable reactions ranging from moderate to advanced degrees (28). Recent studies have revealed that the activation of STAT 3 is related to RCC proliferation and poor survival (29, 30).

Masuda *et al*. showed that in the RCC, STAT 3 mRNA and p53 expression were suppressed. STAT 3 mRNA and p53 also have similar relationship on pathological features and survival rate.

In this study, positive correlation with staining intensity was observed when STAT 3 was evaluated with Fuhrman grading. The correlation was found as r=0.456, p=0.019. This meant that as the Fuhrman grade increased in parallel with the increase in the intensity of the staining.

The relation of Fuhrman grading system with PRCC in the survey is debatable. As for ChRCC, Fuhrman grading is reported to be not correlated with it. For this reason, only CCRCC group was evaluated in this study (38,39)

As a result, in our study it was observed that STAT 3 expression was correlated with Fuhrman grading. Owing to this relation, it is concluded that STAT 3 expression could be a prognostic indicator. The relationship between the RCC subtypes and STAT 3 could not be shown clearly. To show this relationship, studies with larger sample populations are needed.

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