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Predictive role of vascular endothelial growth factor polymorphisms in the survival of renal cell carcinoma patients

Y.-Q. Yang[1] and J. Chen[2]

[1]Medicine Pharmacy, PLA General Hospital, Beijing, China
[2]Children's Nutrition Research Center,
Children's Hospital of Chongqing Medical University, Chongqing, China

Corresponding author: J. Chen
E-mail: chengjie_811@163.com

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ABSTRACT. We conducted a study to investigate the possible role of the vascular endothelial growth factor (VEGF) polymorphisms -2578C/A, -1154G/A and -634C/G and clinical factors in renal cell carcinoma (RCC) prognosis in a cohort of 336 RCC cases. A total of 336 patients with RCC were recruited from PLA General Hospital between January 2004 and December 2005. All patients were followed up until December 2010, and no patient was lost to follow-up. The follow-up time of this study was 60 months. At the time of analysis, a total of 210 died during the follow-up. The median overall survival for patients was 29.1 months (95%CI = 17.1 to 41.3 months), and the 5-year survival rate for the patients was 37.5%. Our study showed that Karnofsky performance status ≥60 could delay death from RCC, with HR (95%CI) of 0.57 (0.39-0.84). Patients with anemia, platelet count >400 x 10^9/L, neutrophilia and lymphocytes >160 g/L had increased risk of death from RCC, with HR (95%CI) of 1.84 (1.18-2.96), 2.01 (1.27-3.25), 1.65 (1.03-2.56) and 1.49 (0.99-2.71), respectively. The VEGF -2578AA and -1154AA genotypes were significantly associated

with a poor overall survival of RCC patients, with HR (95%CI) of 2.41 (1.32-5.13) and 3.77 (1.42-15.67), respectively. In conclusion, our study presented the factors regarding the prognosis of RCC patients, and high platelet and neutrophil counts, low lymphocytes, and VEGF -2578C/A and -1154G/A polymorphisms were shown to be independent factors for a lower prognosis of RCC patients.

Key words: Renal cell carcinoma; VEGF polymorphism; Prognosis; Predictive factors

INTRODUCTION

Renal cell carcinoma (RCC) remains the most frequently occurring cancer in the kidney. Epidemiologic studies suggest a continued rise in the incidence and mortality of renal cell carcinomas worldwide over the last 30 years, particularly in the Western world where it has been among the tumors with the highest upward trend in incidence (Mathew et al., 2002; Hollingsworth et al., 2006). Although most patients with early-stage RCC can be cured surgically, approximately 33% of patients present metastatic disease for which treatment is usually not curative (Motzer et al., 1996). Moreover, about 50% of RCC patients with curative surgery may be expected to develop a recurrence with distant metastases, and the prognosis of RCC patients with metastatic or recurrent diseases is poor, with a 5-year survival of less than 20%. Previous studies have indicated an improved prognosis of RCC by targeting a number of growth factors, such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor and their receptors (Motzer et al., 2007; Patard et al., 2008).

It is reported that VEGF is overexpressed in RCC tissues when compared with normal renal tissues (Langsenlehner et al., 2008). Therapeutic targeting of VEGF has shown preliminary clinical efficacy in RCC (Jäkel et al., 2012; Vrdoljak et al., 2013). Given the possible influence of VEGF levels on treatment efficacy in RCC, VEGF polymorphisms may have an association with risk of RCC patients. Two previous studies conducted in Japan and Spain demonstrated that VEGF polymorphisms may have effects on RCC progression or prognosis (Kawai et al., 2007; Sáenz-López et al., 2013). But the results are inconsistent, and another study has suggested that VEGF gene polymorphisms are not associated with RCC risk (Matthew et al., 2002). Therefore, we conducted a study to investigate the possible role of the VEGF polymorphisms -2578C/A, -1154G/A and -634C/G and clinical factors in RCC prognosis in a cohort of 336 RCC cases.

MATERIAL AND METHODS

A total of 336 patients with RCC were recruited from the PLA General Hospital between January 2004 and December 2005. All RCC patients were histologically diagnosed, had no prior history of cancer, and were absence of significant cardiac disease and no recent surgery. All the patients were followed up until December 2010, and no patient was lost to follow-up. The study was approved by the Institutional Ethics Committee and informed consent for participating in this study was obtained for each case.

Data collection

We collected data regarding demographic and clinical characteristics, baseline biochemical parameters, and date of death or last follow-up. The overall survival was defined as the time from initiation of treatment to the date of death or last follow-up.

Blood samples and genotyping

All participants were asked to provide 5 mL blood, and the blood samples were stored at -20°C. Genomic DNA for VEGF -2578C/A, -1154G/A and -634C/G analysis was extracted using the Qiagen Blood kit (Qiagen, Chastworth, CA, USA) according to manufacturer instructions. Polymerase chain reaction (PCR) combined with restriction fragment length polymorphism assay was used for genotyping. The primers and probes of VEGF -2578C/A, -1154G/A and -634C/G were designed using the Sequenom Assay Design 3.1 software (Sequenom, San Diego, CA, USA). PCR was conducted using 5 ng genomic DNA in a 5-μL reaction mixture in the GeneAmp® PCR System 9700 with Dual 384-Well Sample Block Module (Applied Biosystems, Carlsbad, CA, USA). PCR amplifications were conducted with 1-15 ng DNA from all blood samples, using 1.0 U/mL HotStar Taq DNA polymerase (Qiagen, St. Louis, MO, USA), 0.21 μM forward and reverse primers, and 0.125 mM dNTPs. The amplification protocol was 95°C for 15 min, followed by 35 cycles at 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, with a final extension at 72°C for 10 min. For quality control, we randomly selected 10% of the cases and controls to genotype again by different researchers, and the reproducibility was 100%.

Statistical analysis

All statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS) Version 16.0 software (SPSS Inc., Chicago, IL, USA). Continuous variables are reported as means ± standard deviation (SD), while categorical variables are shown as frequencies and percentages. Distributions of overall survival were estimated by using the Kaplan-Meier method, and median and 2-year overall survival along with 95%CI was reported. Associations between overall survival and potential prognostic factors were assessed by using the log-rank test in unavailable analysis. Variables were found to be significant if the two-sided P value was <0.05 on univariate testing. The Cox proportional hazards model was subsequently applied in multivariable analysis by using a step-wise procedure with a level of 0.25 for entering and removing variables. The variables that reached statistical significant in this model were then deemed to be independent predictors of the outcome concerning the overall survival.

RESULTS

Patient characteristics and outcomes

Baseline characteristics of patients are shown in Table 1. The follow-up time of this study was 60 months. At the time of analysis, 210 patients died during follow-up. The

median overall survival of patients was 29.1 months (95%CI = 17.1 to 41.3 months), and the 5-year survival rate of the patients was 37.5% (Figure 1). Patients who underwent nephrectomy had a lower risk of death from RCC, and patients with anemia, hypercalcemia, neutrophilia and platelet count >400 x 10^9/L as well as Karnofsky performance status (KPS) ≥60 had a higher risk of death. For VEGF polymorphism, we found that those carrying the -2578CA and AA genotypes had increased risk of death from RCC, with HR (95%CI) of 2.17 (1.27-3.73) and 2.99 (1.51-6.09), respectively. Similarly, we found that those carrying the -1154AA genotype had a greatly increased risk of death from RCC, with HR (95%CI) of 5.12 (1.67-20.82).

Table 1. Univariate analysis of demographic and clinic characteristics of study patients.

Variables	Patients		Five-year survival rate (%)	Hazard ratio (95%CI)
	Total (N = 336)	Died (N = 210)		
Age (years)				
<40	47	32	32.6	1.0 (Ref.)
40-49	79	52	34.3	0.98 (0.56-1.74)
50-59	103	61	41.0	0.87 (0.51-1.52)
≥60	107	66	38.7	0.91 (0.53-1.57)
Gender				
Male	247	150	39.2	1.0 (Ref.)
Female	89	60	32.8	1.1 (0.75-1.60)
Nephrectomy				
No	66	60	8.2	1.0 (Ref.)
Yes	270	150	44.7	0.51 (0.37-0.87)
Treatment				
Sunitinib	206	128	38.0	1.0 (Ref.)
Sorafenib	97	55	43.1	0.92 (0.62-1.35)
Bevacizumab	33	27	17.7	1.43 (0.77-2.61)
VEGF targeted therapy				
First line	217	139	35.9	1.0 (Ref.)
Second line	119	71	40.5	0.93 (0.65-1.32)
Anemia				
No	113	52	54.1	1.0 (Ref.)
Yes	223	158	29.1	1.52 (1.04-2.25)
Hypercalcemia				
No	298	175	41.4	1.0 (Ref.)
Yes	38	35	6.5	1.61 (1.02-2.53)
Neutrophilia				
No	294	173	41.3	1.0 (Ref.)
Yes	42	37	10.8	1.57 (1.03-2.41)
Thrombocytosis				
No	284	172	39.4	1.0 (Ref.)
Yes	52	38	27.1	1.20 (0.76-1.88)
Karnofsky performance status				
<60	97	84	13.6	1.0 (Ref.)
≥60	239	126	47.2	0.61 (0.43-0.87)
Histology				
Non-clear cell	25	21	15.8	1.0 (Ref.)
Clear cell	311	189	39.3	0.74 (0.41-1.37)
Platelet count >400 x 10^9/L				
No	72	28	60.6	1.0 (Ref.)
Yes	264	182	31.2	1.77 (1.11-2.90)
Lymphocytes >160 g/L				
No	63	30	51.9	1.0 (Ref.)
Yes	273	180	34.2	1.38 (0.84-2.31)

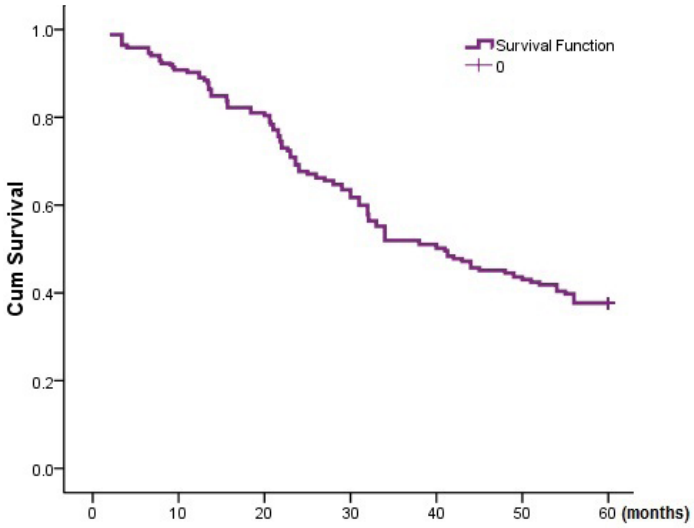

Figure 1. Overall survival probability of renal cell carcinoma patients.

Multivariable analysis results

All the potential predictive values for the survival of RCC are shown in Table 2. Although many factors were associated with individuals with RCC by univariate analysis, only five factors were identified as independent predictors of the outcome by multivariate analysis. Our study showed that KPS ≥60 could delay death from RCC, with HR (95%CI) of 0.57 (0.39-0.84). Patients with anemia, platelet count >400 x 10^9/L, neutrophilia and lymphocytes >160 g/L increased the risk of death from RCC, with HR (95%CI) of 1.84 (1.18-2.96), 2.01 (1.27-3.25), 1.65 (1.03-2.56) and 1.49 (0.99-2.71), respectively. The VEGF -2578AA and -1154AA genotypes were significantly associated with a poor overall survival of RCC patients, with HR (95%CI) of 2.41 (1.32-5.13) and 3.77 (1.42-15.67), respectively.

Table 2. Multivariable analysis of predictive value for the survival of renal cell carcinoma.

Predictive value	Hazard ratio	95%CI	P
Karnofsky performance status			
<60	1.0 (Ref.)	-	
≥60	0.57	0.39-0.84	0.004
Anemia			
No	1.0 (Ref.)	-	
Yes	1.84	1.18-2.96	0.022
Platelet count >400 x 10^9/L			
No	1.0 (Ref.)	-	
Yes	2.01	1.27-3.25	0.013
Hypercalcemia			
No	1.0 (Ref.)	-	
Yes	1.77	1.06-2.92	0.036
Neutrophilia			
No	1.0 (Ref.)	-	
Yes	1.65	1.03-2.56	0.047
Lymphocytes >160 g/L			
No	1.0 (Ref.)	-	
Yes	1.49	0.99-2.71	0.037

DISCUSSION

Genetic factors can affect the development of RCC, which in turn influence the incidence rate of carcinoma or carcinoma progression. Genes implicated in angiogenesis could be candidates for tumor progression and important prognostic factors in various cancers, such as breast cancer and colorectal cancer (Oh et al., 2013; Absenger et al., 2013). VEGF, a growth factor that regulates angiogenesis, is regarded as the most potent stimulatory cytokine stimulating tumor angiogenesis and an important factor for metastasis, survival and spread of the tumor (Salven et al., 1997). Several polymorphisms in the VEGF gene have been identified recently, and -2578C/A, -1154G/A and -634C/G at the promoter region of VEGF may have a role in altering gene transcription and may affect its expression (Oh et al., 2013; Absenger et al., 2013). Our study found that the VEGF -2578C/A and -1154G/A polymorphisms are correlated with tumor stage and tumor size, and associated with shorter survival time of RCC patients.

These results suggest that the VEGF -2578C/A and -1154G/A gene polymorphisms may be associated with increased expression of VEGF, which promotes tumor angiogensis, thus resulting in a higher tumor stage and decreased survival time of RCC patients. Therefore, VEGF gene polymorphisms could play a critical role in altering VEGF expression and influence the progression of RCC and patient survival. Previous studies have reported similar results in line with our study regarding the association between these polymorphisms of the VEGF gene and various diseases (Kawai et al., 2007). Hefler et al. (2007) demonstrated that VEGF -634C/G, -1154G/A, and -2578C/A polymorphisms were associated with increased VEGF expression, and associated with a shortened survival time of ovarian cancer patients. Another *in vitro* study indicated that the -2578C allele was correlated with higher levels of VEGF production (Shahbazi et al., 2002). Our study indicated that VEGF polymorphisms are associated with prognosis of RCC, which is in line with previous studies.

In our study, we showed that the prognostic factors in advanced RCC patients included high platelet, neutrophil and lymphocyte counts. These factors are inflammation markers, and these factors could be better predictors for the survival of RCC patients. A previous study showed that platelets and neutrophils were independent factors for the prognosis of advanced RCC, where patients who had elevated platelet counts had significantly shorter median survival time (8.4 months) than patients who had a normal count (14.6 months) (Choueiri et al., 2006). The mechanism of platelets in the prognosis of RCC could be that platelet overproduction can lead to enhanced adherence of malignant cells to the endothelial wall and penetration, and that platelet granules contain a variety of angiogenic factors, such as VEGF, platelet-derived growth factor, transforming growth factor β, and others that have been implicated in various steps of tumor progression (Mohle et al., 1997; O'Byrne et al., 1999). The increased neutrophils could result in overproduction of interleukins and other growth factors by the tumor (Hollen et al., 1992).

There are two limitations in our study. First, there might be limitations in the generalization of the study results, since our study results are different from studies conducted in the United States and Canada (Choueiri et al., 2006; Heng et al., 2009; Motzer et al., 2009). The different results might have been due to differences in ethnicities, treatment methods, patients' situation or by chance. Therefore, further studies in the Chinese population are warranted. Second, previous studies showed that there may be other genes involved in the prognosis of RCC, and investigation of the association between gene polymorphism and prognosis of RCC is needed.

In conclusion, our study presented the factors regarding the prognosis of RCC patients, and high platelet and neutrophil counts, low lymphocytes, and VEGF -2578C/A and -1154G/A polymorphisms were shown to be independent factors for a lower prognosis of RCC patients. These findings may be helpful in predicting the clinical outcome of patients with RCC. Further studies are needed to confirm their clinical significance.

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