Article is available online at http://www.webio.hu/por/2002/8/2/0129

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Significance of Angiogenesis and Microvascular Invasion in Renal Cell Carcinoma

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The aim of this study is to evaluate the relationship between tumor angiogenesis and microvascular invasion, and the subsequent development of metastatic disease in patients undergoing surgery for renal cell carcinoma (RCC). The study group consisted of 102 patients who underwent surgery for RCC between the years 1990 and 1997 in our institute with a mean follow up period of 81.3 months. Paraffin blocks were stained for Factor VIII- related antigen and CD34 which decorate endothelial cells in order to assess angiogenesis and microvascular invasion and their relevance for developing metastatic disease. When Factor VIII- related antigen staining was used we found that the microvessel count correlated with the development of metastatic disease with a mean count of 49.7 for patients with no evidence of disease and a mean count of 95.5 for patients who developed metastatic disease ($p<0.05$). We also found that microvascular invasion correlated with the development of metastatic disease. It was demonstrated in 55.5% of patients who developed metastatic disease versus 23.8% of patients with no evidence of disease with Factor VIII staining ($p<0.05$), and in 33.3% and 7.1%, respectively ($p<0.05$) with CD34 staining. This study suggest that demonstration of intense angiogenesis and microvascular invasion may be a predictor of a more aggressive tumor mandating closer follow up and consideration of adjuvant therapy. (Pathology Oncology Research Vol 8, No 2, 129–132, 2002)

Keywords: angiogenesis, factor VIII, renal cell carcinoma, prognosis

Introduction

RCC is a relatively rare tumor affecting six of every 100,000 people and accounting for approximately 3% of adult malignancies. It is more common among urban population and among males with a male-to-female ratio of approximately 2:1.[36] The tumor arises from the proximal convoluted tubules of the kidney and is characterised by abundant neovascularization and arteriovenous-venous fistula formation.[14] Radical nephrectomy is the curative treatment option for renal cell carcinoma.[6,17,20,21,22] If the tumor is small and nephron sparing is required, partial nephrectomy is the procedure of choice. Although tumor size, tumor grade and nuclear atypia have all been reported as prognostic factors,[7,8,13,16] the biological behavior of this tumor is still unpredictable. The impact of invasion of the carcinoma cells into large veins on the prognosis of patients remains controversial.[5,9,18,24,25,28,29] Recently, several reports have been published attributing an adverse impact of microvascular invasion and angiogenesis on survival of patients with urologic tumors, particularly in RCC.[1,3,4,11,12,15,23,26,34,38]

In this paper, we evaluated the impact of microvascular invasion and angiogenesis on the survival of patients who underwent surgery for RCC .

Received: April 2, 2002; *accepted:* May 31, 2002

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Materials and methods

Clinical history

Between 1990 and 1997, 132 patients underwent surgery for RCC in our institute. Complete data and follow-up were available for 102 patients; 62 males and 40 females, age range from 47 to 81 years (mean age of 65.1 years). The preoperative evaluation included a chest X-ray and abdominal CT scan. Forty-nine patients underwent radical nephrectomy and two underwent partial nephrectomy. Mean postoperative follow-up was 81.3 months

Table 1. **Tumor histology and progression**

Histological diagnosis	*Patients*		*NED*		*Metastatic disease*	
	No.	*% of all patients*	*No.*	*% of patients*	*No.*	*% of patients*
Clear cell	74	72.5	62	83.8	12	16.2
Tubulopapillar	22	21.6	20	90.9	2	9.1
Chromophobe	4	3.9	2	50	2	50
Sarcomatoid	2	2	0	0	2	100

NED – no evidence of disease

(range 42 to 111 months). Follow-up consisted of performing abdominal ultrasonography and chest X-ray every six months for the first five years and then once a year, and an abdominal CT scan annually over the first five years.

We reviewed the patient's records for tumor size, stage of the tumor (according to the TNM classification)[37] and current status of the patient.

Pathological analysis

All pathological slides were reviewed by the same pathologist for definition of the specific type of the tumor and grade of the tumor (according to the method of Fuhrman).[7] From a representative paraffin embedded block, four microns-thin slices were cut, mounted on glass slides, deparaffinized by xylene and alcohol, rehydrated, incubated in citrate buffer for antigen retrieval at 90°C, in a microwave oven for 10 minutes and stained with monoclonal mouse anti-human CD34 (Signet – Massachusetts) and rabbit anti-human, Factor VIII-related antigen (EURO-DPC – UK Llarberis). The staining process was done with the DAKO Chemmate Detection Kit on the DAKO Chemmate slide processing instrument (DAKO - Danmark). For quantification of angiogenesis we first checked the slides under low magnification (x100) and marked the area with the maximal number of small vessels within the tumor and then counting the vessels in three randomly selected high magnification fields (x 400) within the marked area, and calculating the average number of vessels. For the detection of vascular invasion we checked the periphery of the tumor under high magnification (x 200), counting the vessels that demonstrated vascular invasion. We considered vascular invasion whenever a group of tumor cells was surrounded by endothelial cells which stained positively by Factor VIII- related Ag and/or CD34. The possible correlation between maximal angiogenesis, vascular invasion, and the patient's clinical status has been evaluated.

Statistical analysis

Comparison between groups was made with a Kruskall-Wallis test. Values of $p < 0.05$ were considered stastistically significant.

Results

During the follow up period, six patients (5.9%) died of unrelated disease, with no evidence of metastases, 18 patients (17.6%) died with evidence of metastatic disease and 78 (76.5%) are alive with no evidence of disease. *Table 1.* summarizes the tumor histology and outcome. 74 patients (72.5%) had clear cell carcinoma; 12 (16.2%) died with evidence of metastatic disease. 22 patients (21.6%) had tubulopapillary type; 2 (9.1%) died with evidence of metastatic disease, 4 (3.9%) had chromophobe type; 2 died with metastatic disease and 2patients (2%) had sarcomatoid type. These two last patients subsequently died with metastatic disease. The distribution of tumor stages and grades and patients outcome is given in *Table 2.* and *3.*

Table 2. **Tumor stage and progression**

Stage of disease	*Patients*		*NED*		*Metastatic disease*	
	No.	*% of all patients*	*No.*	*% of patients*	*No.*	*% of patients*
T1	16	15.7	12	75	4	25
T2	66	64.7	58	87.9	8	12.1
T3A	14	13.7	10	71.4	4	28.6
T3B	6	5.9	4	66.7	2	33.3

NED – no evidence of disease

Table 3. **Tumor grade and progression**

Grade of tumor	*Patients*		*NED*		*Metastatic disease*	
	No.	*% of all patients*	*No.*	*% of patients*	*No.*	*% of patients*
G-1	26	25.5	26	100	0	0
G-2	62	60.8	48	77.4	14	22.6
G-3	12	11.7	10	83.3	2	16.7
G-4	2	2	0	0	2	100

NED – no evidence of disease

Angiogenesis has been evaluated by both in CD34 and Factor VIII staining. The results are given for each histologic type separately because we found different figures for the different histologic types. When we analysed the angiogenesis results for Factor VIII staining we found that in the clear cell carcinoma there are twice as many vessels in tumors that eventually metastasized (95.5 ± 50.32) than there were in tumors that did not (49.7 ± 13.7) and this difference is statistically significant ($p<0.05$). In the other histological groups there were not enough patients for statistical evaluation. There was no difference between patients with and without metastatic disease CD34 staining.

Vascular invasion has been evaluated by both CD34 and Factor VIII. Factor VIII staining demonstrated vascular invasion in 30 patients; 20 out of the 84 patients in the NED (no evidence of disease) group (23.8%), and 10 of the 18 patients who died with metastatic disease (55.5%) ($p<0.05$). CD34 staining demonstrated micro vascular invasion in 12 patients, including out of 84 patients in the NED group (7.1%) and 6 out of 18 patients in the metastatic group (33.3%) ($p <0.05$). *Table 4.* summarizes the angiogenesis and vascular invasion results in both stains and the disease outcome.

Discussion

Growth of solid tumors requires angiogenesis.[19] The new proliferating vessels supply oxygen and nutrition for the tumor cells and promote their growth. Experimental evidence shows that the dynamics of hematogenous metastasis are dependent on the access of the tumor cells to the microvasculature.[31] The important role of angiogenesis in human solid tumors have been well reported in recent studies. Many studies have demonstrated that the microvessel count assessed by immunostaining correlateds with the risk of metastasis, recurrence and the prediction of patient survival.[10,32,33]

RCC is a tumor with unpredictable behavior. In this study we evaluated the impact of angiogenesis and microvascular invasion on the prognosis of the patient, using two immunohistochemical staining methods – for Factor VIII and for CD34. We found that the CD34 staining did not discriminate between patients with and without metastatic disease on the basis of angiogenesis. We also found that, in general, vessel count was higher with this antibody than Factor VIII. It is well known that CD34 staining results for microvascular count are usually higher than those of Factor VIII.[2,30] One explanation for this is that CD34 is less specific than Factor VIII and tend to stain nonendothelial cells.[27,35] We believe that another possible explanation is that there was background staining with CD34, thus making the vessel count higher than it really was and masking the difference between the two groups of patients. In the Factor VIII stained sections, we found a statistically significant difference in vessel count between patients with and without metastatic disease in patients with clear cell type RCC. In the other histologic types we did not have enough patients to draw any conclusions. The vessel count in the tubulopapillar type was lower than in the other histologic types.

Microvascular invasion is a good prognosticator with a high risk for the development of metastatic disease when demonstrated. This phenomenon could be demonstrated with both staining methods. Again because of the higher staining with CD34 it was harder to define those tumor cells that were tipically surrounded by endothelial cells in order to diagnose microvascular invasion, and that is probably the reason why microvascular invasion was less frequet in the CD34 staining. It is quiet impressive that inspite these limitations the difference between those who developed metastatic disease and those who did not was statistically significant in both stainings.

Table 4. **Vessels count, microvascular invasion and disease status**

	CD34 staining	*Factor VIII staining*	
Disease status	*MVI (% of patiens)*	*Vascular count**	*MVI (% of patiens)*
NED	7.1	49.7 ± 13.7	23.8
Metastatic disease	33.3	95.5 ± 50.32	55.5

NED = No evidence of disease; MVI = microvascular invasion
*Number per high power field, given in mean ± standard deviation

Conclusions

Patients with RCC demonstrating microvascular invasion or a high degree of angiogenesis are at high risk for developing metastatic disease. These patients deserve closer follow-up and perhaps adjuvant therapy.

Acknowledgements

We are grateful for the editorial and secretarial help of Mrs Gloria Ginzach and Mrs Melanie Kawe.

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