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ARTICLE

Association between Microvessel Density and Histologic Grade in Renal Cell Carcinomas

Nikolaos KAVANTZAS, Helen PARASKEVAKOU, Sofia TSELENI-BALAFOUTA, Kyriaki ARONI, Pauline ATHANASSIADES, George AGROGIANNIS, Efstratios PATSOURIS

Department of Pathology, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece

Angiogenesis seems to contribute to tumor growth and the development of metastases. There may be an association between the vascular density of individual tumors and their prognosis. In the present survey we studied 53 cases of renal cell carcinoma investigating possible relationship between histologic grade and microvessel density (MVD) measured by an image analysis system. According to our results MVD was significantly associated with the histologic grade, higher grades being accompanied with a higher MVD. Further studies are needed to investigate a possible connection of MVD with the prognostic role of grade in RCCs. (Pathology Oncology Research Vol 13, No 2, 145–148)

Key words: renal carcinoma, microvascular density

Introduction

Renal cell carcinoma (RCC) is the most frequent malignancy in the human kidney, representing 80 to 90% of all malignant kidney tumors and 2% of all cancers in adults.

Recent findings of genetic and molecular biologic changes, based on immunohistochemistry and molecular genetic techniques, classified RCC into the following histologic subtypes: conventional clear-cell, papillary, chromophobe, collecting duct carcinoma and unclassified.

Clear-cell/granular renal carcinoma can be defined by mutations or deletions on the short arm of chromosome 3p (by loss of chromosome 3p and particularly by inactivation of the von Hippel-Lindau disease tumor suppressor gene. Papillary renal cell carcinomas have been found to harbor a number of genetic alterations including trisomies of chromosomes 7, 16 and 17. Chromophobe carcinomas demonstrate abnormalities of chromosomes 1, 2, 6, 10, 13, 17 or 21, while collecting duct carcinomas have been reported to show loss of 1q, 13q, 19q, 6p and 8p.[10]

Angiogenesis and endothelial cell proliferation contribute to tumor growth and the development of metastases. Tumor growth has been related to the capacity to induce neo-angiogenesis.[1]. However, the relationship among the vascular pattern, clonality and cell kinetics remains unknown in adrenocortical nodular hyperplasias, adenomas and carcinomas.[2]

In this survey we investigated microvessel density (MVD) based on factor VIII expression, using computerized image analysis, and its possible correlation with grade in RCC.

Materials and Methods

Paraffin-embedded tumor samples from 53 patients with renal cell carcinoma were evaluated in this study. No patients received chemotherapy or radiotherapy before surgery. The original histological slides, stained with hematoxylin and eosin, were reviewed by the study pathologists to confirm the diagnosis, the histologic pattern and tumor grade. From one paraffin block per tumor specimen, two slides were stained to highlight blood vessels, by staining endothelial cells for factor VIII-related antigen (using an anti-factor VIII polyclonal rabbit antibody) using a standard immunoperoxidase technique.

Tumor microvessels included capillaries, small venules and arterioles. In all tumor sections, individual microvessels were counted in the area of the highest vascularity, at 400x magnification (40x objective, 10x ocular lenses) in five randomly selected microscopic fields.

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Correspondence: Dr George AGROGIANNIS, 1st Department of Pathology, School of Medicine, University of Athens, 60 Agias Lavras str., GR-157 73 Athens, Greece.
email: agrogeorge@panafonet.gr

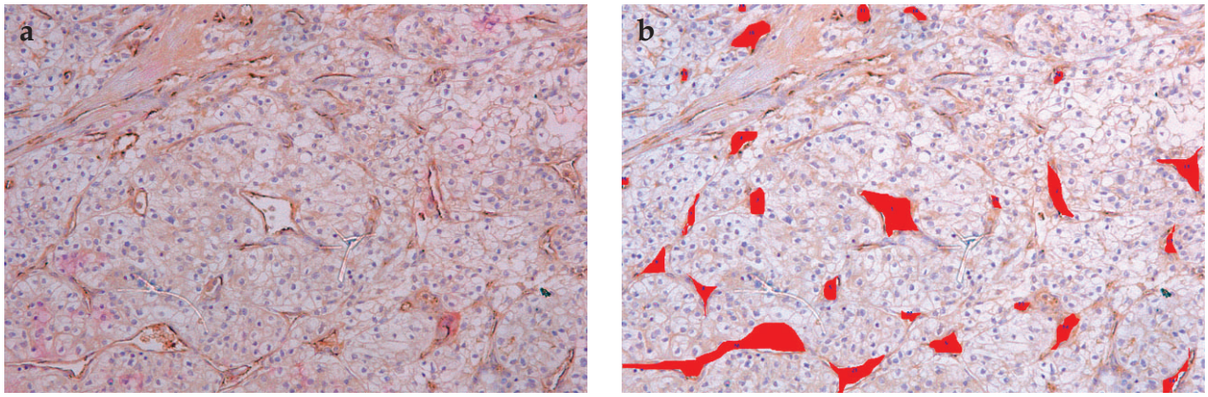

Figure 1. (a) *Digital image of an RCC section immunostained for FVIII to highlight the blood vessels of the tumor.* ***(b)*** *The same image as in (a), elaborated by the appropriate image analysis and morphometry software.*

Images were acquired using a Zeiss Axiolab microscope (Carl Zeiss GmbH, Jena, Germany) with a mechanical stage, fitted with a Sony-Iris CCD video camera (Sony Corp., Tokyo, Japan). The video camera was connected to a Pentium IV personal computer loaded with the appropriate image analysis software (Sigma Scan Pro, Science, Erkrath, Germany). When measuring microvessel density, single endothelial cells were excluded because they cannot be considered as microvessels. The presence of a vascular lumen was not necessary to identify a microvessel. Each count was expressed as the highest number of microvessels present in the selected 100x magnification fields. Extratumoral blood vessels were ignored (*Fig. 1*).

MVD is analyzed through measures of central tendency and dispersity (mean, standard deviation, confidence limits, range). The statistical association between grade and MVD was tested by one-way analysis of variance followed by Bonferroni test for the pair-wise comparisons. All tests were two-sided and the level of statistical significance was set at 5%.

Results

Of a total of 53 cases, 24 (45.3%) belonged to grade I, 18 (34.0%) to grade II and 11 (20.8%) to grade III, respectively. *Table 1* presents data on the microvessel density of the cases according to their grade. Statistical evaluation (ANOVA) of MVD in association with tumor grade shows a statistically significant correlation ($p<0.0001$) between grade and MVD meaning that MVD increases between grade I and grade III. Also, using post-hoc tests of multiple comparisons we found that MVD differs statistically between grades in all possible combinations: grade I vs. II, $p<0.0001$; grade I vs. III, $p<0.0001$; grade II vs. III, $p<0.0001$) (*Fig. 2*).

Discussion

RCC is the most common malignancy of the human kidney, with a variable outcome. The hypoxia-induced pathway has been linked generally to RCC through the von Hippel-Lindau tumor suppressor gene, which is inactivated in the majority of both familial and sporadic clear-cell RCCs. Therefore, RCC may represent one of the best clinical models for directed therapies based on an understanding of the hypoxia-induced pathway. Both VHL-deficient mice and VEGF-knockout mice die in utero because of defective vasculogenesis, a shared phenotype that might be explained by the fact that the VHL protein is tightly linked to angiogenesis via upregulation of VEGF regulation through the action of HIF (hypoxia-inducible factor). Recently, a phase II clinical trial to evaluate the activity of bevacizumab, a neutralizing antibody to VEGF, was performed in patients with metastatic RCC. There was a significant prolongation of time-to-progression in patients receiving high-dose antibody versus placebo. The hypoxia-induced pathway, linked to RCC through the VHL tumor suppressor gene, may serve as the source of new rational treatment strategies based on the design of small molecule inhibitors, as well as vac-

Table 1. **MVD data of RCC cases according their grade**

	N	*Mean*	*SD*	*SE*	*95% CI for mean*	*Minimum*	*Maximum*
Grade I	24	14.04	6.70	1.37	11.21–16.87	5	30
Grade II	18	40.44	12.93	3.05	34.02–46.87	20	67
Grade III	11	93.27	15.65	4.72	82.76–103.79	72	123
Total	53	39.45	32.14	4.41	30.60–48.31	5	123

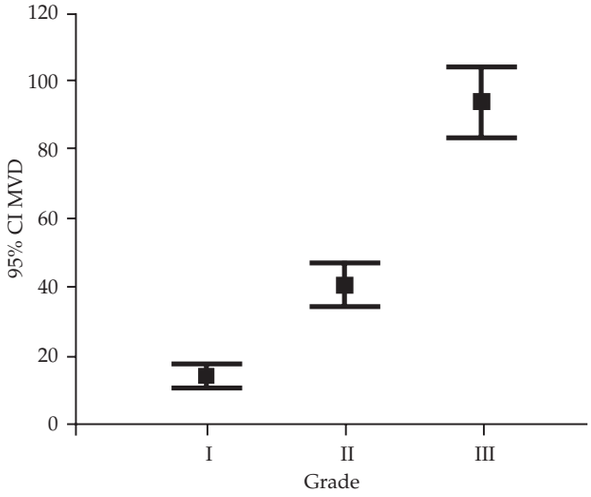

Figure 2. *MVD significantly differs between RCC grades in all possible combinations.*

cine, gene and antibody therapies directed against targets such as VHL, HIF-1α and VEGF.[4,5,13,19]

Until now tumor stage and nuclear grade were identified as the most important prognostic factors for patients with RCC. However, these parameters are insufficient to predict the biological behavior of these tumors. For this reason, a number of biomarkers have been promoted to predict patients' outcome. Recent studies indicate that DNA ploidy, nuclear morphometry, proliferative indices, adhesion molecules and angiogenesis may contribute to the prompt diagnosis of RCC.[6,14,17]

Angiogenesis seems to be necessary for tumor growth and metastasis. Many authors prove an association between the vascular density of individual tumors and the development of metastases in a variety of malignant neoplasms. This relationship may be caused by increased nutrient transfer in hypervascular tumors, promoting the establishment of a more rapidly proliferating clone of tumor cells, as it has been shown for a variety of malignancies that more actively proliferating tumors possess a greater microvessel density.[1,3]

Higher vascular density within a tumor also provides increased opportunity for vascular infiltration by malignant cells leading to metastatic dissemination by either a vascular route or by lymphatics associated with the development of vascular network.[12,20]

Renal cell carcinoma is a strongly vascular tumor. According Rioux-Leclercq et al[15] two different vascular patterns were found in RCC. One of them is characterized by a decrease in MVD, corresponding to a decrease in crowded small vessels with an increase in a smaller number of large-diameter vascular channels, resulting in a total decrease in the number of vessels.

According to our findings, MVD was significantly associated with the histologic grade. Higher grade was accompanied with a higher MVD. The size of vessels observed in our cases was rather small-medium with a wall slightly thicker than the sinusoidal pattern referred to by Rioux-Leclercq et al.[15] An important number of other studies on microvessel density in RCC found no association between MVD and tumor grade,[9,11,16,18] or found an inverse correlation.[7,8,12] The disagreement of these results and the findings of our study could mainly be due to the different methodology of MVD quantification, including differences in microscope magnification, the number of selected microscopic fields and finally the type of vessels selected for quantification. Also, in our study microvessels were counted in the so-called "hot spot" areas, areas of the highest vascularity by a semiautomatic method. It means that the selection of vessels was performed manually by an expert pathologist, whereas the total value of MVD was measured automatically by the appropriate image analysis software. The difference between automatic and semiautomatic methodology leads to a disagreement of the results, often a common major problem of quantitative methods used in pathology.

Further studies are needed to investigate a possible association of MVD with the prognostic role of grade in RCCs.

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