Tumour-associated Angiogenesis and Intermediate Blood Vessels in Renal Cell Carcinoma

MARIUS RAICA¹, ANCA MARIA CIMPEAN¹, OVIDIU CATALIN FERICIAN² and ADELA MARIA FERICIAN²

¹Department of Microscopic Morphology/Histology, Angiogenesis Research Center, Victor Babes University of Medicine and Pharmacy, Timisoara, Romania; ²Department of Urology, Victor Babes University of Medicine and Pharmacy, Timisoara, Romania

Abstract. Background/Aim: Renal cell carcinoma is strongly vascularized, and formation of new blood vessels is a complex and multi-step process. In this study, we analysed the subtypes of intermediate blood vessels, as shown by double immunohistochemistry. Materials and Methods: Tumour-associated blood vessels were identified by double immunostaining based on CD34 and smooth muscle cell actin. Blood vessels were classified both quantitatively and qualitatively based on the expression of the aforementioned two markers. The main criteria to sub-classify intermediate blood vessels was the presence, distribution, and arrangement of perivascular cells. Results: We described three subtypes of intermediate blood vessels found particularly in the tumour area: Subtype 1 lacked perivascular cells, subtype 2 showed scattered pericytes attached to the vascular wall, and subtype 3 showed a continuous layer of perivascular cells on one side. Conclusion: We describe for the first time three subtypes of renal cell carcinoma-associated intermediate blood vessels, which could be important in prognosis and as potential targets for anti-vascular therapy.

Renal cancer is a significant public health problem due to its increasing incidence, unpredictable nature of metastases, and random response to chemotherapy and/or radiotherapy. Renal cell carcinoma is highly vascularized in both tumour and peri-tumour areas. The blood vessels close to the tumour have been long thought to be pre-existing, dilated blood vessels (BVs), and those found within the tumour are suggested to be induced by the growth factors secreted by neoplastic cells. Many concepts in the field have been changed after the demonstration of the role of angiogenesis in tumour progression and spreading (1). Tumour cells and certain cells of the tumour microenvironment have the ability to secrete growth factors, which induce formation of new BVs, a process that has actually been demonstrated in the majority of solid tumours (2). Tumour growth strongly depends on the presence of BVs and virtually, any type of tumour cannot grow over 2 to 3 mm without vascular supply (3). Newly formed BVs are different from the mature BVs, in terms of the fine structure and molecular profile (4). This is why the tumour-associated BVs have become an attractive target for therapy, and specific inhibitors of endothelial cell (EC) proliferation have already been identified more than 20 years ago (5). Tumour-associated BVs were originally classified as immature, intermediate, and mature, based on the proliferation of ECs and the presence or not of perivascular cells (6). Immature BVs are non-perfused, have high rate of proliferation, and lack pericytes. Mature BVs comprise both non-proliferative ECs and perivascular cells. Intermediate BVs provide the link between immature and mature BVs; are perfused, have a low proliferation index, and usually lack pericytes. This classification of BVs could have an important value not only in prognosis but also in anti-vascular therapy. The maturation of renal cell carcinoma-associated vasculature is a dynamic process, and intermediate vessels are numerous in the tumour area. In the current work, we present different types of intermediate BVs that have not been previously described in the literature.

Materials and Methods

We investigated specimens from 90 cases of renal cancer. Of them, 71 were clear cell renal cell carcinoma, 11 papillary renal cell carcinoma type 1 and 2, 2 chromophobe renal cell carcinomas, 1
collecting duct carcinoma, and 5 sarcomatoid renal cell carcinomas. The pathological diagnosis and grade of differentiation were analysed using haematoxylin-eosin-stained slides. Three μm thick sections were used for immunohistochemistry, performed with the Leica Bond-Max automation system (Leica Biosystem, Newcastle upon Tyne, UK). We used anti-CD34 (clone QBend10) to identify ECs, and anti-smooth muscle cell actin for perivascular cells. The Bond polymer refine detection system (Leica Biosystem) was used as working system. The final product of the reaction was visualized as a brown colour for endothelial cells and as a red colour for perivascular cells. Examination was performed using the Leica Axio Imager A2 microscope (Leica Biosystem), and types of tumour-associated intermediate BVs were assessed in relation to the pathological type and grade.

Results

We identified several types of BVs in all cases included in the study. Immature BVs expressed CD34 only and lacked perivascular cells. They were more numerous in well differentiated clear cell renal cell carcinoma (RCC) and almost absent in high grade tumours. Mature BVs were found in the peri-tumour and tumour areas in high grade RCC. In most of the cases with clear cell RCC the three types of BVs (immature, intermediate, and mature) coexisted in a heterogeneous manner. The difference between immature and mature BVs was based on the presence, number, and distribution of perivascular cells as a continuous or discontinuous outer layer. On this basis, we described three types of intermediate BVs as follows: intermediate subtype vessel 1 (ISV1), as perfused vessels, with strong CD34 staining but without perivascular cells (Figure 1A). ISV2, with discontinuous layer of perivascular cells on only one side (Figure 1B), and ISV3 with a continuous layer for perivascular cells on at least 50% of the contour (Figure 1C).

All these three subtypes of intermediate BVs predominated over large areas in clear cell RCC, and mature vessels were identified particularly in the thick connective tissue of the tumor stroma and peritumoral space, and only rarely in the tumor area. The aspects described above were found mainly in clear cell RCC, characterized by a network of vessels with intermediate predominance. In the cases of clear cell RCC with a loose vascular network the majority of BVs consisted of intermediate subtype 1, completely devoid of perivascular cells. We found no particular relation between these subtypes of intermediate BVs and papillary and chromophobe RCC. In sarcomatoid carcinoma, over 90% of the identified vessels were of the mature type and most likely represent pre-existing vessels of the stroma invaded by the tumour.

Discussion

In the current work, we described three subtypes of RCC-associated intermediate BVs, a sub-classification not previously reported. ISV1 lacked perivascular cells and only endothelium was detected by double immunohistochemistry; ISV2 had only few perivascular cells attached to endothelial cells, and ISV3 displayed a continuous layer of perivascular cells. Most probably, these subtypes reflect some steps in the continuous maturation and stabilization of newly formed BVs. A remarkable heterogeneity has already been noticed in the tumour area of RCC, and usually, all main types may be found even in the same microscopic field. The
distribution and behaviour of the perivascular cells are important not only for the identification of newly formed BVs but also for predicting a potential resistance of RCC to anti-vascular therapy (7-9). After antivascular therapy, most of the BVs become mature and less responsive to this type of therapy (4). According to Nina et al. (10), normalization of perivascular cells by using a vaccine against the DLK1 (delta-like 1 homologue antigen) surface antigen will lead to normalization of the vascular domain (by decreasing vascular permeability and intratumoral hypoxia), which is associated with enhanced immune response and suppression of tumour growth.

According to Chen et al. (11), in renal cell carcinoma treated with tyrosine kinase inhibitors, the structure of BVs and microvessel density are affected, although the tumour size, nuclear grade, and stage of the disease show no significant changes. At the same time, from a pathological point of view, the specimens treated with tyrosine kinase inhibitors showed modified vessels at the tumor level near the necrosis areas. In the experimental model, treatment with the tyrosine kinase inhibitor sunitinib induced major changes in the tumour-associated vasculature, and vasculopathy is thought to be an unrecognized anti-tumour effect (12).

Using double immunohistochemistry we subclassified intermediate vessels, in addition to mature and immature BVs, as: ISV1, pure intermediate vessels devoid of perivascular cells; ISV2, intermediate vessels with the presence of pericytes at one of the walls; and ISV3 with the presence of perivascular cells on an area comprising at least 50% of the vascular contour. Until now, these subtypes of intermediate vessels have not been mentioned in the literature. When taking into account the lack of response to anti-vascular therapy that depends to a large extent on the presence of perivascular cells, we consider this subclassification as having a potential therapeutic impact. No similar data currently exist in the literature. At the present time, it cannot be concluded that the subtypes of intermediate vessels will improve the prognostic relevance of microvessel density or whether they will be an attractive target for therapy together with immature BVs.

Our data suggest that the vessels in the tumor area undergo a rapid process of morphological maturation, which would explain the low number of tumor cells related to each branch, and the failure of efficiency of the anti-vascular therapy in experimental models of solid tumours. The higher the density of the vascular network in the tumor area, the more numerous are the mature vessels (13). The identification of perivascular cells and indirectly of vascular subtypes requires future studies. Perivascular cells can also represent a prognostic marker of survival and response to anti-vascular therapy.

**Conclusion**

We identified three particular subtypes of intermediate BVs (ISV1, ISV2, and ISV3) in the tumor area of RCC with potential implications for the effectiveness of anti-vascular therapy. The distribution of the network of mature and immature intermediate vessels depends on the histopathological type but with relatively large variations especially in clear cell RCC.

**Conflicts of Interest**

The Authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest, or non-financial interest in the subject matter or materials discussed in this manuscript.

**Authors’ Contributions**

AMF and OCF performed surgery, harvested tumor biopsies, designed and performed the study, and wrote the manuscript; MR evaluated tumor specimens by microscopy for histopathologic diagnosis and immunohistochemistry; AMC was involved in data interpretation and supervised manuscript writing.

**Acknowledgements**

The Authors are grateful to Onica Ciprian and Amalia Raluca Ceausu for their excellent technical assistance.

**References**


Received April 11, 2021
Revised April 21, 2021
Accepted April 22, 2021