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Vascular architecture of human urinary bladder carcinoma: a SEM study of corrosion casts

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Abstract The vascular architecture of five advanced invasive papillary tumours of the urinary bladder was investigated using corrosion casting and scanning electron microscopy. The superficial vasculature was composed predominantly of capillary systems of two types: dense flat networks with numerous interconnections and tightly packed tortuous loops, forming multiple irregular folds that reflected the papillary morphology of the tumours. The capillaries were supplied and drained by numerous straight nonanastomosing arterioles and venules, which arose by way of multiple branching of larger vessels originating from the mucosal plexus of the bladder. Differences between the tumours in the spatial arrangement of these vessels probably reflect different growth dynamics. The intramural parts of the tumours contained a chaotic network of straight, uniform capillaries with numerous sprouts, which was very different from the superficial capillary system. It is postulated that different angiogenesis-targeted growth factors may be expressed in the phases of exophytic growth and muscularis invasion of the tumour, leading to the formation of different microvascular patterns.

Key words Bladder cancer · Blood vessels · Angiogenesis · Corrosion casting

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Introduction

The urothelium is constantly exposed to a variety of exogenous and endogenous chemical compounds, including carcinogens. It may respond to such exposure with transformation into hyperplastic, metaplastic or premalignant lesions, which can initiate the process of carcinogenesis [4]. In human pathology, bladder cancer is the most common tumour of the urinary system, and the fourth most common cancer in men [7].

The vast majority of bladder tumours have an epithelial origin, and about 90% are urotheliomas (transitional cell carcinomas, TTC). They are divided according to their growth pattern into papillary (exophytic) and nonpapillary (endophytic, solid, tumour in situ) types. Both can be noninvasive or invasive, although superficial papillary lesions, constituting about 70% of bladder tumours, are less invasive than nonpapillary lesions [4, 13, 16].

Vascular proliferation and neoangiogenesis are prerequisites for tumour growth and progression [10]. Increased vascular density correlates with higher incidence of metastases and poor prognosis in many tumours, including urinary bladder carcinoma [9, 14]. Anti-angiogenic drugs inhibit development of chemically induced bladder tumours in animal models [27], and these observations stress the need for further studies on blood vessels.

The most common approach to the assessment of tumour vasculature and angiogenesis has been light microscopic examination of tumour sections in which the blood vessels are either stained routinely or highlighted by immunocytochemical techniques [11]. Studies aimed at visualization of the entire complex architecture of tumour vessels are few. In human urinary bladder cancer, only one paper has described the vascular system of papillary tumours, and a relatively old-fashioned technique, microangiography, had been used in the study reported [26].

The method of vascular corrosion casting followed by scanning electron microscopy is especially well suited to detailed morphological analysis of both normal and pathologically altered vascular systems, since it offers quasi-three-dimensional images of relatively high resolution, in

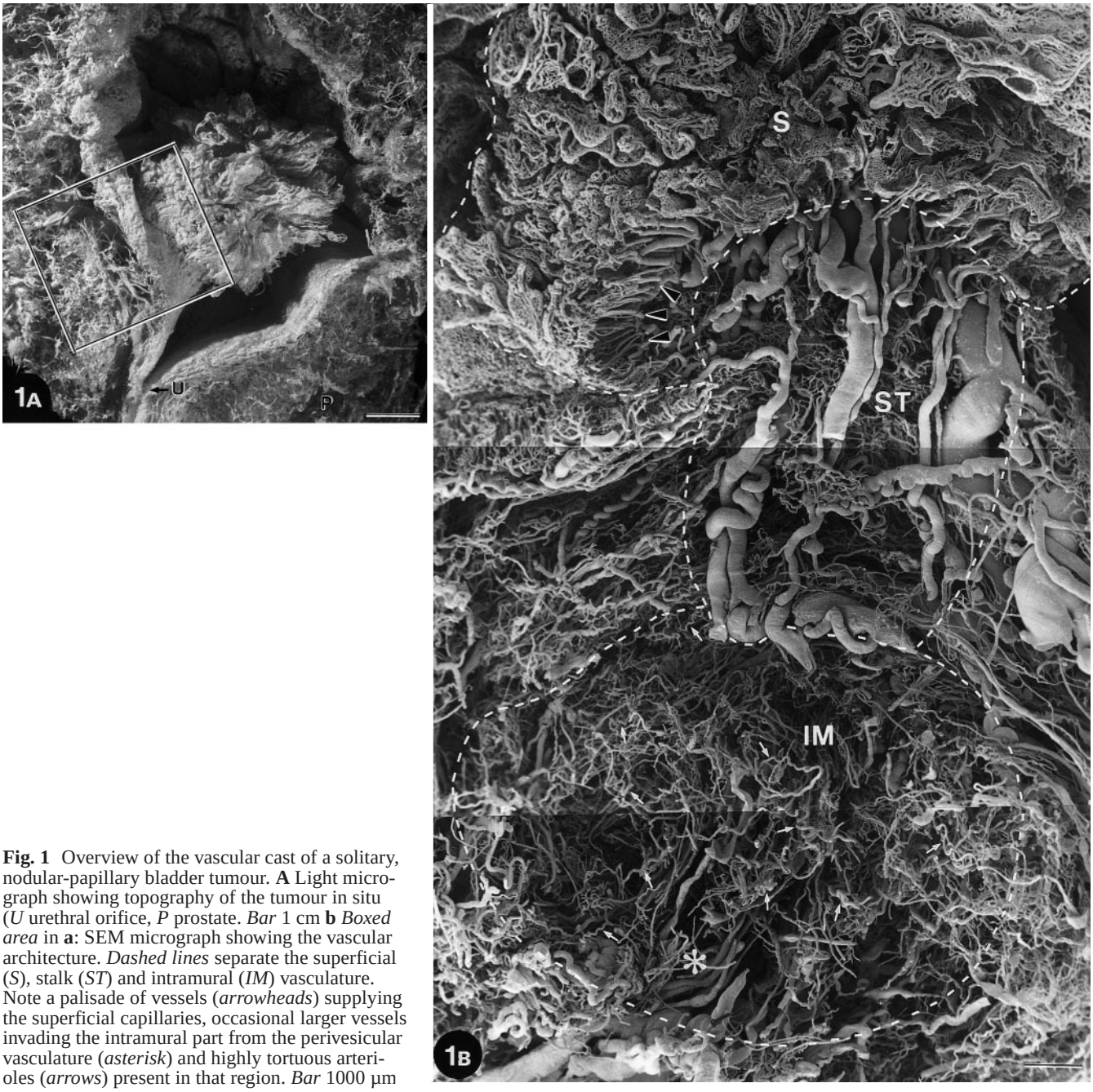


Fig. 1 Overview of the vascular cast of a solitary, nodular-papillary bladder tumour. **A** Light micrograph showing topography of the tumour in situ (*U* urethral orifice, *P* prostate. *Bar* 1 cm **b** *Boxed area* in **a**: SEM micrograph showing the vascular architecture. *Dashed lines* separate the superficial (*S*), stalk (*ST*) and intramural (*IM*) vasculature. Note a palisade of vessels (*arrowheads*) supplying the superficial capillaries, occasional larger vessels invading the intramural part from the perivesicular vasculature (*asterisk*) and highly tortuous arterioles (*arrows*) present in that region. *Bar* 1000 μm

which arteries, veins and capillaries and also their spatial distribution can be discerned with high accuracy [19].

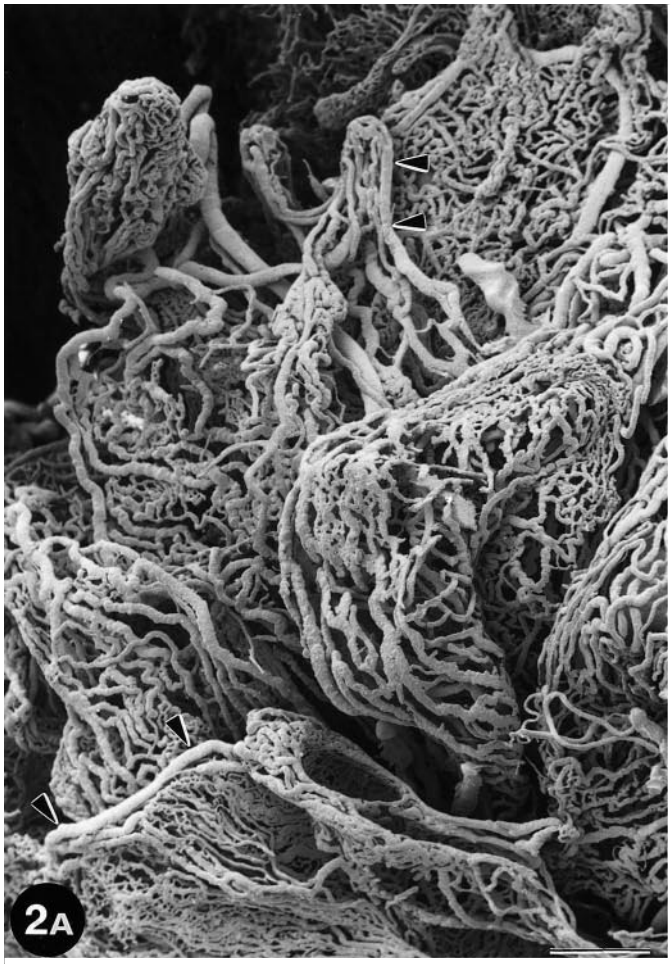
The aim of the present study was to investigate the vascular architecture of human urinary bladder cancer by means of the corrosion casting technique.

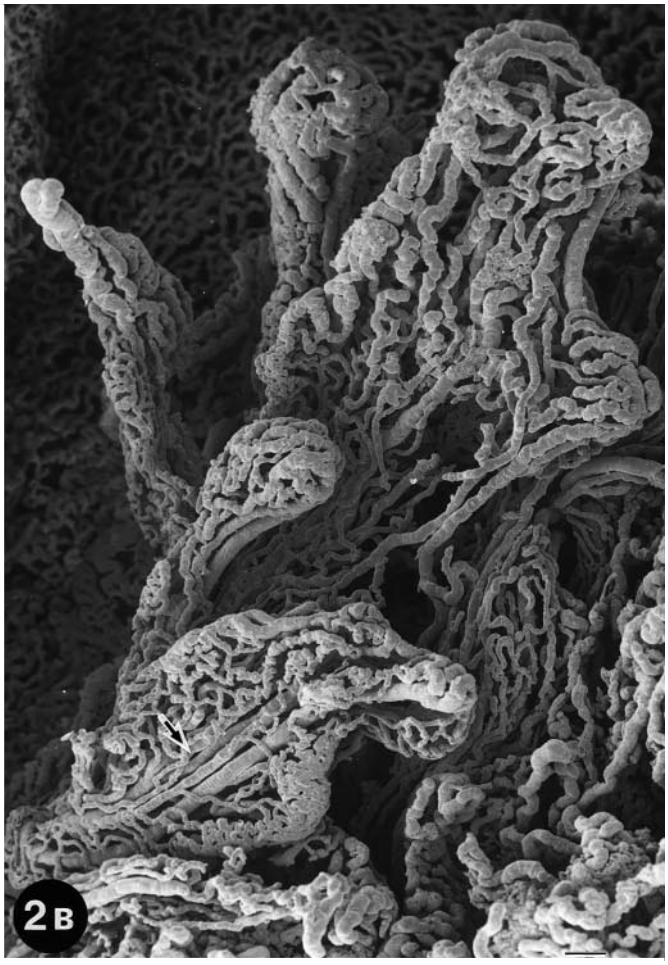
Materials and methods

Five urinary bladders containing advanced tumours that had been removed surgically (total cysto-prostato-vesiculotomy) from male patients were obtained from the Department of Urology, Jagiellonian University School of Medicine, Kraków, Poland. Two solitary nodular-papillary and three multiple papillary tumours were assessed according to the TNM classification (4th edition, 1992) as T3a–T3bN0M0 and confirmed histopathologically (by preoperative biopsies and examination of small fragments of tumours collected prior to the corrosion casting procedure) as urothelial carcinoma (TCC G1, 1 case; G2, 3 cases; G3, 1 case).

Following surgery, the bladders were immediately transferred to prewarmed (37°C), heparinized saline (12.5 IU/ml). At least four arterial vessels of each bladder, branches of the anterior trunk of the internal iliac artery, were cannulated and perfused with approximately 800 ml of the same saline solution additionally containing 3% Dextran, mol. wt. 70 kDa. The catheter introduced into the bladder prior to surgery was retained and used for gentle washing of the lumen with the saline and for its final filling (80 ml) to keep the bladder slightly distended.

After about 15 min, the bladder was fixed by perfusion with 300 ml of 0.66% paraformaldehyde/0.08% glutaraldehyde in





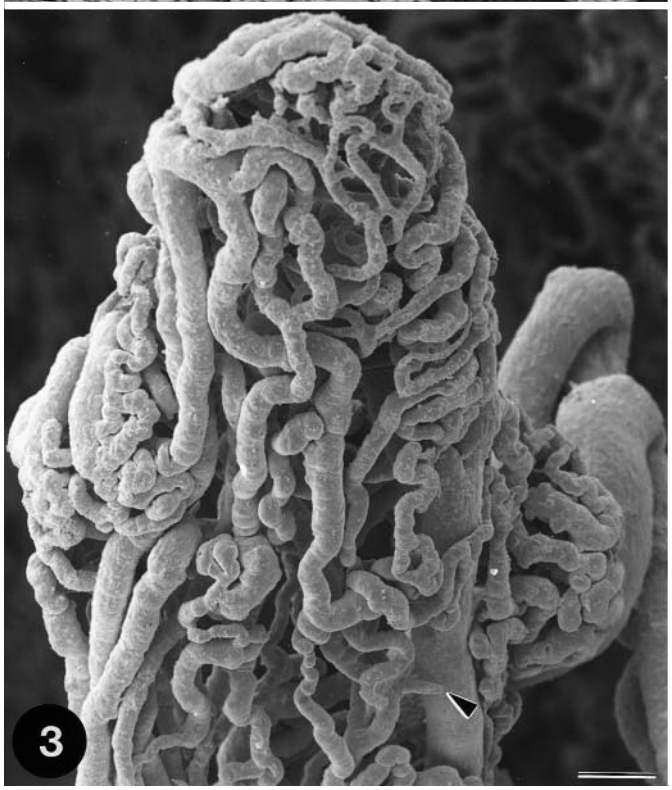


Fig. 2A, B Folds formed by the superficial vasculature of the tumour. **a** Flat folds composed of extremely dense meshworks of capillaries with numerous interconnections. Draining venules often run along the margins of folds (arrowheads). *Bar* 500 µm **b** Flat and finger-like folds with tortuous capillary loops. In one fold arteriole and venule are located along its central axis (*arrow*). *Bar* 100 µm

Fig. 3 Typical capillaries of the superficial plexus, showing uneven contours with constrictions and dilatations. Blindly ending sprouts are only rarely seen (*arrowhead*). *Bar* 100 µm

0.1 M cacodylate buffer, pH 7.3, supplemented with 0.5% lignocaine [24]. Then the vascular system was injected with 160–180 ml of Mercox CL-2R/2B resin (Vilene, Tokyo, Japan) containing 0.0625 mg/ml MA initiator and left in a warm water-bath (56°C) for several hours to allow polymerization and tempering of the resin. When the polymerization was completed, the bladder was macerated by repeated baths in 10% sodium hydroxide at 37°C followed by washing with warm (56°C) running tap-water for 5–6 days [2].

The vascular casts of whole bladders obtained were washed for the next 4–5 days in multiple changes of distilled water under mild vacuum, cleaned in 3% formic acid for 1–2 days, washed again in distilled water for 2–3 days and freeze-dried. They were then embedded in molten water-soluble Aquax wax (Gurr, U.K.) at 56°C and, after its solidification at room temperature, cut along the mid-sagittal plane to expose the vascular casts of the luminal bladder surface and of the tumours. After a thorough wash in multiple baths of distilled water changed frequently to remove the wax, the casts were again freeze-dried and photographed in an Olympus SMZ stereomicroscope. Finally, they were mounted on copper plates using colloidal silver and "conductive bridges" [18] and coated with gold. Owing to the large size of the casts and copper plate mounts, specimen holders especially made in our laboratory were used, to which the plates were glued with colloidal silver. The casts were examined in JEOL SEM 35-CF scanning electron microscope at 20–25 kV.

After the microscopic examination and photographic documentation, casts of tumours were sectioned longitudinally into halves with microsurgical scissors to unveil the internal vasculature and were then examined again.

Results

Since all the tumours investigated invaded the bladder wall, three distinct regions could be distinguished: a superficial vascular network covering the tumour protruding into the lumen of the bladder, a vascular "stalk" composed of larger vessels originating at the level of mucosal lamina propria and supplying the superficial network, and an intramural vasculature occupying the part of the tumour invading the muscularis (Fig. 1A, B).

The papillary and/or nodular morphology of the tumours was reflected by the presence of distinct superficial vascular plexuses having the form of branched flat or curved fold- or finger-like protrusions of the vascular network. Smaller folds were composed entirely of capillary meshworks, while larger units were mostly supplied by a single central arteriole branching off into the capillary network, which was drained by venules located either centrally or along the margin of the plexus (Fig. 2A, B).

Two types of capillary plexuses could be distinguished in the superficial vasculature. Flat folds usually showed the presence of an extremely dense network of capillaries, with very fine meshes and short distances between capillary interconnections (Fig. 2a). Finger-like folds contained highly tortuous capillaries forming tightly packed loops with fewer interconnections (Fig. 2b). The capillaries most frequently observed in the tumours had a relatively large diameter (10–20 μm) and uneven contours with local dilatations and constrictions (Fig. 3). Blindly ending sprouts were observed occasionally, but they were few in number.

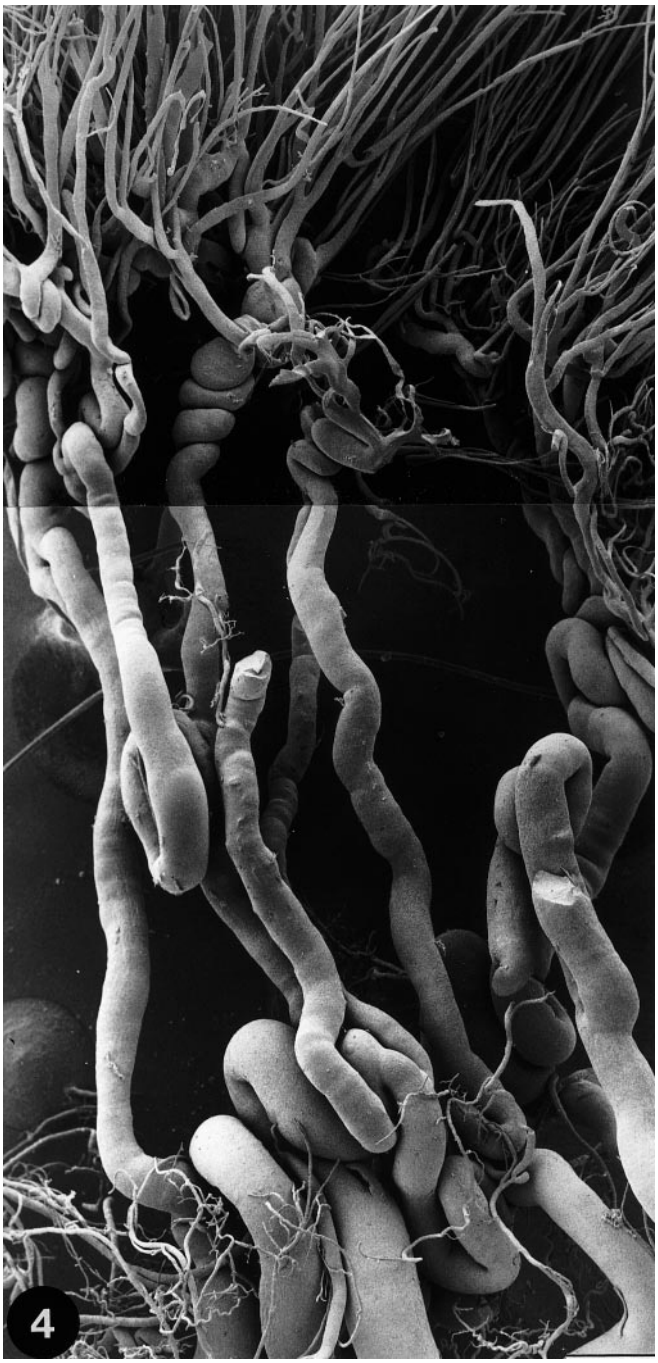


Fig. 4 The stalk region of a papillary tumour. Large vessels with coiled segments at their distal ends extensively branch into a dense array of arterioles and venules showing "osier bed" appearance. *Bar* 500 μm

The two types of tumour differed in the architecture of their vessels located in the stalk region, supplying and draining the superficial vasculature. In the solitary, nodular-papillary tumours, large, tortuous, sometimes coiled vessels located in the stalk region branched into a palisade of relatively short, straight and nonanastomosing arterioles and venules, which supplied and drained, respectively, the superficial plexuses (Figs. 1b, 7). In the multi-

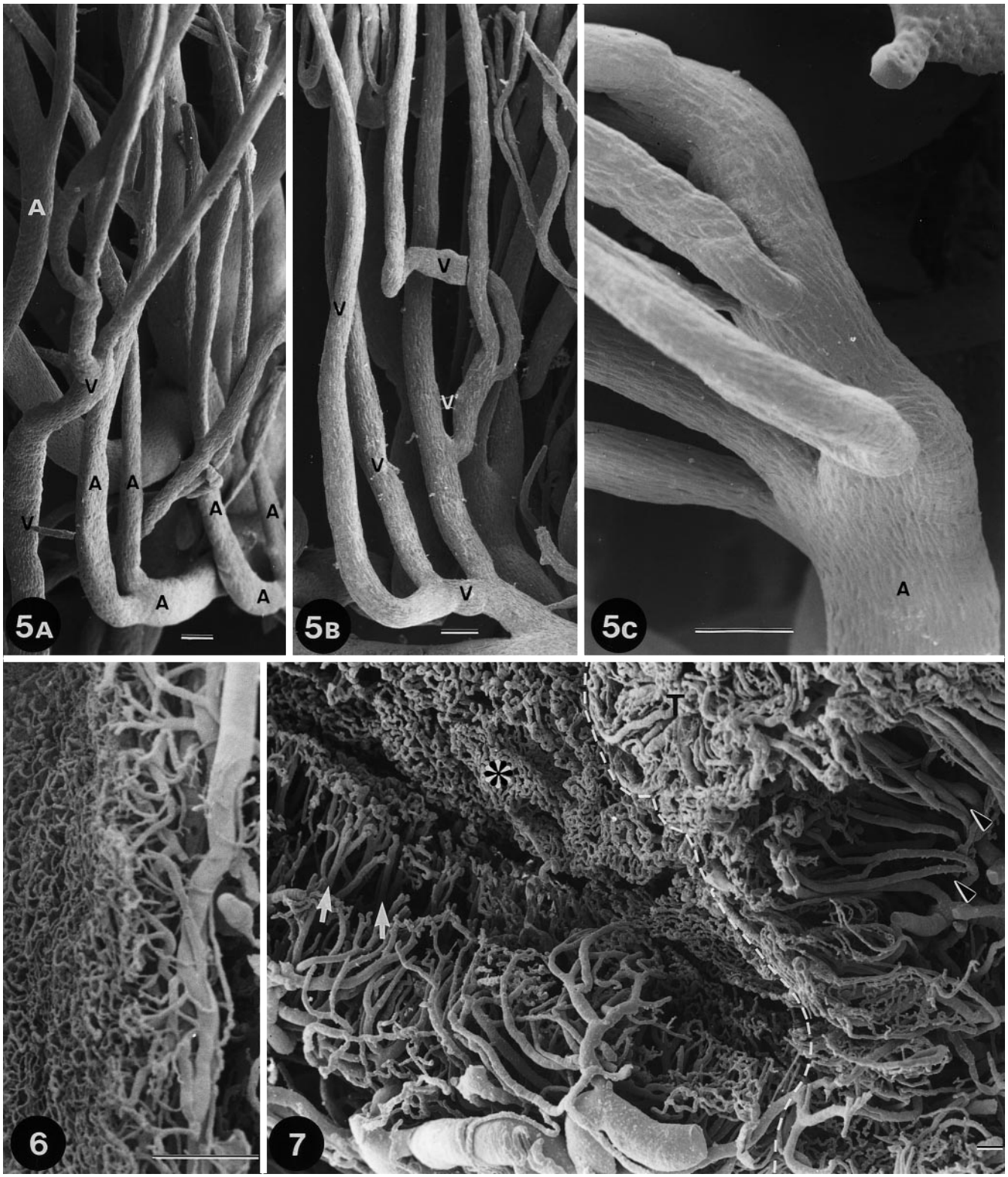


Fig. 5A, B, C Examples of vessel branching at the base of the "osier bed" region in papillary tumours (*A* artery, *V* vein). *Bars* 100 µm

Fig. 6 Vascular architecture of the unchanged region of the urinary bladder mucosa. Note relatively smooth surface of the subepithelial capillary network and irregular mucosal plexus. *Bar* 500 µm

Fig. 7 Vascular architecture of mucosa adjacent to the tumour. *Dashed line* separates the vasculature of the tumour (*T*). Note uneven surface of the subepithelial capillary network, with elevations and depressions (*asterisk*) and palisade-like array of its supplying and draining vessels (*arrows*) similar to that present in the tumour (*arrowheads*). *Bar* 100 µm

ple, papillary tumours the larger vessels formed two distinct zones. The proximal zone consisted of long, contorted, roughly parallel arteries and veins possessing short coiled segments at their distal ends. Immediately above the coils, the vessels branched extensively at about the same level, to form a conspicuous, dense array of long, parallel, nonanastomosing arterioles and venules that were nearly identical in diameter (about 50–60 µm) and had hardly any branches (Fig. 4). This array, located inside the protruding part of the tumour and similar in appearance to an osier bed, was completely devoid of any capillary networks, which were formed only at its distal margin as superficial plexuses. Tri- tetra-, penta- or multifurcations of single blood vessels were often observed in case of both arteries and veins (Fig. 5a–c).

The vascular bed of the intramural part of the tumour (Fig. 1b) was composed almost exclusively of a dense, chaotic network of thin capillaries, which were quite different from those present in the superficial plexuses in that they mostly had a straight course and uniform diameters. Larger vessels, originating mainly from the perivesicular vasculature, were very rare and short, branching into infrequent arterioles and venules that mostly had a highly tortuous course. In contrast to the protruding part of the tumour, the intramural part was characterized by numerous capillary sprouts.

Comparison with the vascular architecture of apparently normal mucosa far distant from the tumour (Fig. 6) disclosed some modifications of the mucosal blood vessels in the immediate vicinity of the tumour base (Fig. 7). The superficial, subepithelial capillary network was still regular but had an uneven surface with elevations and depressions. The most striking difference concerned the arterioles and venules supplying and draining the capillary bed; instead of a plexiform arrangement, as observed in normal mucosa, they were elongated and formed a palisade-like array similar to that observed in the tumour.

Discussion

The patterns of superficial capillary organization observed in this study were very similar to the type 1 (very dense network of capillaries with numerous short terminal branches) and type 3 (loops formed by tortuous, dilated capillaries) vascular foci described in chemically induced rat bladder tumours, which have also been examined by corrosion casting and scanning electron microscopy [8, 28]. This may indicate a common mechanism of angiogenesis in experimentally induced and spontaneously arising bladder tumours. The authors [8, 28] have postulated an association of type 1 foci with inflammatory and necrotic processes, and of type 3 with epithelial proliferation.

The large diameter of the superficial capillaries of the bladder tumours and their irregular contours, with constrictions and dilatations, seem to be features common to many tumours [15]; they were also observed in primary human tumours studied in our laboratory [6, 17, 20]. Aharinejad and Böck [1] have suggested that such features are characteristic of fenestrated capillaries and may serve for their identification. Fenestrated capillaries were indeed found in type 3 plexuses of rat bladder tumours [29]. As demonstrated recently, new vessels induced by vascular endothelial growth factor (VEGF) are fenestrated [25], and its receptors show considerably inreased expression in human bladder tumours [5].

Angiogenesis-targeted growth factors may also be responsible for the striking difference between the capillary networks of the protruding and the intramural parts of the tumours. In contrast to well-differentiated plexuses in the superficial vasculature, capillaries in the intramural part had a chaotic arrangement; moreover, they were uniform and straight, corresponding to the continuous (nonfenestrated) type [1]. According to O'Brien et al. [22], two distinct angiogenic pathways can be distinguished in human urinary bladder neoplasia: the superficial tumours have elevated levels of VEGF, while the invasive tumours show very high expression of PDECGF (platelet-derived endothelial cell growth factor), which is correlated with stage progression [21]. The angiogenetic process in the invasive tumours may also be co-stimulated by basic fibroblast growth factor (bFGF) released from the stromal tissue following degradation of the extracellular matrix [23].

It seems therefore that in the advanced T3 tumours examined, the different capillary patterns reflect the history of the tumours: they first develop as exophytic papillary outgrowths with neoangiogenesis stimulated by VEGF, but with time they invade mucosa and muscularis [3, 13]. The invasion may be associated with a VEGF/PDECGF–bFGF switch, resulting in different capillary architecture.

The much larger number of blind sprouts in intramural than in superficial capillaries may also indicate different predominant angiogenic pathways in the two regions of the advanced tumour: sprouting in the former and elongation of tortuous capillary loops in the latter. Moreover, the intramural tumour vasculature contained only a few larger vessels, suggesting that it developed mainly by way of incorporation and remodelling of the preexisting capillary network of the muscularis [30].

Although at first sight the vascular architecture of the stalk region in multiple papillary tumours seems to be different from that in the solitary nodular-papillary tumours with more irregular large vessels and palisaded arterioles and venules, a careful comparison of the two arrays reveals some similarity: a palisade of vessels directly supplying and draining the superficial vasculature of the solitary tumours may be regarded as a variant of the "osier bed" area of the multiple tumours, although in the former type the vessels are shorter and the levels of their origin not so clearcut.

The occurrence of long, straight, nonanastomosing vessels indicates their rapid, unidirectional growth together with the surrounding tissue, while extensively coiled vessels suggest growth within a limited space, under physical constraints created by the surrounding tissue [26, 31]. We conclude that the development of multiple papillary tumours includes at least two phases of rapid exophytic growth (reflected by the relatively straight seg-

ments of large vessels and by the osier bed pattern of arterioles and venules), possibly separated by a phase of growth that has left its vascular manifestations in the form of terminal coils of large vessels preceding the zone of extensive branching. According to this concept, the vascular arrangement of the solitary nodular-papillary tumours displays a growth pattern that is more uniform in all directions – as a result the large vessels are more tortuous, the phase of branching is more diffuse and the extensive development of multiple superficial folds with their capillary networks at the periphery of the protruding part of the tumour limits the length of the arteriolar and venular branches directly supplying the folds.

Palisade-type vessels supplying and draining the subepithelial capillary network were also observed in our material in the mucosa adjacent to tumours. Together with the uneven surface of that area, the vascular array indicated local hypertrophy of the mucosa in the direct vicinity of the tumour base. Such hypertrophic areas were demonstrated around papillary tumours; they may indicate the origin of more dysplastic, invasive lesions of the tumour in situ type [4, 16]. The appearance of a palisade pattern of arterioles and venules might perhaps be one of the first signs of the angiogenic switch postulated at the transition between hyperplasia and tumourigenesis [12].

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