

SEMINAR**Current Concepts of Tumor-Induced Angiogenesis**

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Tumor induced angiogenesis is responsible for the nutrition of the growing tumor and can also increase the probability of hematogenous tumor dissemination. Data obtained from morphological analysis of tumor angiogenesis can contribute to the development of new anti-angiogenic therapies. Based on in vitro and in vivo observations several models of angiogenesis were introduced, explaining the mechanism of lumen formation and the timing of basement membrane deposition. (1) Lumen is formed either by cell body curving or by fusion of intracellular vacuoles of nonpolarized endothelial cells. New basement membrane is deposited after lumen formation. (2) Slit-like lumen is immediately formed by migrating polarized endothelial cells. Basement membrane is continuously deposited during endothelial cell migration, only cellular processes of the endothelial cell migrating on the tip of the growing capillary are free of deposited basement membrane material. (3) Development of transluminal bridges in larger vessels – a process called intussusceptive growth –

Key words: tumor, metastasis, angiogenesis, morphology

leads to the division of the vessels. These models, however, describe angiogenesis in tissues rich in connective tissue. Different processes of angiogenesis take place in organs – such as liver, lungs, adrenals, which are the most frequent sites of metastasis – having high vessel density without sufficient space for capillary sprouting. In the case of liver metastases of Lewis lung carcinoma the proliferation of endothelial cells was elicited only by direct contact between tumor and endothelial cells, leading to the development of large convoluted vessels inside the metastases. These vessels were continuous with the sinusoidal system, suggesting that these metastases have dual blood supply. This observation, among others, is in contrast to the generally accepted view that liver tumors have arterial blood supply. The increasing number of data demonstrating the dual or venous blood supply of liver metastases should be taken into consideration in the therapy of liver metastasis. (Pathology Oncology Research Vol 4, No 1, 62–75, 1998)

Introduction

Two, morphologically different processes take part in the development of new vessels. During angiogenesis new vessels arise from preexisting ones, contrary to vasculogenesis, which occurs at early embryonic development, when vessels are organized from primordial endothelial cells.^{1,3}

Tumor induced angiogenesis has two effects on malignancy. On the one hand the developing vasculature feeds

the tumor, on the other hand it increases the probability of tumor dissemination via the vascular system. Although tumors generally lack lymphatic vessels and lymphangiogenesis has not been observed during tumor progression,⁴ recent results have shown that VEGF-C transgenic mice develop a hyperplastic lymphatic capillary system in the skin, raising the possibility of lymphangiogenesis also in the case of tumors.⁵

It is generally accepted that tumors cannot grow beyond the size of 2 mm in diameter without eliciting angiogenic response. In case of tumor cells lodged in distant organs, the temporary lack of the angiogenesis inducing ability, can lead to the development of dormant metastases.^{1,2}

There are ample data showing inverse correlation between tumor vascularity and patient survival.⁶ Opposite

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results suggest, however, that simple vessel counting (determination of vessel number in places of highest vessel density, so called hot spots) does not represent the more complex relationship between tumor vascularity and metastasis.⁷ Instead, the detection of disseminated tumor cells at the invasion front, or determination of blood or lymph vessel invasion appear to be parameters that – although more difficult to determine – are more closely related to the metastatic process.^{8,9}

Tumor vasculature has two important further effects on the therapy of tumors. It can itself be a target for therapy (anti-angiogenic therapy), and it is responsible for delivering therapeutic agents to tumor cells.^{10,11} Morphological analysis of *in vivo* angiogenesis as well as determination of the spatial distribution of cell-cell, cell-matrix adhesion molecules, extracellular matrix components, matrix remodelling enzymes and angiogenesis factors during the process can lead to development of new anti-angiogenic strategies.

Tumor angiogenesis is initiated by disturbing the balance between angiogenesis activators and inhibitors, already in the stage of dysplasia or *in situ* carcinoma before the progression to invasive tumor stage.¹² Positive mediators of this process are the angiogenic factors produced directly by tumor cells, or by host cells recruited to the peritumoral space. Numerous polypeptide and small molecular weight angiogenic factors has been identified, recently reviewed by Bouck et al.¹¹ Similarly, extracellular matrix molecules and their degradation products has been shown to induce angiogenesis alone, or in combination with the above factors.¹³

Angiogenesis *in vitro*

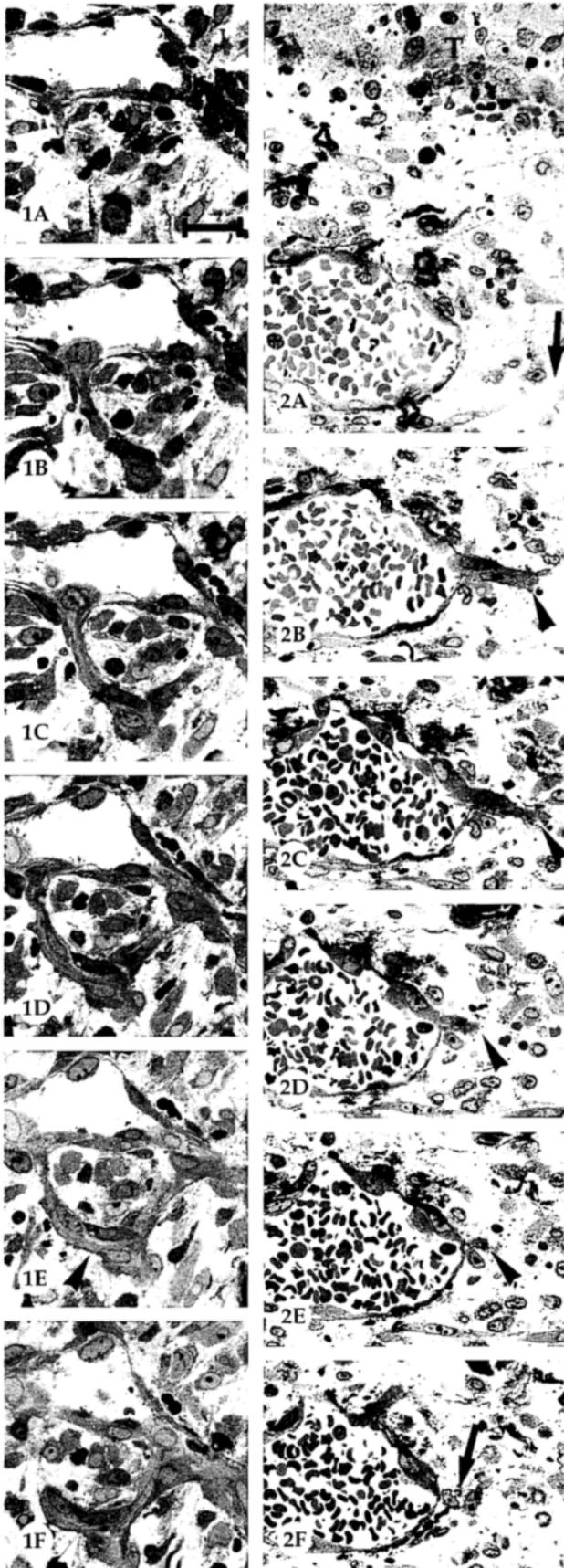
The effect of angiogenesis factors and extracellular matrix components on endothelial cell behaviour has been extensively studied using *in vitro* cultured endothelial cells of different origin. Endothelial cells can undergo a process called *in vitro* angiogenesis cultured on different matrices. Tubular structures consisting of several endothelial cells were found to develop on surfaces coated with gelatin or fibronectin in overconfluent cultures.¹⁴⁻¹⁶ Similar structures were seen to arise rapidly, after seeding endothelial cells on a gel consisting of basement membrane components (Matrigel).^{17,18} Although the cords on the surface of the extracellular matrix were formed by numerous elongated endothelial cells, and in cross section the cells enclosed a primitive lumen sealed by intercellular junctions,¹⁴⁻¹⁸ the lumen often contained extracellular matrix material or cellular debris and the basal-apical polarization of the endothelial cells was not demonstrated.^{14,17} Tube formation proved not to be an exclusive characteristic of endothelial cells, because many other cell types were able to form cords and networks on basement membrane matrix

gel.¹⁹ With decreasing malleability of the gel the cord formation diminished, suggesting that cord formation is more dependent on the mechanical properties of the matrix than on the cell type.¹⁹ This manner of endothelial cell organization is more resemblant to the process of vasculogenesis, since angiogenesis is characterized by migration of endothelial cells into the matrix (a process called sprouting) from a polarized layer of endothelial cells. To examine angiogenesis *in vitro*, it is more relevant to study the *in vitro* model systems using endothelial cells cultured to confluency on the top of collagen I gel or human amniotic membrane or aortic rings explanted into fibrin gel.²⁰⁻²⁴ In the former case the treatment of such cultures with FGF or PMA resulted in invasion of the gel, or the basement membrane by the endothelial cells which process depended on the production of collagenase IV and plasminogen activator.²⁰⁻²² Tube-like structures appeared in the collagen matrix, which showed a lumen delineated by endothelial cells, connected by intercellular junctions.^{20,21} More importantly, polarized deposition of the basement membrane was observed in these structures.²⁰ Similar polarized deposition of fibronectin, laminin, and collagen IV was observed around the developing capillaries in the case of aortic rings explanted into fibrin gel.²⁴

Angiogenesis *in vivo*

Angiogenesis in primary tumors

Fibrin and collagen I are the components of the provisional extracellular matrix during tumor development and wound healing *in vivo*.²⁵ Fibrin gel was shown to induce angiogenesis when implanted subcutaneously.²⁶ The development of the fibrin containing stroma in and around the tumor is controversial. Numerous ultrastructural studies on the angiogenic vasculature of tumors and healing wounds have suggested that leakiness of the vessels is caused by open interendothelial junctions.²⁷⁻³⁰ It is well known that tumor vessels usually show abnormal structure, such as fragmented or ultrastructurally not detectable basement membrane and the absence of pericytes.^{31,32} The lumen of the tumor vessels is often covered by extremely thin fragile endothelial cells showing fenestrations, or vascular spaces are present delineated by tumor cells. It is not clear, however, whether these alterations are characteristic to the angiogenic process or are caused by the invasive activities of the tumor cells. The possibility that tumor invasive activities play role in the enhanced leakiness of tumor vessels is supported by the observation – made on corrosion casting specimens of colon tumors – that resin leakage was observed only in carcinomas and not in adenomas.³³ Examination of the angiogenic vasculature under normal condition demonstrated that angiogenic vessels are not permeable for molecules larger than



20 kD.³⁴ Other ultrastructural reports have also shown the absence of open interendothelial junctions during tumor angiogenesis, although tumor vasculature showed leakiness for larger 70-150 kD molecules.³⁵ The leakiness of tumor vessels, as suggested by Dvorak's group, is mediated by vesiculo-vacuolar organelles (VVO) present in endothelial cells of the neovasculature induced by VEGF.³⁶ However, recent results revealed that the endothelial cells in the vasculature of VEGF transfected tumor are fenestrated and contain open interendothelial junctions.³⁷

The developing tumor matrix also contains other extracellular matrix proteins, such as fibronectin, vitronectin and thrombospondin. The former two proteins have a positive effect on tumor angiogenesis by inducing chemotaxis of endothelial cells. The role of thrombospondin is more complicated, since its ability to induce angiogenesis indirectly *in vitro* as well as elevated mRNA level and deposition around breast carcinomas^{41,42} seems to contradict its pronounced ability to inhibit angiogenesis *in vitro* and *in vivo*.¹¹

The morphology of *in vivo* angiogenesis has been studied most frequently in subcutaneous tissue, rabbit cornea and chick chorioallantoic membrane, in normal and pathological conditions such as inflammation, wound healing and tumor development.^{3,28-31,43-45}

The main pitfall in studying angiogenesis is the misidentification of vessel structures as tips or distal parts of growing capillaries. To avoid this, serial sectioning must be performed. The situation is less complicated in case of avascular tissues where the growing new vessels are not mingled with preexisting or older vessels, but serial sectioning cannot be avoided when examining, for example, tumor angiogenesis in subcutaneous tissue. As shown in *Figure 1A-F*, sections of a small capillary loop can easily be misidentified as a tip. In contrast, a real tip as shown on *Figure 2A-F* is only discernible in serial sections.

According to ultrastructural studies, the most commonly used description of the angiogenic process (which is considered to be valid also in case of tumor induced angiogenesis) can be summarized as follows (*Figure 1A,B*): *a*. dilatation of postcapillary venules situated around the tumor; *b*. local degradation of the basement membrane on the side of the vessel located more closely to the angiogenic stimulus; *c*. weakened intercellular contacts between

Figure 1. A-F. Serial sections of a short loop of a capillary (arrowheads). This loop does not show an opened lumen and returns to the original vessel. X 280.

Figure 2. A-F. Serial sections of a short newly formed capillary (arrowheads). Arrows in *Figure 2A* and *2F* points to regions, which show that the capillary does not continue into the connective tissue. X 280.

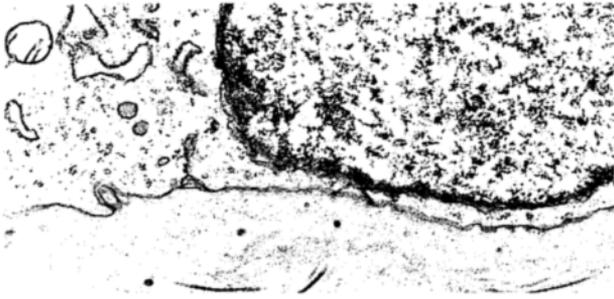


Figure 3. Part of a dilated venule near the tumor. Beneath the endothelial cell there is no basement membrane. X 9000.



Figure 4. Similar vessels as shown on Figure 3. Basement membrane material reacting positively for laminin can be detected beneath the endothelial cell. X 9000

endothelial cells and the start of emigration into the connective tissue, toward the angiogenic stimulus; *d*. the formation of a solid cord by endothelial cells following each other arranged in bipolar fashion, with mitotic endothelial cells observed in the middle of the sprout; *e*. formation of the lumen, occurring either by fusion of intracellular vacuoles, or by cell body curving of a single endothelial cell; *f*. loop formation by fusion of different sprouts; *g*. appearance of pericytes along the sprout and the synthesis of a new basement membrane.

The main disadvantage of this model is its inability to identify the nature and origin of the stimulus necessary for lumen formation. It also assumes that dedifferentiation and redifferentiation take place during the process, manifest in the loss and regaining of luminal-basal polarity. A large number of publications dealing with the effect of Matrigel on the behavior of endothelial cells suggest that stimulus necessary for lumen formation derives from the developing basement membrane.^{17,18} According to this model the importance of the basement membrane in the

process of lumen formation is rather questionable, because basement membrane deposition occurs after lumen formation, which presumes the existence of basal-luminal polarity. If basement membrane synthesis occurs before lumen formation it must proceed around the whole circumference of a nonpolarized cell, excluding that lumen formation occurs later by cell body curving. Lumen formation by fusion of intracellular vacuoles allows basement membrane deposition around the cell, but to increase lumen size in such capillaries the cells must undergo transversal division, implicating a change in cell polarity. Seamless type endothelial cells have very rarely been found *in vivo*, and it has been suggested that they are present only where growing capillaries meet preexisting vessels during loop formation.⁴⁶

A different model was suggested based on data obtained from ultrastructural examination of angiogenesis, using a rat nonmetastatic pancreatic adenocarcinoma cell line growing subcutaneously (*Figure 14C*).⁴⁷ The first step in this type of angiogenesis was the alteration of the base-

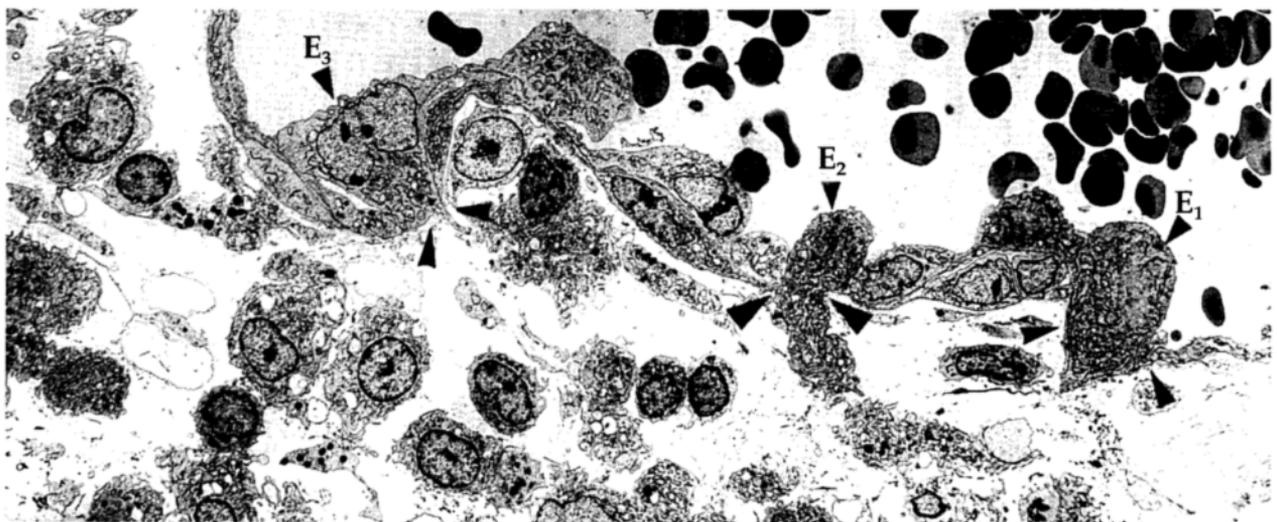


Figure 5A. In the wall of the extended venule three endothelial cells (E1, E2, E3) are seen in different stages of emigration into the connective tissue (arrowheads). X 1400.

ment membrane over the entire circumference of venules, characterized by loss of electron density (Figure 3), but basement membrane components (laminin, collagen IV) could be detected by immunoelectronmicroscopy (Figure 4). Interestingly, fibronectin was also detectable in these basement membranes. This so called gel-sol transition of the basement membrane, probably mediated by matrix metalloproteinases or plasminogen activator, can be partly responsible for the initiation of cell division and migration (Figure 5A,B).⁴⁸ Conversely migrating endothelial cells express elevated level of uPA.⁴⁹ It has been shown that gelatinase A can be activated by membrane type metalloproteinase and also by uPA/plasmin,^{48,50} and bFGF, VEGF and TNF can increase the expression of uPA and its receptor.⁵¹ Partial and regulated proteolysis of the base-

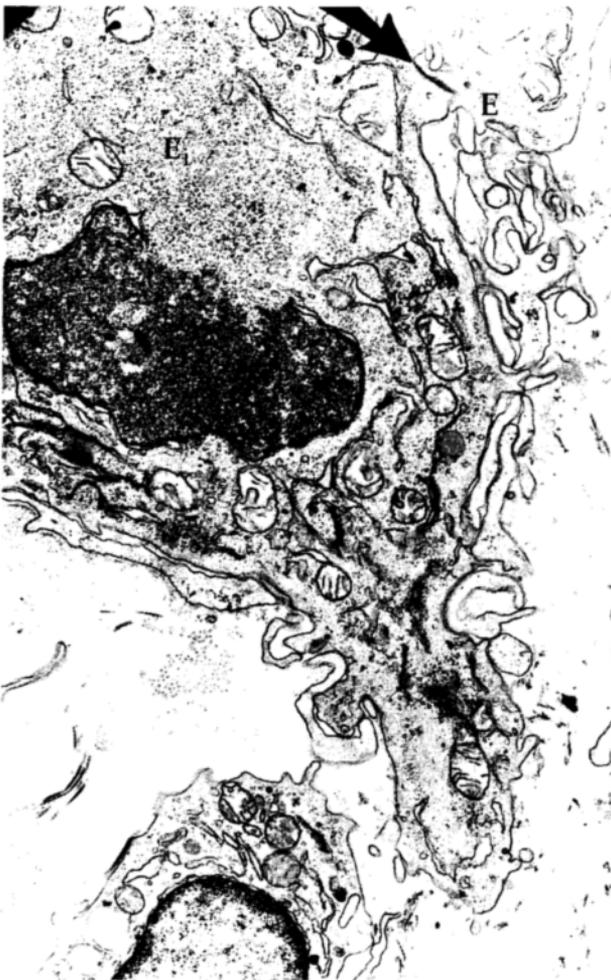


Figure 5B. Detail of the region shown in Figure 5A at another plane of sectioning. The emigrating endothelial cell (E1) is dividing, the nucleus is reconstituted. The endothelial cell is in connection with another endothelial cell (E) via interendothelial junction (arrow). Around the leading process of the endothelial cell and beneath the other endothelial cell (E) no basement membrane can be observed. X 6900.

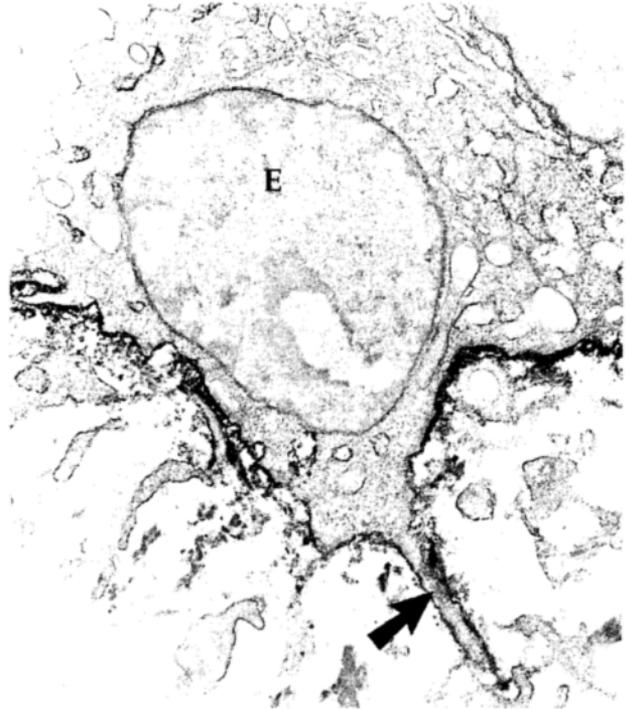


Figure 6. The process (arrow) of the endothelial cell (E) projecting into the connective tissue is free of deposited basement membrane material. Other parts of the subendothelium stains positively for laminin. X 9000.

ment membrane can lead to exposition of different domains of laminin, for example SIKVAV, which has been shown to induce endothelial cell proliferation and angiogenesis, and has also been able to enhance the production



Figure 7. Cross section of a newly formed capillary. The slit-like lumen (arrow heads) is sealed by intercellular junctions (arrows). Basement membrane cannot be identified around the endothelial cells. X 11000.

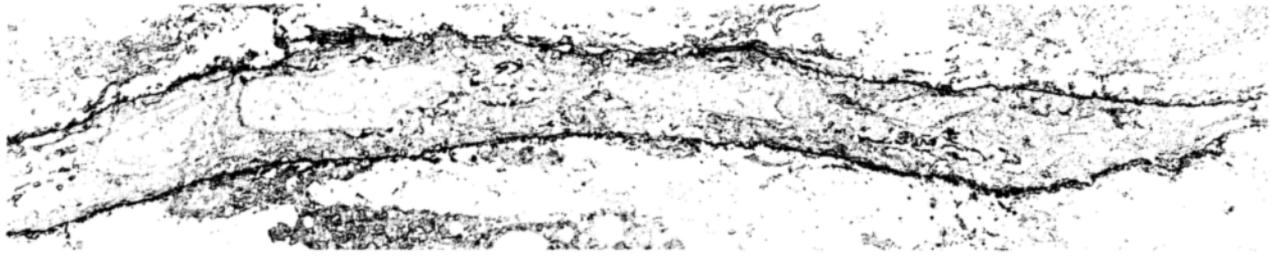


Figure 8. Longitudinal sections of a long newly formed capillary. Dilated lumen and pericytes cannot be observed. Basement membrane material staining positively for laminin is deposited around the capillary. X 2400

of proteases.⁵² bFGF can also be deliberated from the endothelial basement membrane during this process.⁵³ The regulated manner of basement membrane degradation is further supported by the observation that small vessels express high levels of TIMP-1 mRNA.⁵⁴ Loosening of intercellular contacts was not observed, suggesting that loss of contact inhibition is not responsible for initiation of cell division and migration (*Figure 5B*). Immunoelectron-microscopy showed complete degradation of basement membrane only at places where cellular processes were projecting into the connective tissue (*Figure 6*). During further migration, endothelial cells were arranged in parallel maintaining their polarity (basal-luminal), consequently a slit-like lumen was immediately formed between the endothelial cells (*Figs. 7-9*). This lumen was continuous with the lumen of the original vessel and was sealed by intact interendothelial junctions (*Figure 7*). This type of premature capillaries reached several hundred microns

in length (*Figure 8*). Similar structures containing slit-like lumen were observed by others suggesting that this type of angiogenesis also occurs in normal and other pathological conditions.⁴³⁻⁴⁵ The development of a slit-like lumen makes it easier to overcome the resistance of the connective tissue during tip advancing. Immunocytochemical analysis of the distribution of PECAM-1 during angiogenesis showed that this cell adhesion molecule is evenly distributed along the basal and luminal surfaces of endothelial cells in normal vessels, but is relocalized to intercellular junctions and to the apposed surface of the endothelial cells.⁵⁵ These results are in accordance with the observation that intact intercellular junctions are necessary for polarized migration of endothelial cells, at the same time, they also raise the possibility that during capillary growth the slit-like lumen is at least partially sealed (PECAM-1 homotypic interaction), preventing the dilatation of the lumen.

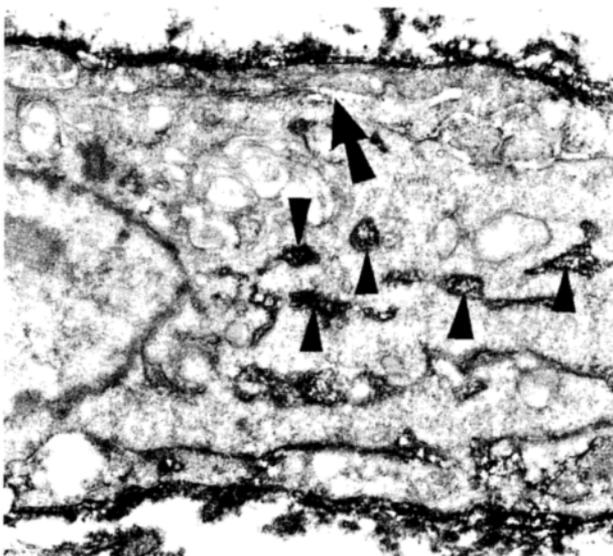


Figure 9. Detail of a capillary with slit-like lumen (arrow). Amorphous laminin positive material can be observed around the capillary. Note the laminin positive material in the endoplasmic reticulum cisternae of the endothelial cell (arrow heads). X 11000.

Basement membrane was found deposited immediately by the polarized endothelial cells and only cellular processes of cells migrating on the very tip of the growing capillary were seen to be free of basement membrane material. According to this model no stimulus is necessary for induction of lumen formation and the retained polarity of endothelial cells allows the continuous deposition of the basement membrane. Interstitial collagens were shown to enhance proliferation of endothelial cells, whereas other results suggested an increased deposition of collagen I by differentiating capillary tubes during *in vitro* angiogenesis.^{56,57} In this model, endothelial cells are separated from interstitial collagens by basement membrane during capillary growth, which phenomenon questions the importance of these molecules in the growth and differentiation of endothelial cells. The role of gelatinases has been extensively studied during angiogenesis, however, in the former model its role was restricted to the initial degradation, contrary to this model where it can contribute to the maintenance of the sol state of the basement membrane. As it has been shown, only processes of endothelial cells located at the tip of growing capillary are in contact with interstitial collagens. This phenomenon suggests an important role for interstitial collagenases during tip advancing.



Figure 10. Immunoelectronmicroscopy of a young capillary composed of three endothelial cells. Amorphous fibronectin-positive material is deposited around the capillary. There is no positive material in the endoplasmic reticulum cisternae of the cells. Material staining positively for fibronectin can be observed in the connective tissue (arrows). X 8100.

Supportive of this is the observation that VEGF can induce interstitial collagenase expression in endothelial cells.⁵⁸ As mentioned above the extracellular matrix surrounding the tumor frequently contains fibrin, fibronectin, vitronectin and thrombospondin. The wide substrate specificity of the plasminogen activator/plasmin system implicates an important role for these enzymes in angiogenesis. It has also been shown that uPA can enhance cellular migration independent of its proteolytic activity⁵⁹ and recently it has been demonstrated that uPAR is a receptor for vitronectin,⁶⁰ which is present in the peritumoral matrix and probable in the immature basement membrane of the growing capillaries. The importance of uPA in tumor angiogenesis has further been supported by the observation that anti-uPA antibody was able to inhibit network development in vitro and more importantly, uPA antagonist or mutant uPA expression was able to inhibit tumor growth.^{61,62}

Vast number of data suggest that integrins play a pivotal role in cell migration, differentiation and apoptosis.^{63,64} Recently it was demonstrated that specific integrins are

expressed in the newly developed growing capillaries. The most important among them is the $\alpha\beta3$ integrin which, as it was suggested, mediates the migration of endothelial cells in the fibrin containing tumor matrix.⁶⁵ This suggestion is in concert with the first model, but does not disagree with the second model either, where it was shown that the synthesized new basement membrane contains fibronectin (Figure 10). Since fibronectin immunoreactivity could not be observed in the endoplasmic reticulum of endothelial cells (Figure 10), in contrast to laminin and collagen IV (Figure 9), it is probable that this molecule is not synthesized by endothelial cells, but rather incorporated into basement membrane from the tumor matrix (Figure 10), providing a ligand for $\alpha\beta3$ integrin. Beside mediating migration of endothelial cells on the developing basement membrane, $\alpha\beta3$ can also play a role in the maintenance of the sol state of the basement membrane as a consequence of its ability to bind the matrix metalloproteinase MMP-2.⁶⁶ Interference with the ligand binding activity of this integrin by antibodies or RGD peptides was able to induce apoptosis of endothelial cells in growing capillaries,⁶⁷ and inhibit wound healing and tumor growth.⁶⁸ Some discrepancy exists, however, between the

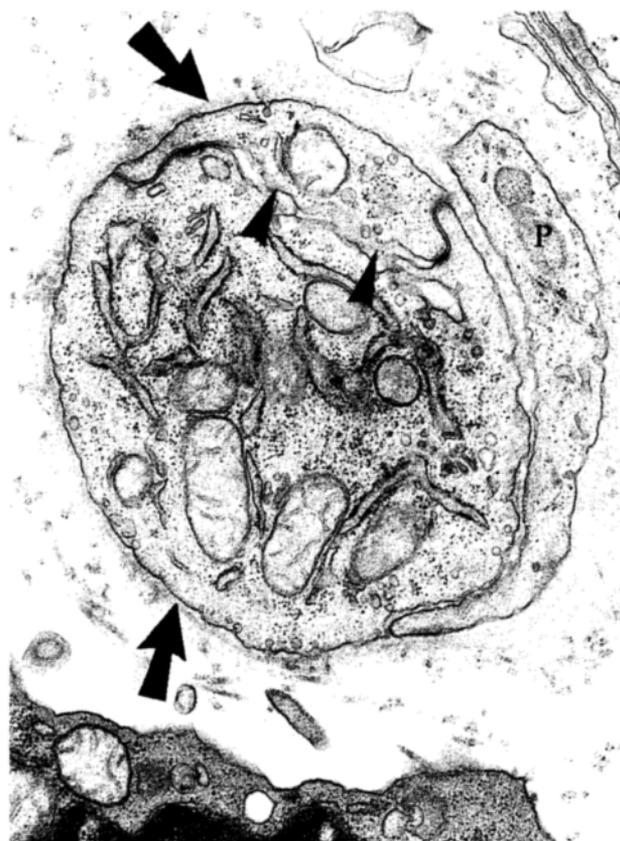


Figure 11. Newly formed capillary still showing slit-like lumen (arrow heads) but well defined basement membrane (arrows) and pericyte (P) can be observed around the capillary. X 11000.

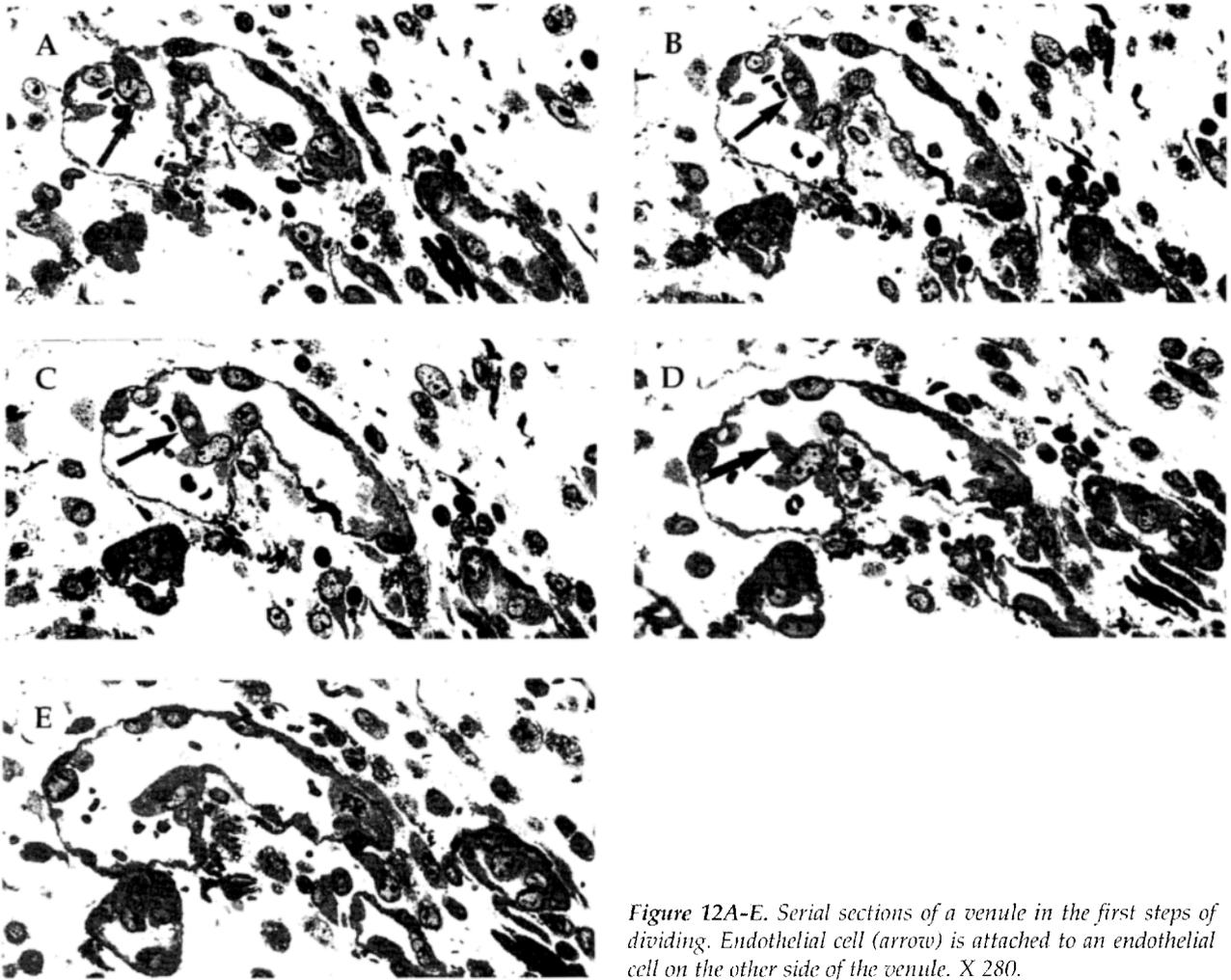


Figure 12A-E. Serial sections of a venule in the first steps of dividing. Endothelial cell (arrow) is attached to an endothelial cell on the other side of the venule. X 280.

second model and the proposed role of $\alpha v \beta 3$ integrin, since it was shown that Matrigel down regulates the expression of this integrin.⁶⁹ The selectivity of therapies based on the exclusive expression of $\alpha v \beta 3$ on newly growing capillaries is somewhat questioned by a recent observation detecting $\alpha v \beta 3$ integrin on parent vessels as well.⁷⁰ Other integrins may also be a target for anti-angiogenic therapies such as the laminin binding $\alpha 6 \beta 4$ integrin, detected on the entire length of the capillary sprout, and also in the parent vessels.⁷¹ In contrast to these results reduced staining for $\alpha 6$ integrin was observed in growing capillaries *in vivo*, which is in agreement with the well known notion that cell migration needs reduced adhesivity.⁵⁵ The positive effect of the reduction of adhesion on angiogenesis is supported by the observation that anti-integrin antibodies, enhanced, instead of reduction, the formation of tubules *in vitro*.⁷² Interesting results pointed out the important role of the tumor derived extracellular matrix in angiogenesis. Tumor cells deficient in fibronectin receptor or $\beta 1$ integrin developed a defective vasculature, characterized by small irregular leaky vessels, which phe-

non resulted in reduced tumor growth as well.^{73,74} One possible explanation for this could be, the defective anchorage of the basement membrane deposited by the endothelial cells to the surrounding extracellular matrix. This is further supported by earlier observations that inhibition of crosslinks between collagen molecules caused regression of newly growing capillaries.⁷⁵

Regular basement membrane and pericytes appeared at later stage of capillary maturation (Figure 11). Increasing deposition of collagen IV,⁷⁶ the sol-gel transition of the basement membrane and the appearance of pericytes may all contribute to the cessation of endothelial cell proliferation and migration. It has been shown that latent TGF- β is activated by uPA in cocultures of endothelial cells and pericytes,⁷⁷ where the former down regulates the VEGF receptor on endothelial cell.⁷⁸

Numerous data suggest that the growth of capillary sprouts is not oriented towards the tumor, instead the process yields a high density anastomosing network of capillaries at the tumor periphery, a process leading to the observed phenomenon that vessel density is higher

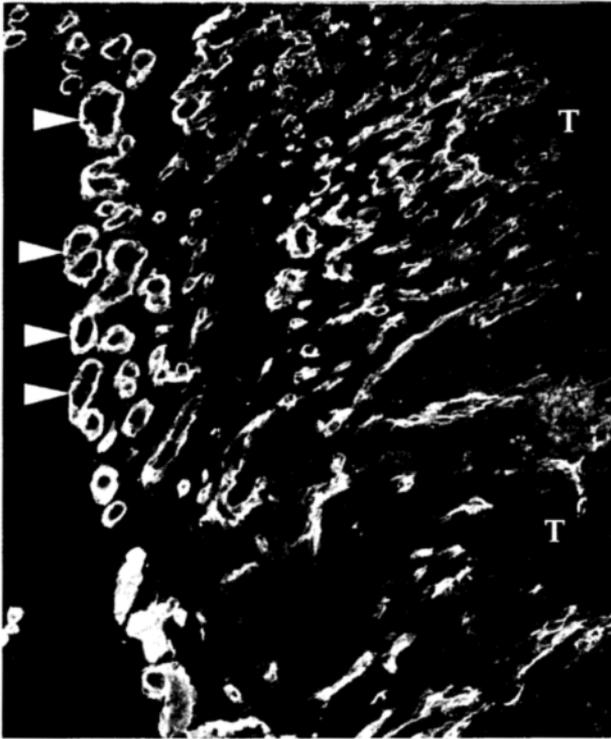


Figure 13. Numerous large caliber vessels (arrow heads) are stained positively for laminin outside the capillary sprouting area. T; Tumor. X 110.

around the tumor than inside of it. A possible explanation is that the continuously growing tumor incorporates the vessels at the tumor periphery, thereby thinning out the network, whereas a new network develops at the advancing tumor border.⁷⁹

A third type of angiogenesis involving larger vessels has been described recently. This process is called intussusceptive growth of vessels, culminating in the division of vessels (Figure 14D).^{80,81} The process is not completely known, but it starts with the projection of an endothelial cell into the lumen of the vessel and the attachment to endothelial cells on the other side (Figure 12A-E). Finally transluminal connective bridges develop, dividing the vessel into two or more parts. The process -which yields a high number of large caliber vessels situated outside the area of the active capillary growth (Figure 13) – probably does not contribute to the nutrition of the tumor significantly, but rather provides more sites for sprouting.

Angiogenesis in metastases

The above described models of angiogenesis are based on observations made in tissues that contain high amounts of connective tissue fibers, and can also be valid in case of tumors such as melanomas, skin-, breast-, colon cancer. The connective tissue has two important

roles in angiogenesis: it allows the build-up of a gradient of the angiogenic factor and provides space for vessel sprouting. The question arises, what kind of angiogenesis takes place in highly vascularised organs such as liver, lungs or adrenals, which in humans are the most frequent sites of metastasis ?

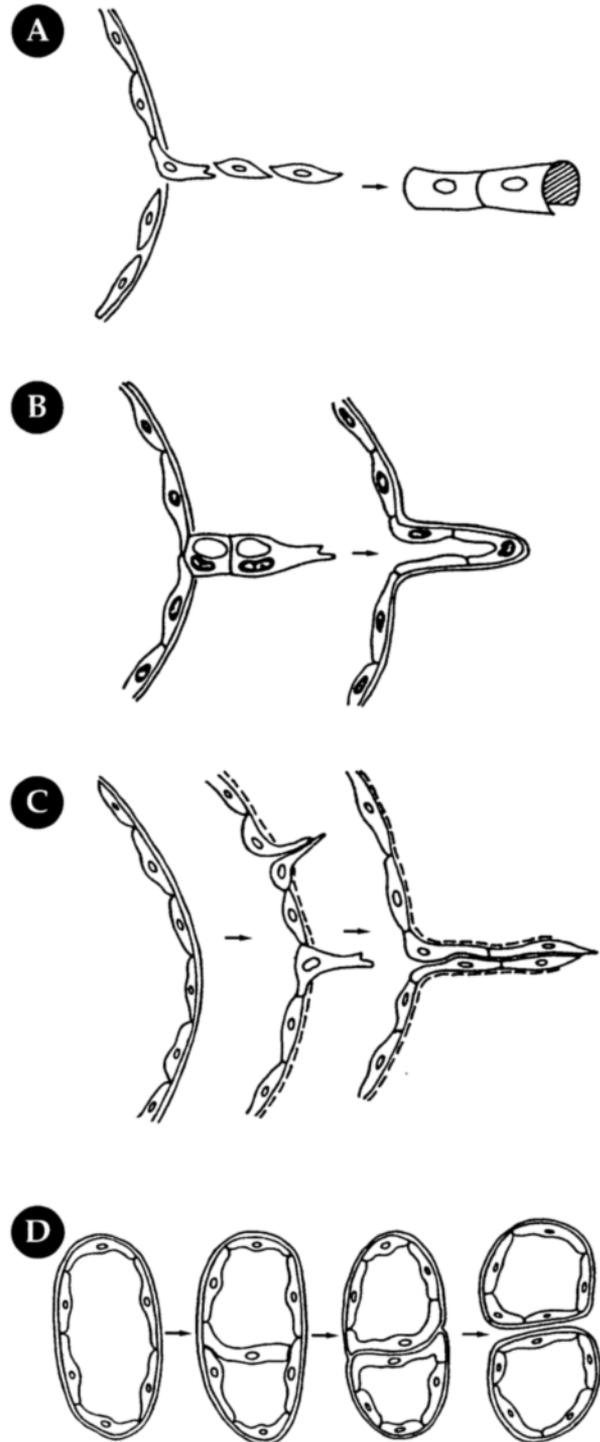


Figure 14A-D. Schematic representation of the different models of angiogenesis.

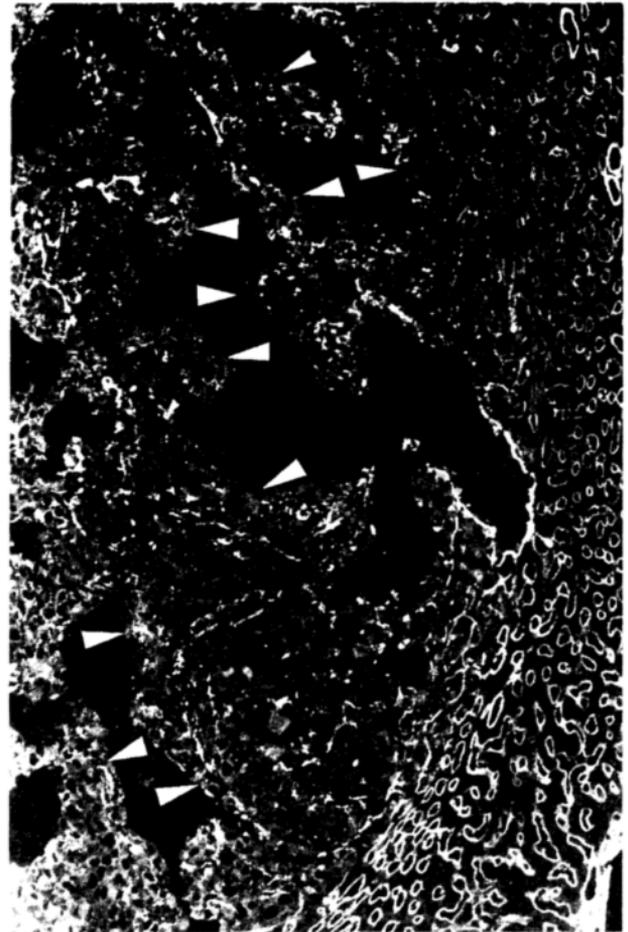


Figure 16. Cryosection of a liver metastasis stained for laminin. The walls of the large convoluted vessels stain negatively for laminin (arrow heads). X 75.

A process of angiogenesis, was described in case of liver metastases of Lewis lung carcinoma, showing remarkable differences to the models described above.⁸² Neither dilatation, or increase in the number of the vessels, nor enhanced proliferation of endothelial cells could be observed around the metastases, suggesting the absence of sprouting activity in this region. This indicates that the angiogenic stimulus could not reach the endothelial cells around the tumor, which can be explained by the high vascularity of the liver being responsible for diluting or flushing out the factor. The sparse connective tissue cannot provide sufficient space for diffusion of the angiogenic factor, and also impedes the migration of endothelial cells, thereby the development of new capillaries. Initiation of endothelial cell proliferation was caused by direct contact

Figure 15A-D. Serial sections of a liver metastasis of the 3LL-HH tumor. Large convoluted (V) and smaller vessels (asterisks) can be seen in the metastasis. Two sinusoids of the liver are continuous with these vessels (arrows). X 90.

between tumor cells and endothelial cells (Figure 18). Invading tumor cells migrated along the basement membrane of sinusoids and larger vessels, detaching the endothelial cells from their own basement membrane (Figures 18, 19). Interestingly the basement membrane was not degraded and remained on the surface of hepatocytes, even when these cells became enclosed into the tumor (Figure 20). The observation that proliferative activity is restricted to endothelial cells situated inside the metastases can be explained by results demonstrating that mRNAs of VEGF receptors (KDR, flt-1) were more strongly expressed in metastases of colorectal tumors than in the surrounding liver tissue.⁸³

The proliferating endothelial cells formed large convoluted vessels deeply penetrating into the metastases (Figures 15B, 16). These vessels were even lacking an immunohistochemically detectable basement membrane (Figures 16, 17). This observation is rather unusual

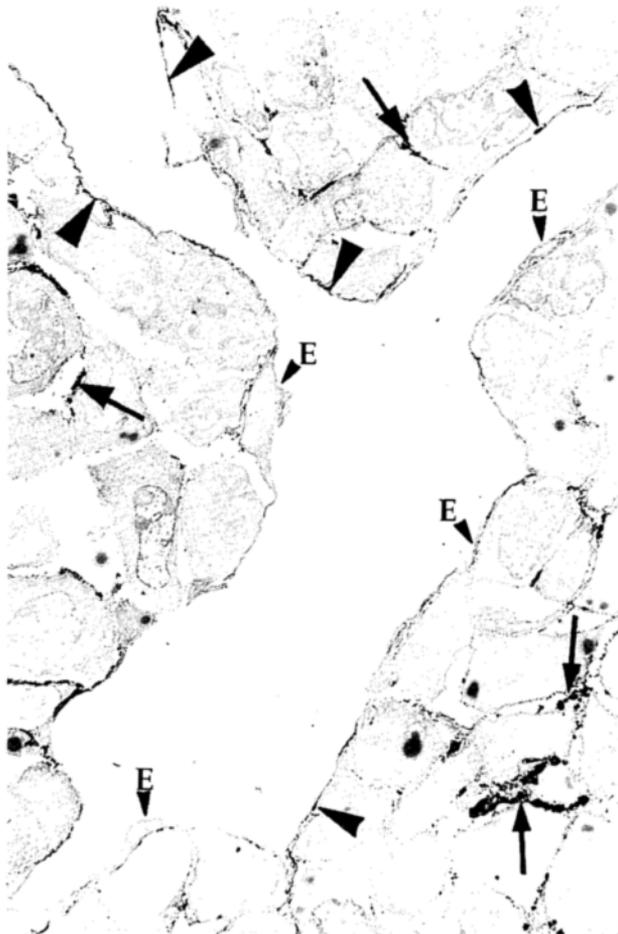


Figure 17. Immunoelectronmicroscopy of a part of a convoluted vessel inside a metastasis. Under the endothelial cells (E) laminin positive material can be observed only in small spots (arrow heads). Similar material is deposited in some areas by the tumor cells (arrows). X 1.000.

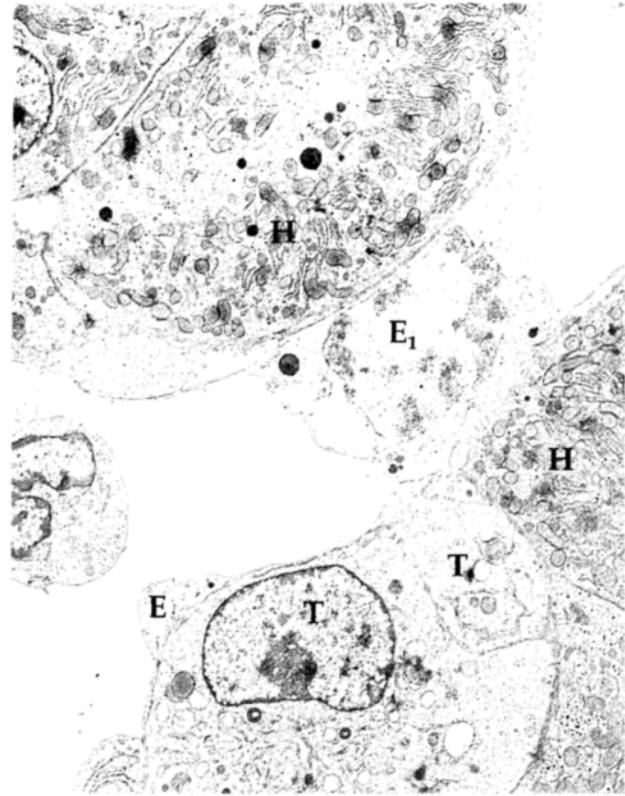


Figure 18. 3LL-HH tumor cells (T) migrating between the endothelial cells (E) and hepatocytes (H) in a sinusoid at the periphery of a metastasis. The endothelial cell (E1) in the vicinity of the tumor cells shows chromatin condensation. X 3.000.

although lack of electron density is commonly observed in case of liver sinusoids, lymphatic capillaries or tumor vessels. Complete loss of basement membrane (loss of anchorage) – as was mentioned earlier – leads to apoptosis and vessel regression. It must be noted, however, that in 3LL tumors – although having a very poorly developed stroma – the tumor cells deposited some laminin containing material, which could serve as substrate for attachment of endothelial cells in these new vessels. This attachment took place only at some spots on the surface of tumor cells, resulting weak anchorage which can be responsible for the high fragility of these vessels (Figure 17). In serial sections these vessels were continuous with the sinusoidal system of the liver consequently supplied mainly by the portal vein (Figure 15A-D).

Another less frequently observed type of 3LL-HH liver metastases showed a different pattern of vasculature. These metastases contained small vessels with detectable basement membranes, and were located in the vicinity of portal tracts. Their vasculature probably raised by sprouting from the peribiliary plexus, in the periportal connective tissue, suggesting that blood supply was originating mainly from the hepatic artery.

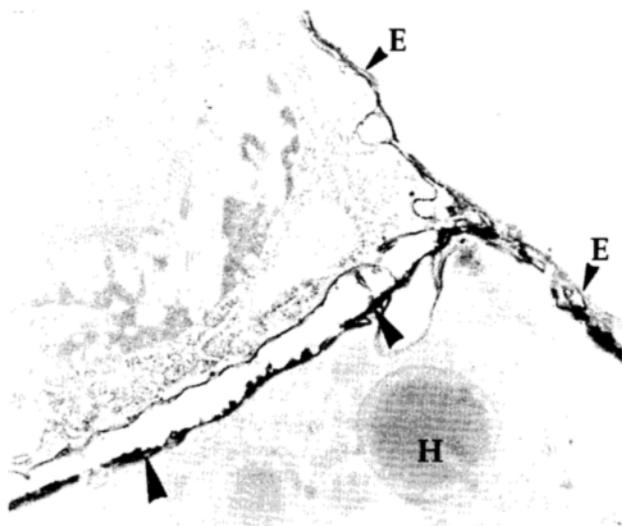


Figure 19. Invading tumor cell (T) in a sinusoid detaches the endothelial cell (E) from the basement membrane (arrow heads). The laminin positive material remains associated with the hepatocyte (H). X 10,000.

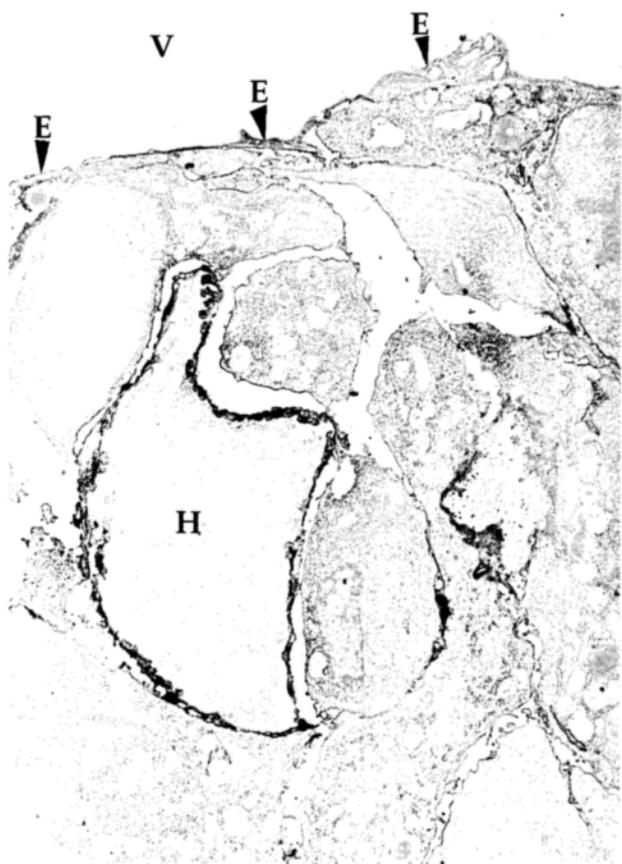


Figure 20. The surface of a hepatocyte (H) enclosed into the metastasis still stains positively for laminin. E; Endothelium, V; Vessel lumen. X 1.300.

Therapeutic considerations

The above results strongly suggest that the type of vasculature and blood supply can be dependent on the localization of metastases in organs having both arterial and venous blood supply. Accordingly, these and other observations support the possibility that the tissue architecture of organs, in which angiogenesis is taking place, has an important impact on the process of angiogenesis and consequently on the structure of the developing neovasculature. On the other hand the properties of tumor cells can also determine the outcome of the angiogenic response. For example the previously described process of angiogenesis in liver metastases can be valid in case of anaplastic tumors having high invasive ability and poorly developed tumor stroma. Well differentiated tumors, however, showing the same tissue architecture in primary tumors as in metastases, probably elicit a different type of angiogenesis. The widely used methods in the therapy of primary and secondary liver tumors (hepatic artery infusion, hepatic artery ligation and chemoembolization) are based on the notion that most tumors in the liver have an arterial blood supply.⁸⁴⁻⁸⁶ In contrast to this, it is generally accepted that during angiogenesis, new vessels originate mainly from postcapillary venules.^{2,3} Several authors suggest, however, that new capillaries can originate from arterioles or large veins, containing elastic laminae and smooth muscle cells, demonstrating the fact that such structures cannot prevent the emigration of endothelial cells.^{87,88} A number of data suggest that a considerable portion of human and experimental liver metastases have portal blood supply,^{89,91} and even vessels, originating from hepatic sinusoids can contribute to the nutrition of the entire metastatic nodule, not only to the supply of the tumor periphery.⁹² According to these facts, more attention should be paid in the future to the portal blood supply of liver metastases in the design of locoregional therapy.

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