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ORIGINAL ARTICLE

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In situ expression of β1, β3 and β4 integrin subunits in non-neoplastic endothelium and vascular tumours

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Abstract Endothelial cells play an important role in adhesive interactions between circulating cells and extracellular matrix proteins. In vitro studies have shown that many of these processes are mediated by a superfamily of $\alpha\beta$ heterodimeric transmembrane glycoproteins called integrins. The distribution patterns of $\beta1$, $\beta3$ and $\beta4$ integrin subunits in endothelial cells (EC) in situ were examined immunohistochemically on serial frozen sections of a wide range of non-neoplastic tissues and of vascular tumours, both benign and malignant. Expression of the $\beta1$ subunit was a constitutive feature of EC. Among the $\beta1$-associated α subunits, $\alpha5$ and $\alpha6$ were broadly distributed in EC, irrespective of vessel size and microenvironment. The $\alpha3$ subunit displayed intermediate levels of expression with a slight preference for small vessel EC. Presence of $\alpha1$ was confined to EC of capillaries and venules/small veins. Expression of $\alpha2$ in EC was inconsistent. With rare exceptions, the $\alpha4$ chain was absent in EC. The $\beta3$ and αv subunits were expressed in most EC, though not always concomitantly. In contrast to the $\beta1$ chain, however, these integrin subunits were absent in EC of glomerular capillaries and were expressed variably in sinusoidal EC. The $\beta4$ chain was evenly present in the great majority of EC, except for those of large vessels. In vascular tumours, the patterns of $\beta1$ and $\alpha1$ to $\alpha6$ subunit expression generally corresponded to those found in their non-neoplastic counterparts. Expression of $\beta3$, αv and $\beta4$ chains, however, decreased in neoplasia, especially in angiosarcomas. These data show that EC dispose of broad and at the same time differential repertoires of integrin subunits that presumably reflect vessel-type associated functional differences among these cells. In vascular tumours, the orthologous distribution patterns of $\beta1$ and $\alpha1$ to $\alpha6$ chains are conserved in most instances while the amounts of $\beta3$, αv and $\beta4$ subunits expressed in EC tend to decrease in the course of malignant transformation.

Key words Endothelium · Vascular tumours · Integrins Immunohistochemistry

Dedicated to Prof. Dr. med. Dres. h.c. Wilhelm Doerr on the occasion of his 80th birthday

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Introduction

Endothelial cells (EC) form a functionally important ubiquitous interface between the blood stream and the surrounding tissues. They display a remarkable extent of heterogeneous differentiation with respect to vascular type, size and tissue microenvironment. Central functional elements of EC are closely linked to complex mechanisms of homotypic and heterotypic cell/cell together with cell/matrix interactions.

In the last decade, a plethora of adhesion molecules have been described which mediate cell/cell and cell/matrix interactions. They comprise four major families, cadherins, immunoglobulins, selectins, integrins, and many other yet unclassified molecules [1]. While cadherins, immunoglobulins and selectins mediate cell/cell adhesion [1], integrins are involved in both cell/matrix and cell/cell interactions [12, 31].

Integrins are a superfamily of non-covalently associated transmembrane $\alpha\beta$ heterodimers. Based on different β subunits, the integrins are subdivided into protein subfamilies. At present, eight β and 15 α subunits are known which build at least 21 different $\alpha\beta$ heterodimers [12, 28, 31]. Some α subunits combine with more than one β chain indicating promiscuity within the integrin system. The integrins function as receptors for a comprehensive set of extracellular matrix and basement membrane proteins [32] and some of them interact with cell

adhesion molecules. Most integrins bind to more than one ligand thus implying redundancy in the integrin system. However, the highly diverse sequences of the cytoplasmic domains especially of the α subunits and the existence of alternative splicing of several integrin subunits indicate that each subunit might contribute to discrete intracellular functions [12]. In addition to their functions in cell/cell and cell/matrix interactions, recent studies provided evidence for a signal transducing role of these molecules [13] even confering mitogenic signals to various normal and transformed cells [20].

Our knowledge of the repertoire and function of integrins on EC is mainly derived from in vitro studies of isolated EC in pure culture. It is generally accepted that EC dispose of a comprehensive set of integrin subunits which are expressed either constitutively or which are inducible on their cell surface by a variety of physiologic mediators such as cytokines or growth factors [2, 3, 6, 7, 9, 17, 19]. These in vitro studies, however, cannot deal with the anatomical diversity of endothelial cells.

We therefore performed a detailed study on the distribution patterns of β1, β3 and β4 integrin subunits in a wide range of normal endothelial cells (EC) of different vascular beds and tissue sites in situ. These data were compared with the integrin immunoprofile of neoplastic endothelium in a variety of vascular tumours both benign and malignant.

Materials and methods

Except for cerebral cortex, which was drawn from autopsy material, the non-neoplastic human tissues and the vascular tumours were obtained from fresh surgical specimens or biopsies within 2 h after removal. Non-neoplastic tissues examined were: skeletal muscle (2), heart (1), colon (2), fallopian tube (1), skin (2), lung (1), kidney (2), liver (2), spleen (2), reactive tonsil (1), reactive lymph node (1), pancreas (1), adrenal (2), thyroid (1), cerebral cortex (1), femoral bundle (1), telangiectatic granuloma (1), ulcer of the stomach (1), placenta (1), and umbilical cord (1). Vascular tumours studied comprised: 9 haemangiomas (2 capillary, 6 cavernous, 1 venous), 1 glomangioma, 1 infantile haemangioendothelioma, 4 haemangiopericytomas, and 5 angiosarcomas. The tissues were snap-frozen in liquid nitrogen and stored at –70° C until use. Serial frozen sections of about 1 cm^2 and a thickness of 4 to 6 µm were air-dried overnight, acetone-fixed at room temperature for 10 min, immediately stained or stored at –20° C for 1 to 3 weeks.

The following primary monoclonal antibodies (mAbs) to β1, β3 and β4 integrin subunits were used in this study: MAbs K20 (anti-β1), Gi9 (anti-α2), HP2/1 (anti-α4), SAM1 (anti-α5), GOH3 (anti-α6), SZ.21 (anti-β 3) and AMF7 (anti-αv) were obtained from Dianova (Hamburg, Germany); mAb TS2/7 (anti-α1) was purchased from T Cell Sciences (Cambridge, Mass., USA); mAbs P1B5 (anti-α3) and 3E1 (anti-β4) were obtained from Telios Pharmaceuticals Inc. (San Diego, Calif., USA). MAb SG134 (CD31) was also obtained from Dianova. Except for mAb GOH3 which was of rat origin, the mAbs were produced in mice. A polyclonal biotinylated sheep antibody to mouse Ig and, for detection of rat-derived mAb GOH3, a polyclonal biotinylated sheep antibody to rat Ig, and a streptavidin-biotinylated peroxidase complex, all obtained from Amersham (High Wycombe, UK), served as detection system for the primary mAbs. 3-Amino-9-ethylcarbazole (AEC) and N´N-dimethylformamide (DMF) were obtained from Sigma Chemical Company (St. Louis, Mo., USA).

For immunohistochemistry following rehydration with phosphate-buffered saline solution (PBS; pH 7.5), the frozen sections were incubated for 1 h with primary mAbs. Ascites preparations were diluted 1:2000 in PBS, purified reagents were used in a protein concentration of about 10 µg/ml PBS. The sections were then incubated with biotinylated anti-mouse or anti-rat Ig (1:100) and streptavidin/biotin-peroxidase complex (1:100) for 30 min, respectively. All incubation steps were carried out in a humid chamber at room temperature. Washing was done in PBS. Using AEC as the chromogen (0.4 mg/ml in 0.1 mol/l acetate buffer, pH 5.0, with 5% DMF and 0.01% hydrogen peroxide for about 20 min), the peroxidase reaction caused an intense red precipitate. The sections were then rinsed in tap water, counterstained with Harris' haematoxylin, and mounted with glycerol gelatin. Negative PBS controls were performed by omitting the primary mAbs. No staining was observed except for scattered granulocytes. This staining was due to endogenous peroxidase which was not blocked for the benefit of optimal antigenicity. In cases in which EC were unstained, strongly stained stromal cells and/or lymphocytes and/or epithelial cells were present, serving as positive intrinsic control for the respective integrin subunit under study.

The staining of non-neoplastic and of neoplastic EC was evaluated as follows: +, all cells strongly positive; (+), all cells weakly positive; –, all cells negative. Furthermore, a semi-quantitative evaluation was carried out. Formalized, A/B indicated a bimodal reactivity; A>B indicated that reaction pattern A clearly prevailed; B>A, vice versa. The integrin subunit staining in EC was compared to that obtained for CD31 which is known to be a reliable marker for endothelium [27].

Results

The distribution patterns of β1, β3 and β4 integrin subunits in non-neoplastic EC are detailed in Tables 1 to 3. The integrin immunoprofile of vascular tumours is given in Table 4.

Non-neoplastic tissues

The β1 subunit was consistently expressed in EC of all vessel types studied, irrespective of size and tissue site (Fig. 1).

Apart from fenestrated EC in renal glomeruli, the capillary endothelia examined were $\alpha1^+$ (Fig. 2). Furthermore, EC of epithelioid venules (Fig. 3) and of the majority of venules/small veins studied expressed the α1 subunit. In contrast, EC of arterioles/small arteries, of medium-sized and of large vessels were $\alpha1^-$ (Fig. 3).

The α2 chain was variably and inconsistently expressed in EC. Within capillaries, presence of the α2 subunit was confined to at least an EC subset of the colon, exocrine pancreas, lung and placenta (Fig. 4) and to fenestrated EC of the endocrine pancreas, adrenal and thyroid. The distribution pattern of α2 in EC of small and medium-sized arteries and veins ranged from a consistent over a focal presence at some tissue sites to a complete absence at others. EC of large femoral and umbilical veins were $\alpha2^+$ while EC of the corresponding arteries were almost entirely $\alpha2^-$ (Fig. 5a, b).

With rare exceptions such as a subset of capillary EC of the tonsil and lymph node, the endothelium of all capillaries studies was $\alpha3^+$ (Fig. 6). Furthermore, the endothelium of epithelioid venules and of most arteries/small arterioles and venules/small veins expressed the α3 subunit. In central arteries of the spleen and in portal arteries of the liver, however, some EC were $\alpha3^-$, and central

Table 1 Expression of $\beta1$, $\beta3$ and $\beta4$ integrin subunits in endothelial cells of blood capillaries

Vessel type and tissue	$\beta1$	$\alpha1$	$\alpha2$	$\alpha3$	$\alpha4$	$\alpha5$	$\alpha6$	$\beta3$	αv	$\beta4$
Continuous capillaries										
Skeletal muscle	+	+	–	+	–	+	+	+	+	–>+
Cardiac muscle	+	+	–	+	–	+	+	+	+	+
Colon (l. muscularis propria)	+	+	–/(+)	+	–	(+)	+	+	+	+
Pancreas (exocrine parenchyma)	+	+	(+)	+	–>(+)	+	+	+>–	+	+
Skin (papillary dermis)	+	+	–	+	–	+	+	+	+	+
Tonsil	+	+	–	–	–	+	+	+	+	+
Lymph node	+	+	–	+>–	–	+	+	+	+	+
Lung	+	+	–>+	+	–	+	+	na	na	+
Cerebellar cortex	+	+	–	+	–	+	+	+	+	+
Placenta (villi)	+	+	+	+	+	+	+	+	+	–>(+)
Fenestrated capillaries										
Kidney (glomeruli)	+	–	–	+	–	–	–	–	–	–
Colon (l. mucosae)	+	+	–	(+)	–	+	+	+	+	+
Pancreas (islands)	+	+	(+)	(+)/–	–	+	+	+>–	+	+
Adrenal	+	+	+/–	–	–	+	+	+/–	+/–	–
Thyroid	+	+	+/–	–	+>–	+	+	na	na	–>+
Sinusoidal capillaries										
Lymph node	+	+	–	(+)	–	(+)	–	–	–	–
Spleen	+	+	–	–	–	–	+	–	(+)	+
Liver	+	+	–	–	–	+	–	(+)>–	(+)>–	–

Scoring of endothelial cell (EC) reaction: +, all cells strongly positive; (+), all cells weakly positive; –, all cells negative. *A/B*, bimodal reactivity; *A>B*, reaction pattern A clearly prevailing; *B>A*, vice versa; *na*, not analysed

Table 2 Expression of $\beta1$, $\beta3$ and $\beta4$ integrin subunits in endothelial cells of small blood vessels

Vessel type and tissue	$\beta1$	$\alpha1$	$\alpha2$	$\alpha3$	$\alpha4$	$\alpha5$	$\alpha6$	$\beta3$	αv	$\beta4$
Arterioles/Small arteries										
Skeletal muscle	+	–	(+)/–	+	–	+	+	+	+	+
Cardiac muscle	+	–	–	+	–	+	+	+	+	+
Lymph node	+	–	+	+	–	+	+	+	+	+
Tonsil	+	–	–	+	–	+	+	+	+	+
Spleen (central arteries)	+	–	+>–	+>–	–	+	+	+	+	+
Liver (portal arteries)	+	–	–	+/–	–	+	+	+	(+)	+
Lung	+	–	+	+	–>(+)	+	+	+	(+)	+
Fallopian tube	+	–	–>(+)	+	–	+	+	+	+/–	+
Colon	+	–	–	+	–	+	+	+	+	+
Pancreas (exocrine parenchyma)	+	–	–>(+)	+	–	+	+	(+)	+	+
Thyroid	+	–	–>+	+	–>+	+	+	+	+	+
Venules/small veins										
Skeletal muscle	+	+/–	+>–	+	–	+	+	+	+	+
Cardiac muscle	+	+	–>+	+	–	+	+	+	+	+
Lymph node	+	–	+	+	–	+	+	+	+	+
Tonsil	+	–	–>+	+	–	+	+	+	+	+
Liver (central veins)	+	+	–	–	–	+	–	+	(+)	–
Liver (portal veins)	+	+	–	+	–	+	–	+	(+)	–
Lung	+	+	+	+	–>(+)	+	+	+	+	+
Fallopian tube	+	–	(+)>–	+	–	+	+	+	+/–	+
Colon	+	+/–	–>(+)	+	–	+	+	+	+	+
Pancreas (exocrine parenchyma)	+	+/–	(+)/–	+	–	+	+	+	+	+
Thyroid	+	–	(+)>–	+	–>+	+	+	+	+	+
Epithelioid venules										
Lymph node	+	+	(+)/–	+	–	+	+	+	(+)	+
Tonsil	+	+	–	+	–	+	+	+	(+)	+
Inflammatory teleangiectasia[a]	+	+	–	+	–	+	+	+	+	+

Scoring of EC reaction: +, all cells strongly positive; (+), all cells weakly positive, –, all cells negative; *A/B*, bimodal reactivity; *A>B*, reaction pattern B clearly prevailing; *B>A*, vice versa. [a] Studied in one each case of pyogenic granuloma and of chronic ulcer of the stomach

vein endothelium of the liver lacked the $\alpha3$ chain. In medium-sized vessels, $\alpha3^+$ and $\alpha3^-$ EC were found in variable amounts. The endothelium of umbilical arteries and vein was $\alpha3^+$ throughout while EC of the femoral artery and vein were only partly $\alpha3^+$. Presence of the $\alpha4$ subunit was restricted to continuous capillary EC of the placenta and exocrine pancreas, to fenestrated capillary EC of the thyroid (Fig. 7) and to EC of some small and medium-sized vessels of the thyroid and lung that expressed variable amounts of $\alpha4$.

Table 3 Expression of $\beta 1$, $\beta 3$ and $\beta 4$ integrin subunits in endothelial cells of medium-sized and large blood vessels

Vessel type and tissue	$\beta 1$	$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 4$	$\alpha 5$	$\alpha 6$	$\beta 3$	αv	$\beta 4$
Medium-sized arteries										
Lung	+	–	+>–	–	(+)/–	+	+	+	+	+
Spleen (trabecular arteries)	+	–	–	–>+	–	+	+	+	+/–	+/–
Fallopian tube	+	–	(+)/–	–	–	+	+	+	(+)/–	(+)/–
Kidney	+	–	–>(+)	–	–	+	(+)/–	+	–	–
Adrenal	+	–	–>+	+/–	–	+	(+)	+	–	–>(+)
Thyroid	+	–	–>(+)	+	(+)/–	+	(+)	+	(+)/–	(+)/–
Medium-sized veins										
Lung	+	–	+>–	+/–	(+)/–	+	+	+	+	+
Spleen (trabecular veins)	+	–	–	–>+	–	+	+	+	–	+
Fallopian tube	+	–	–	–	–	+	+	+	(+)/–	+
Kidney	+	–	–	(+)/–	–	+	+	+	–	–>(+)
Adrenal	+	–	–	+/–	–	+	+	+	–	+
Thyroid	+	–	+	+	–	+	+	+	(+)/–	+
Large arteries										
Femoral artery	+	–	–>+	–>+	–	+	(+)/–	+	+	–
Umbilical arteries	+	–	–	+	–	+	+	+	+	–>(+)
Large veins										
Femoral vein	+	–	+	+/–	–	(+)	(+)	+	+	(+)/–
Umbilical vein	+	–	+	+	–	(+)	(+)/–	+	–	–

Scoring of EC reaction: +, all cells strongly positive, (+), all cells weakly positive; –, all cells negative. *A/B*, bimodal reactivity; *A>B* reaction pattern A clearly prevailing; *B>A*, vice versa

Table 4 Expression of $\beta 1$, $\beta 3$ and $\beta 4$ integrin subunits in neoplastic cells of vascular tumours

Tumour type		n	$\beta 1$	$\alpha 1$	$\alpha 2$	$\alpha 3$	$\alpha 4$	$\alpha 5$	$\alpha 6$	$\beta 3$	αv	$\beta 4$
Haemangioma (capillary)		2	2	2	(1)	2	–	2	2	1 (1)	2	(1)
Haemangioma (cavernous)		6	6	2 (3)	(4)	4 (2)	–	6	6	6	4 (2)	5 (1)
Haemangioma (venous)		1	1	(1)	(1)	1	–	(1)	1	(1)	(1)	(1)
Glomangioma	EC	1	1	1	(1)	1	–	1	1	1	(1)	1
	GC	1	1	(1)	–	1	–	(1)	–	(1)	1	–
Haemangioendothelioma (infantile)		1	1	1	(1)	1	(1)	1	1	(1)	1	–
Haemangiopericytoma	PC	4	4	1	1 (1)	2	1	3	1	1	2 (1)	–
Angiosarcoma		5	5	3 (1)	1 (4)	2 (2)	–	5	4 (1)	(3)	2 (1)	(1)

Figures without parentheses indicate the number of cases in which all neoplastic cells expressed the corresponding antigen, those with parentheses indicate the number of cases in which the respective antigen was detectable in at least a minor neoplastic population. –, absence of the respective molecule in all tumours, *EC*, neoplastic endothelial cells, *GC*, neoplastic glomus cells; *PC*, neoplastic pericytes

Fig. 1 Cerebral cortex. Capillary EC are $\beta 1^+$ contrasting to $\beta 1^-$ brain parenchyma (×125; immunoperoxidase staining on frozen section, faint haematoxylin counterstain. Same magnification and same technique for all photographs)

Fig. 2 Spleen. Sinusoidal endothelium is $\alpha 1^+$ while EC of the central artery (*arrows*) lack the $\alpha 1$ subunit

Fig. 3 Chronic ulcer of the stomach. A strong staining for $\alpha 1$ is observed in the proliferating venules that exhibit epithelioid features

Fig. 4 Placenta. In placental villi expression of $\alpha 2$ is confined to capillary EC

Fig. 5 Umbilical cord. Umbilical vein endothelium (*arrow heads*) is $\alpha 2^+$ (A) while EC of the accompanying arteries (*arrow heads*) lack the $\alpha 2$ chain (B)

Fig. 6 Reactive lymph node. Sinusoidal endothelium is weakly $\alpha 3^+$ when compared to strongly $\alpha 3^+$ capillary EC

Fig. 7 Thyroid. The majority of capillary EC but not the thyreocytes are $\alpha 4^+$

Fig. 8 Liver. EC of both the sinuses and the central vein express the $\alpha 5$ subunit

Fig. 9 Pancreas. Capillary EC of the exo- and endocrine parenchyma are strongly $\alpha 6^+$. Acinar epithelia show a weak staining for $\alpha 6$

Fig. 10 Spleen. EC of the sinuses, of a central and of a trabecular artery are evenly $\alpha 6^+$

Fig. 11 Skin. EC of the papillary plexus are $\beta 3^+$. The squamous epithelium is $\beta 3^-$

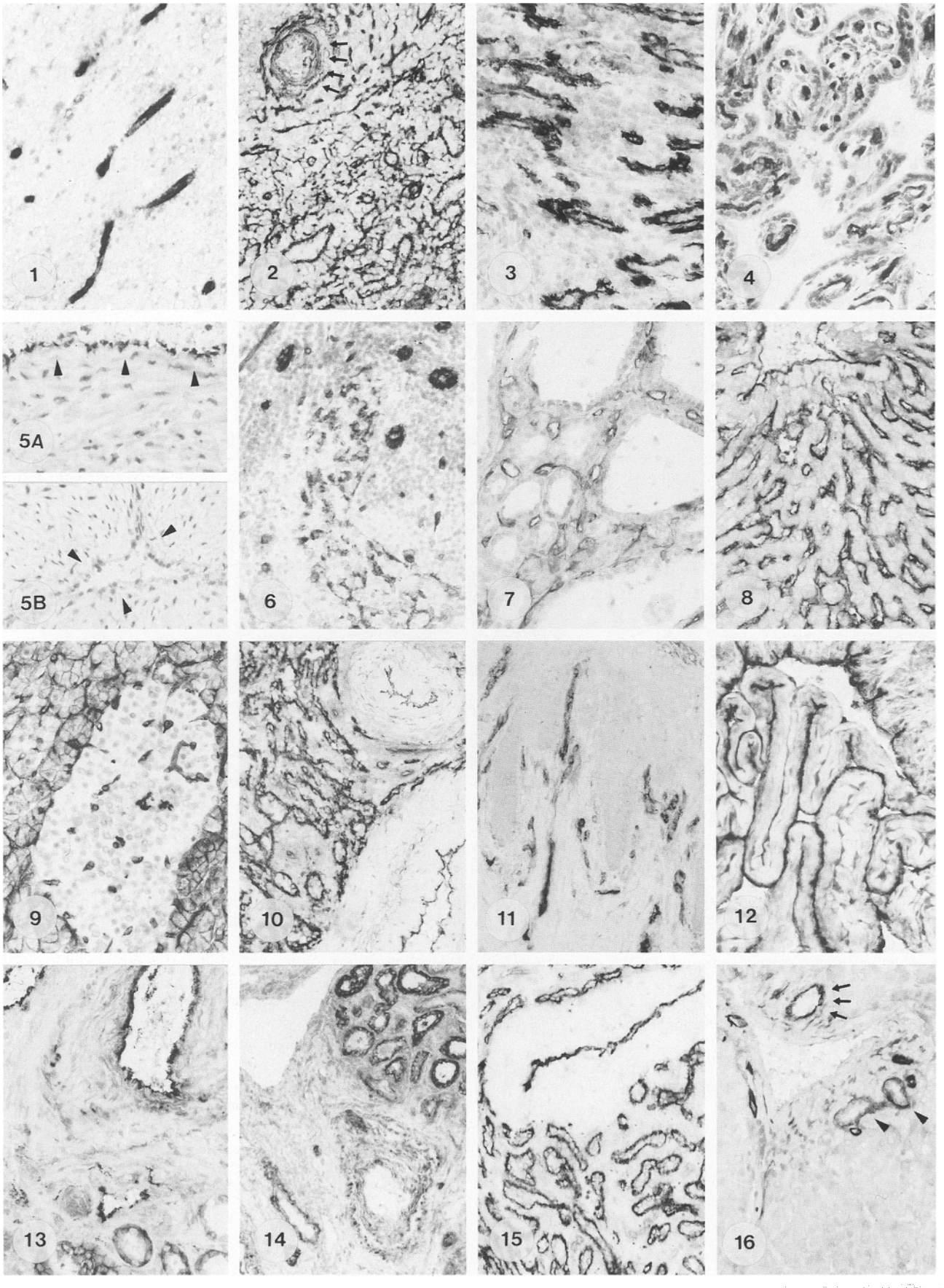
Fig. 12 Femoral vein side branch. The endothelium of a collapsed venous valve is $\beta 3^+$

Fig. 13 Kidney. EC of medium-sized vessels are strongly $\beta 3^+$. Some tubules show a faint staining for the $\beta 3$ subunit

Fig. 14 Kidney. The αv chain, by contrast, is absent from EC. Strongly αv^+ tubules serve as an intrinsic positive control of the immune reaction

Fig. 15 Spleen. Similarly to $\alpha 6$, the $\beta 4$ chain is expressed in EC of the sinuses and of a trabecular artery

Fig. 16 Liver. EC of the portal artery are $\beta 4^+$ (*arrows*) while portal vein EC and hepatocytes are $\beta 4^-$. The basolateral surface of two biliferous ductules is equally $\beta 4^+$ (*arrow heads*)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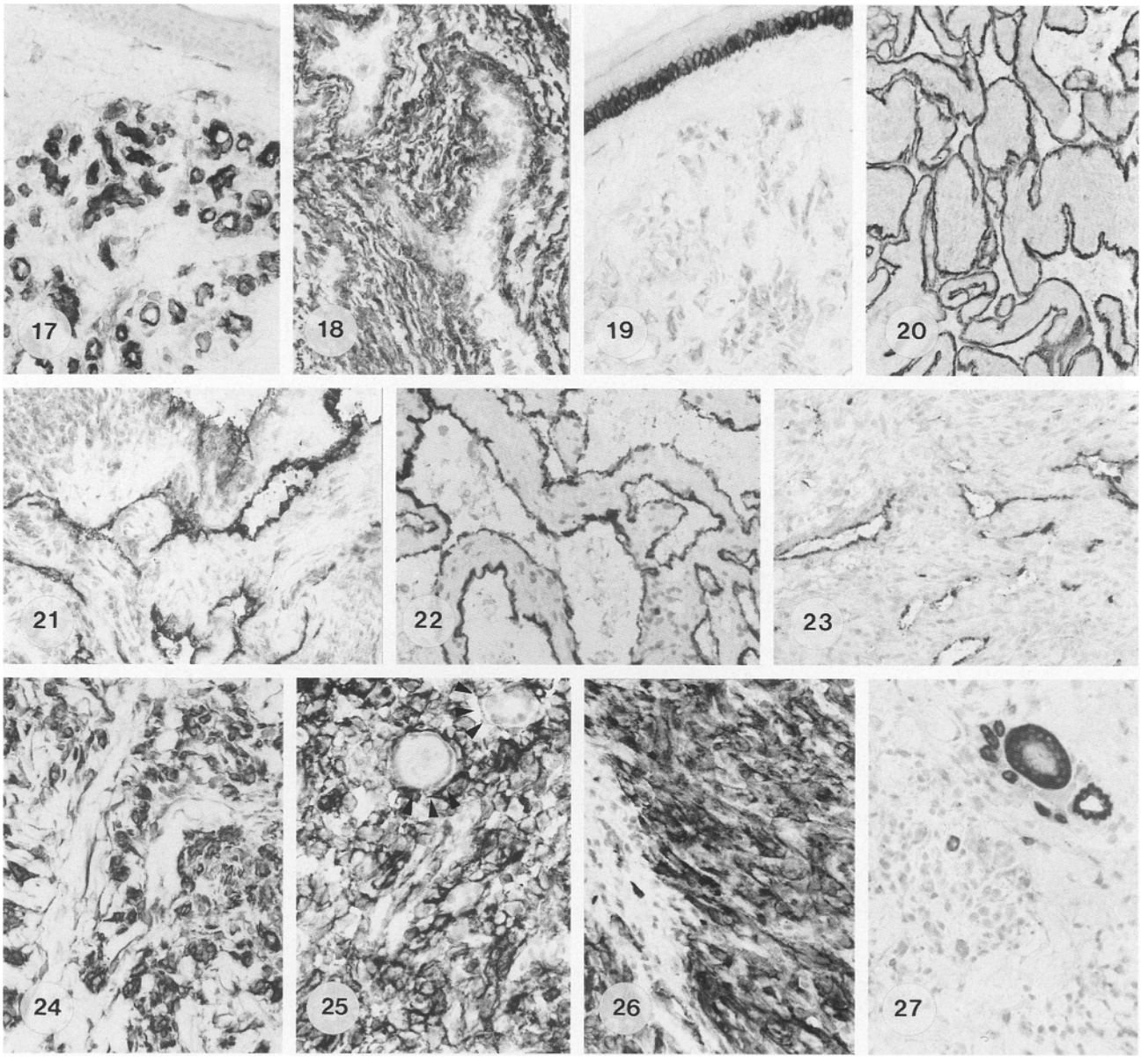

Fig. 17 Capillary haemangioma (skin). EC of the intradermal capillary proliferates are $\alpha 1^+$. The overlying epidermis is $\alpha 1^-$

Fig. 18 Cavernous haemangioma (liver). The $\alpha 1^-$ endothelial lining of the cavernous spaces contrasts with the $\alpha 1^+$ perivascular stroma

Fig. 19 Capillary haemangioma (skin). The neoplastic EC lack the $\alpha 2$ subunit. The squamous epithelium displays an $\alpha 2^+$ basolateral surface

Fig. 20 Cavernous haemangioma (liver). Conversely to $\alpha 1$, the $\alpha 6$ subunit is strongly expressed in neoplastic EC whereas the surrounding stroma is $\alpha 6^-$

Fig. 21 Glomangioma (skin). The neoplastic EC are $\beta 3^+$. The glomus cells are $\beta 3^-$ in the depicted area

Fig. 22 Cavernous haemangioma (liver). The $\beta 4$ chain is evenly expressed in neoplastic EC

Fig. 23 Haemangiopericytoma (neck). The $\alpha 2$ subunit is absent in the pericytic tumour cell population while EC of reactive blood vessels are $\alpha 2^+$

Fig. 24 Well-differentiated angiosarcoma (Stewart-Treves syndrome). The capillary-sized vascular proliferates that infiltrate and dissect the dermal collagen are lined by $\beta 1^+$ neoplastic EC

Fig. 25 Poorly differentiated angiosarcoma (thyroid). The solid tumour cells are $\alpha 3^+$. Small follicular remnants (*arrow heads*) are $\alpha 3^-$

Fig. 26 Poorly differentiated angiosarcoma (liver). The $\alpha 5$ chain is expressed in the neoplastic population while fibrous septae are $\alpha 5^-$

Fig. 27 Well-differentiated angiosarcoma (Stewart-Treves syndrome). The neoplastic endothelium of infiltrating capillary-sized vessels lacks the $\beta 4$ subunit. Dermal adnexae are $\beta 4^+$

The $\alpha 5$ subunit was broadly distributed in EC. Apart from fenestrated EC of renal glomeruli and from sinusoidal EC of the spleen, the endothelia were evenly $\alpha 5^+$ (Fig. 8).

The $\alpha 6$ subunit paralleled the expression pattern of $\alpha 5$ in capillary EC of the continuous and fenestrated type (Fig. 9). Unlike $\alpha 5$, however, $\alpha 6$ was present in discontinuous EC of the splenic sinuses (Fig. 10) while liver and lymph node sinuses were $\alpha 6^-$. With few exceptions like the portal veins of the liver, medium-sized arteries of the kidney, and umbilical vein, EC of medium-sized and large blood vessels were $\alpha 6^+$ throughout.

The great majority of EC were evenly $\beta 3^+$ (Figs. 11–13). Absence of $\beta 3$ was restricted to fenestrated EC in glomerular capillaries and to sinusoidal EC in the lymph node and spleen. A subset of capillary EC in the exo- and endocrine pancreas, adrenal and liver was also $\beta 3^-$. Except for endothelium of the renal glomeruli and lymph node sinuses and for an EC subset in liver sinuses, capillary EC were αv^+. EC of arterioles/small arteries, venules/small veins and epithelioid venules were αv^+ while EC of medium-sized arteries and veins showed a variable expression of αv thus slightly differing from $\beta 3$ (Fig. 14). EC of femoral and umbilical arteries and of the femoral vein were αv^+ but umbilical vein endothelium was αv^-.

The $\beta 4$ subunit was present in the majority of capillary EC studied. Similarly to $\alpha 6$, the $\beta 4$ chain was expressed in capillary endothelium of splenic sinuses (Fig. 15) while being absent in sinusoidal EC of the lymph node and liver (Fig. 16) and in fenestrated EC of renal glomeruli. With rare exceptions, namely EC of hepatic central and portal veins (Fig. 16), arterioles/small arteries, venules/small veins and epithelioid venules were evenly $\beta 4^+$ throughout. The amount of $\beta 4^+$ EC was low in various medium-sized arteries and in medium-sized renal veins. In large vessels, expression of $\beta 4$ was restricted to an EC subset o the femoral vein and the umbilical arteries.

Vascular tumours

All benign and malignant vascular tumours examined were consistently $\beta 1^+$ (Fig. 24). The glomus cells of one glomangioma and the pericytic tumour cells of four haemangiopericytomas studied were also $\beta 1^+$.

The neoplastic EC in four out of nine haemangiomas (which included two of the capillary and two of the cavernous type) and in the single cases of glomangioma and infantile haemangioendothelioma were $\alpha 1^+$ throughout (Fig. 17). Three haemangiomas comprised $\alpha 1^+$ and $\alpha 1^-$ neoplastic EC in variable amounts and one further case lacked the $\alpha 1$ subunit (Fig. 18). The glomus cells were partly $\alpha 1^+$. A consistent expression of the $\alpha 1$ chain was found in three out of five angiosarcomas, one tumour comprised $\alpha 1^+$ and $\alpha 1^-$ neoplastic cells in variable amounts, and one case was completely $\alpha 1^-$. In one haemangiopericytoma, the pericytic tumour cells were consistently $\alpha 1^+$.

The $\alpha 2$ subunit was variably and inconsistently expressed in vascular tumours. The neoplastic EC in six haemangiomas, in the glomangioma and in the infantile haemangioendothelioma were focally $\alpha 2^+$ in the absence of $\alpha 2$ in two further haemangiomas (Fig. 19). The glomus cells were $\alpha 2^-$. One angiosarcoma was $\alpha 2^+$ throughout, and four cases comprised $\alpha 2^+$ and $\alpha\ 2^-$ tumour cells in variable amounts. In one haemangiopericytoma all and in one further case a subset of neoplastic pericytes expressed the $\alpha 2$ subunit but the pericytic tumour cell population was $\alpha 2^-$ in two cases (Fig. 23).

Seven haemangiomas and the single cases of glomangioma and infantile haemangioendothelioma were $\alpha 3^+$ throughout. Two further haemangiomas were partly $\alpha 3^+$. In two angiosarcomas all neoplastic cells expressed the $\alpha 3$ (Fig. 25), two cases comprised $\alpha 3^+$ and $\alpha 3^-$ tumour cells in variable amounts, and one case lacked the $\alpha 3$ subunit. In two haemangiopericytomas the pericytic tumour cell population was entirely $\alpha 3^+$.

Expression of the $\alpha 4$ chain was confined to a neoplastic subset of a single case of infantile haemangioendothelioma studied and to the entire pericytic tumour cell population of one haemangiopericytoma.

Apart from one haemangioma in which a minor neoplastic subset was $\alpha 5^-$, the vascular tumours examined were $\alpha 5^+$ throughout (Fig. 26). The pericytic tumour cells of three haemangiopericytomas were also consistently $\alpha 5^+$.

The entire neoplastic EC population was $\alpha 6^+$ in all haemangiomas (Fig. 20), in the single cases of glomangioma and infantile haemangioendothelioma, and in four angiosarcomas. One angiosarcoma was partly $\alpha 6^+$. Glomus cells were $\alpha 6^-$. In one haemangiopericytoma the pericytic tumour cells were $\alpha 6^+$ throughout.

In seven haemangiomas and in the glomangioma, the neoplastic EC population was evenly $\beta 3^-$ (Fig. 21). The glomus cells of the glomangioma, and the neoplastic EC of two haemangiomas and of the infantile haemangioendothelioma were partly $\beta 3^+$. In angiosarcomas, expression of the $\beta 3$ chain was restricted to a tumour cell subset of three cases. Pericytic tumour cells were $\beta 3^+$ in one case.

Six haemangiomas and the infantile haemangioendothelioma were consistently αv^+, three haemangiomas comprised αv^- neoplastic EC in variable amounts. In the glomangioma, a subset of neoplastic EC was αv^+. The glomus cells were entirely αv^+ in this case. Two angiosarcomas were αv^+ throughout, one case comprised an αv^+ tumour cell subset, and two cases lacked the αv subunit. The pericytic tumour cells of two haemangiopericytomas were evenly αv^+, one case expressed the αv subunit in a neoplastic subpopulation.

The $\beta 4$ chain was consistently expressed in five haemangiomas (Fig. 22), three cases were focally $\beta 4^+$. One case and the infantile haemangioendothelioma were $\beta 4^-$. In the single case of glomangioma studied, expression of $\beta 4$ was confined to neoplastic EC. A mere of tu-

mour cells was $\beta4^+$ in one angiosarcoma, four cases lacked the $\beta4$ subunit (Fig. 27). The pericytic tumour cells were evenly $\beta4^-$.

Discussion

This study revealed that EC in situ not only dispose of a broad repertoire of integrin subunits but show also considerable diversity in the distribution patterns of these molecules depending on the specific vascular microenvironment. In vascular tumours, the orthologous situation was essentially conserved.

The consistent expression of the $\beta1$ subunit in all EC studied corresponds to data from the literature [2, 7, 17, 19] and is expected in view of the presence of most $\beta1$-associated α subunits in endothelium. Expression $\beta1$ was evenly detected in all vascular tumours.

Confirming and extending in situ and in vitro data reported by Defilippi et al. [7], the distribution pattern of the $\alpha1$ subunit in EC showed a clear-cut association with the vessel size. EC of nearly all capillaries and of most venules/small veins examined in situ expressed the $\alpha1$ chain while EC of medium-sized and large vessels lacked the $\alpha1$ subunit. Additionally, we found absence of $\alpha1$ in arteriolar EC. The $\alpha1$ chain was the only integrin subunit that shared the consistent presence in sinusoidal EC of different microenvironments in common with the $\beta1$ chain. Expression of $\alpha1$ in sinusoidal endothelium in the spleen and liver is in accordance with reports from other investigators [5, 37]. Neoplastic EC paralleled generally the $\alpha1$ subunit profile of non-neoplastic endothelium. Thus, for example, the absence of $\alpha1$ in EC of larger vessels was retained in endothelium of cavernous spaces of some haemangiomas while EC of capillary proliferates were $\alpha1^+$ in an orthologous manner.

In vitro studies have shown that human umbilical vein endothelial cells (HUVEC) express the $\alpha2$ subunit and that $\alpha2$ serves as a receptor for laminin, collagen and even for fibronectin in these cells [15, 19]. In situ, $\alpha2$ was evenly expressed in umbilical and femoral vein EC. Similar to findings made in capillary endothelium of breast tissue in situ [38] and in microvascular EC of the foreskin in vitro [17], most EC studied showed an inconsistent staining for $\alpha2$ as did vascular tumours.

The $\alpha3$ chain differed from all other $\beta1$-associated α subunits in its expression in fenestrated EC of glomerular capillaries. Although being present in EC of some medium-sized and large vessels, $\alpha3$ was expressed preferentially in EC of small vessels. In accordance with these findings in situ, larger amounts of $\alpha3$ were found in microvascular when compared to macrovascular endothelium in vitro [7]. The frequent presence of $\alpha3$ in vascular tumours corroborates data presented by Miettinen et al. [24].

Except for the weak expression of $\alpha4$ in capillary EC of the placenta corresponding to data from others [16] and for some $\alpha4^+$ EC in the thyroid, lung and exocrine pancreas, the $\alpha4$ subunit was absent in endothelium. In agreement with in vitro data [2, 19, 36], umbilical cord endothelium was $\alpha4^-$ in situ. Recently, however, Massia et al. [21] reported that $\alpha4$ is present in HUVEC and functions as a receptor for REDV (arg–glu–asp–val)-mediated adhesion to the IIICS regions of plasma fibronectin. Growth factors like retinoic acid and basic fibroblast growth factor (bFGF) have been shown to change the integrin repertoire of EC in vitro [7, 9]. It might therefore be argued to the reported expression of $\alpha4$ in HUVEC results from the retinal-derived growth factor supplemented to the culture medium in this respective study. In contrast to the neo-expression of $\alpha4$ observed in tumours of myo- and neurogenic origin [22, 23], the physiological absence of $\alpha4$ was generally conserved in vascular tumours.

Disregarding the $\beta1$ subunit, the $\alpha5$ chain was most broadly distributed within the EC studied. Unlike reported findings [37], however, $\alpha5$ was absent in the sinusoidal endothelium of the spleen and also in capillary EC of renal glomeruli. The $\alpha5$ chain is part of the „classical" fibronectin receptor, $\alpha5\beta1$, which binds fibronectin in an RGD-dependent fashion [30]. In vitro, fibronectin has been shown to promote EC adhesion, spreading, and formation of adhesion plaques in an RGD-dependent manner [8]. It is meanwhile accepted that monolayer organization of EC is mediated via $\alpha5$ [18]. Given the consistent expression of $\alpha5$ in nearly all vascular tumours, it seems conceivable that an interaction between fibronectin and $\alpha5$ also supports neoplastic vascular growth. In this context it is worth mentioning that fibronectin was shown to stimulate the proliferation of quiescent melanoma cells and that $\alpha5$ and $\beta1$ subunits are required for this process [25].

Like $\alpha5$, the $\alpha6$ subunit was frequently observed in EC. In continuous and in fenestrated capillary endothelium, the $\alpha5$ and $\alpha6$ chains were expressed in parallel while sinusoidal EC showed a complementary distribution pattern of these molecules. Although in vitro studies yielded low amounts of $\alpha6$ in microvascular when compared to macrovascular EC [7], we found only a focal expression of $\alpha6$ in some medium-sized and large vessels while most capillary EC were consistently $\alpha6^+$. Moreover, contrasting to a decrease in $\alpha6$ subunit expression during malignant transformation of neuroectodermal and various epithelial cells [26, 29], expression of $\alpha6$ in neoplastic EC was generally conserved.

The first integrin described on EC in vitro was the vitronectin receptor ($\alpha v\beta3$; [4, 10]). Except for some capillary EC, the $\beta3$ subunit was consistently expressed in non-neoplastic EC in situ. Unexpectedly, however, some $\beta3^+$ EC lacked the αv subunit. Since, according to the current view, the $\beta3$ subunit combines only with the αv and αIIb subunits and since expression of αIIb is known to be absent from EC [34], it cannot be excluded that in some EC the $\beta3$ chain associates with another, even yet unknown α subunit. Unlike members of the $\beta1$ integrin subfamily, $\beta3$ and αv subunits were expressed at

lower amounts in some vascular tumours (especially in angiosarcomas) when compared to non-neoplastic EC in situ. However, microvascular EC treated with cytokines in vitro displayed an increase in $\beta 3$ and αv expression indicating the involvement of these molecules in neoplasia-independent pathophysiological events like inflammation and wound healing [35].

Unlike the initial report of a restricted distribution of $\beta 4$ in human tissues [33], the $\beta 4$ subunit was frequently detected in EC. In addition to its presence in EC of medium-sized vessels also observed in mice [14], $\beta 4$ was found in EC of various capillaries and small vessels. At present, the only α chain known to combine with the $\beta 4$ subunits is $\alpha 6$ [11]. In line with this view, all EC expressing the $\beta 4$ subunit were evenly $\alpha 6^+$. Remarkably, the $\alpha 6$ and $\beta 4$ subunits were both absent in EC of portal and central veins of the liver and in lymph node and liver sinuses and were co-expressed at high levels in sinusoidal EC of the spleen. In contrast to $\alpha 6$, however, expression of the $\beta 4$ chain was reduced in some vascular tumours. Similar findings were observed in neural tumours compared with reactive neural tissue [23].

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