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Alpha 6 integrin distribution in human embryonic and adult tissues

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Abstract Alpha 6 integrin is an adhesion molecule that connects cells with extracellular matrix molecules of the laminin family. The laminin interaction seems to be essential for cell differentiation during embryogenesis and for the subsequent maintenance of tissue integrity in the adult. Alpha 6 integrin can also interact with laminin-independent cellular ligands and in this way plays a role in homing of leucocytes. Furthermore, in cancer biology α6 integrin has an important role in metastasis and as a possible new prognostic factor; exact knowledge of α6 integrin distribution in normal human tissues is therefore a crucial element. By immuno-histochemical methods we have screened α6 integrin expression of representative human tissues from the adult and the embryonic organism. All tested epithelia were α6 integrin positive, except for the endocrine cells of the pancreas and the adrenal glands. Heterogeneous staining was found on non-epithelial tissues. Strong staining was evident in peripheral nerves (Schwann cells), germ and Sertoli cells, endothelia, and smooth muscle cells of the myometrium. Weak staining was found in nerve cells of the stratum granulosum, the microglia, Kupffer's cells and stromal cells of the ovary. All fibroblasts, striated muscle cells and astrocytes were negative. The tissue distribution of α6 integrin and the semi-quantitative estimation of their expression level should provide a better understanding of α6 integrin function under normal and phathological conditions, in particular in tumour progression.

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Introduction

Integrins are adhesion molecules that are involved in many biological processes including embryonic development, the maintenance of tissue integrity, wound healing, cancer development and metastasis (Albelda and Buck 1990; Hynes 1992). In vertebrates, twenty different integrins have been detected so far, of which all are heterodimeric molecules composed of an α and a β subunit. The actual repertoire results from the combination of fourteen α and eight β chains. The α6 integrin chain is synthesized as a single transmembrane protein (1050 amino acids), which is proteolytically cleaved into a heavy and a light chain, both chains being covalently linked by disulphide bonds in the extracellular domain (Hogervorst et al. 1991). It contains three binding sites for divalent cations and nine potential *N*-linked glycosylation sites. It is encoded by two alternatively spliced RNAs which translate two variant proteins, called α6A and α6B, differing in their cytoplasmic domains (Hogervorst et al. 1991; Cooper et al. 1991; Hierck et al. 1993).

It has been shown that the α6 integrin chain combines with either β1 or β4 subunits (Quaranta 1990). Both α6 integrins can bind to laminin, a family of extracellular matrix molecules (Tryggvason 1993). While laminin adhesion by α6β1 integrin could be demonstrated with cell lines originating from different tissues, α6β4 mediated adhesion was restricted to a colon carcinoma cell line (Lee et al. 1992; Sonnenberg et al. 1990). This suggested that the function of α6 integrins was tissue specific. There is growing evidence from work with mice that α6 integrins play a role as adhesion molecules during early development. It is already present on mouse embryonic stem cells (ES) and has been shown to serve as a laminin receptor on mesenchymal cells during gastrulation (Burdsal et al. 1993). In the developing kidney, α6 integrin is absent from the mesenchyme but is essential for the formation of polarized epithelial cells, in particular in the tubules (Sorokin et al. 1990).

Recently, it has been shown that α6 integrins can also mediate cell-cell adhesion in a laminin-independent

fashion. This interaction may play a role in leukocyte homing, i.e. the adhesion of these cells to the vascular endothelium (Lenter et al. 1993). Interestingly we could show that this mechanism was also used by melanoma cells during the process of metastasis (Ruiz et al. 1993). During neoplastic transformation of human fibroblasts α6 integrin was upregulated (Lin et al. 1993). In fact, it becomes more and more evident that a high level of α6 integrin expression can accompany the tumorigenic conversion of certain normal cells (Wolf et al. 1990). Exact knowledge of the α6 integrin expression pattern in normal tissues is therefore a necessity. For rodents, the α6 integrin distribution is relatively well known, but for human tissue this information is rather fragmentary (Hogervorst 1993; Sonnenberg and Linders 1990). Here we present a catalogue of α6 integrin distribution of the most representative human tissues. We performed immuno-histochemistry on frozen tissue sections, using an antibody that recognises the α6 integrin subunit. Keeping the incubation times of all reagents constant allowed a semiquantitative estimation of the expression level of α6 integrin by the different tissues.

Materials and methods

Tissues

Normal human tissue was taken from biopsies after surgery or was obtained less than 6 h postmortem at the Klinik der Justus-Liebig-Universität, Giessen. Patients or deceased persons were between 30 and 55 years old. Embryonic tissue was obtained from the Department of Gynaecology, Universität Giessen. The different tissues were snap frozen in liquid nitrogen and stored at −70° C until use.

Anit-α6 integrin antibody

The antibody EA-1 was generated in rats against cell surface proteins of mouse endothelioma cells. The antibody, an IgG_{2a}, recognizes the α6 chain of mouse and human α6β1 and α6β4 integrins. This has previously been confirmed by immunoprecipitation followed by *N*-terminal micro-sequencing of the precipitated protein and by preclearing experiments using another anti-α6 integrin antibody GoH3 (Ruiz et al. 1993).

Immunohistologcial staining

The alkaline phosphatase anti-alkaline phosphatase (APAAP) staining technique was applied according to Cordell et al. (1984) with the following modifications: frozen tissue sections were incubated in 1% bovine serum albumin (BSA) in Dulbecco's phosphate buffered saline (DPBS) for 10 min at room temperature (RT), loaded with EA-1 monoclonal antibody (mAb; 10 µg/ml) and incubated at RT for 1 h in a humidified chamber. Sections were washed twice in 1% BSA in DPBS and rabbit anti-rat antibody (Dako A/S, Glostrup, Denmark), which crossreacted with mouse IgG, was used as a secondary reagent. After 30 min incubation at RT, sections were washed twice and alkaline phosphatase/rat monoclonal anti-alkaline phosphatase complexes (Dako A/S, Denmark) were loaded onto the sections. Sections were incubated at RT for 1 h, then washed and as a developing reagent the following solution was used: 10 mg naphthol AS-BI-phosphate sodium salt (Sigma, St. Louis, Mo., USA) was added to 500 µl *N*,*N*-dimethylformamide (Merck, Darmstadt, Germany) and 20 ml Michaelis buffer (19.44 g sodium acetate, and 29.42 g 5,5-diethyl-barbituric acid sodium salt in 1 l water adjusted to pH 9.2–9.8); 20 mg Fast Red TR salt (Sigma) and 10 mg levamisole were dissolved in 30 ml Michaelis buffer; both solutions were mixed and filtered before use. Staining of sections was developed for 30 min at RT and after washes in 1% BSA in DPBS and water for 10 min, were counterstained using Mayer's haemalum solution. Sections were mounted in Mowiol (Bayer, Zürich, Switzerland) and analysed using a Zeiss (Jena, Germany) Axiophot microscope equipped with a CCD video camera (Zeiss, Oberkochen, Germany) and a Sony videoprinter UP-5000P. The immunostaining results were evaluated semi-quanitatively and classified on a 5-point scale, as indicated in the tabular presentation of the data.

Results

General observation

Alpha 6 integrins were expressed by most epithelial cells. Often the expression was polarized with larger amounts on the cellular plasma membrane adjacent to the extracellular basement membrane (see e.g. Figs. 1c and 2i). In other tissues α6 integrin expression was not polarized; it was often also found within the cell and not only on the plasma membrane (see e.g. endothelia in Figs. 1a nd 2b). Since α6 integrin expression varied enormously among different tissues of the adult and embryonic human body, we decided to quantify α6 integrins for cells of the most representative tissues; the results are presented in Tables 1–4.

Expression of α6 integrin in human fetal tissues at 10 weeks of development

Most epithelial tissues already showed strong α6 integrin expression at this early time of development (Table 2). Often epithelial tissues were composed of α6 integrin positive and negative cell layers. The main exception was the epithelium of the adrenal gland which

Table 1 Immunohistochemical staining of α6 integrin in human fetal tissues

Nervous tissue:	Peripheral neurons	+++
Eye retina	Outer neuroblast cells	(+++)
	Inner neuroblast cells	(+)
Lymphatic tissues:		
Lymph node	Lymphocytes	(+)
Thymus	Thymocytes	(++)
	Hassall's corpuscles	++
Connective tissue	Fibroblasts	−
Muscle tissues	Muscle cells	+++
	Heart muscle	−
Other	Endothelium	+++
	Endothel of kidney glomerulus	+
	Chondrocytes	(++)

+++, Strong staining in all cells; ++, strong staining in about 2/3 of the cells; +, strong staining in about 1/3 of the cells; ( ), weak stainings; −, no staining

Table 2 Immunohistochemical staining of α6 integrin in human fetal epithelial tissues

Eye retina	Pigment epithelium	+ + +
Skin:		
Epidermis	Basal cell layer	+ + +
	Intermediate layer	+ + +
	Epitrichium	−
Trachea	Basal epithelial cells	+ + +
	Luminal epithelial cells	−
Lung:		
Proximal tubules	Basal cells	+ + +
	Luminal cells	−
Distal tubules		+ + +
Gastrointestinal tract	Stomach	+ + +
	Small intestine	+ + +
	Large intestine	+ + +
Liver	Hepatocytes	+ + +
Adrenal gland		−
Kidney	Blastem	+ + +
	Proximal tubules	+ + +
	Distal tubules	+ + +
	Collecting tubules	+ + +
Urinary bladder	Transitional cells	+ + +
Mesonephric duct		+ + +
Paramesonephric duct		+ + +

For definition of the categories of immunohistochemical staining see footnote to Table 1

Table 3 Immunohistochemical staining of α6 integrin in human adult tissues

Nerve tissues	Astrocytes	−
	Microglia	+
	Nerve cells (other)	−
	Stratum granulosum/cerebellum	+ +
	Ependymal cells	−
	Epithelium of choroid plexus	+ + +
	Epithelial cells of arachnoid	+
	Peripheral neurons	+ + +
Lymphatic tissues:		
Lymph node	Germinal centre cells	+ +
	T cell region	+ + +
	Sinus cells	+
Thymus		
epithelial cells	Cortical	+ +
	Medullary	+ + +
	Hassals corpuscules	+ +
Lymphocytes	Thymocytes	(+ + +)
Muscle tissues	Smooth	(+)
	Striated	−
	Heart	(+)
	Myometrium	+ + +
Other tissues:		
Fibroblasts		−
Endothelium	Large arteries	(+ + +)
	Large venules	+ + +
	Microvessels	+ + +
Kupffer's cells		+
Stromal cells of ovarii		+
Stromal cells of endometrium		+ +
Germinal cells of testis		+ + +
Leydig's cells		−
Sertoli cells		+ + +

For definition of the categories of immunohistochemical staining see footnote to Table 1

was totally negative. In the skin the basal and intermediate epithelial layers were positive but the outermost layer, the epitrichium, was α6 integrin negative (Fig. 1a). This polarized tissue expression was also found in the hair follicles, the trachea, the gut, kidney tubules, the ureter and even the Müllerian duct (Fig. 1b–k). The basal epithelial layer in the trachea showed only intermediate α6 expression over the entire cell body, allowing a very clear visualization of the α6 integrin positivity of the intimate contact region of the epithelia with the basement membrane (Fig. 1c).

Depending on the tissue, α6 integrin expression was seen to be regulated during embryogenesis. In the developing lung, the luminal epithelial cells of differentiated proximal tubules were negative, whereas in non-differentiated distal tubules these cells were positive (Fig. 1d). In the gut, however, differentiated epithelia of small intestinal villus and the non-differentiated cells of the crypt were both positive for α6 integrin staining, forming an intensity gradient in a basal to luminal direction (Fig. 1e, f). Homogeneous, non-polarized but strong staining was found on hepatocytes in the liver, on germ cells in the gonads and on muscle cells (Fig. 1g, l and n). Homogeneous staining over the plasma membrane was also found on nerve tissues, for instance the outer neuroblast cells of the developing eye and the peripheral neurons (Schwann cells, Fig. 1m, o). Very strong staining was generally found on all types of endothelial cells, capillaries, venules and arterioles (Fig. 1a–f and h, n, p).

Expression of α6 integrin in human adult tissues

The finding of significant α6 integrin expression on embryonic epithelia was also encountered in adult tissues. A typical example was found in the skin, where the mitotic basal and spinous cell layers were positive for α6 integrins. However, when these epithelial cells were encountered towards the granular and transitional cell layers, a gradual loss of α6 integrin expression occurred (Fig. 2e, f). The same effect was even more evident for the oesophagus (Fig. 2l). More α6 integrin expression was observed in the adult than in the embryo in certain tissues that have developed pronounced specialization, such as the respiratory epithelium of the trachea (compare Figs. 1c and 2k). In general, polarized α6 integrin expression was observed in adult epithelia of skin, hair follicle, sweat gland, duct of salivary gland, mammary gland, oesophagus, mucosa of the stomach, gut, kidney, endometrium and prostate gland (Figs. 2e–o and 3b–f). There were two α6 integrin negative epithelia, the islet of Langerhans cells of the pancreas and the cells of the adrenal gland (Fig. 3a). Interestingly, both are highly specialized endocrine cells.

Table 4 Immunohistochemical staining of α6 integrin in human epithelia

Skin:		
Epidermis	Basal cell layer	+++
	Spinous cell layer	+++
	Other cell layers	−
Sweat glands	Acinus cells	+++
	Ducts	+++
Sebaceous gland	Acinus cells	+++
Salivary gland	Acinus cells	+++
	Intercalated portion	+++
	Ducts	+++
Thyroid gland	Follicular cells	++
Mammary gland:		
Ducts	Epithelial cells	+++
	Myoepithelial cells	+++
Lung	Respiratory epithelium	+++
	Pneumocytes	+++
Oesophagus		+++
Gastrointestinal tract	Stomach	+++
	Small Intestine	+++
	Large Intestine	+++
Liver	Hepatocytes	+++
	Bile ducts	+++
Pancreas	Acinus cells	+++
	Centroacinar cells	+++
	Intercalated ducts	+++
	Ducts	+++
	Islets of Langerhans	−
Adrenal gland		−
Kidney	Glomerulus (endothel)	(++)
	Bowman's capsule	(++)
	Proximal tubules	+++
	Distal tubules	+++
	Collecting tubules	+++
Urinary bladder	Transitional cells	+++
Uterus	Glands of endometrium	+++
Ovary	Follicular epithelium	+++
	Germinal epithelium	+++
Prostate gland	Glands	+++
	Ducts	+++
Epididymis:		
Ductuli efferentes	Basal cells	+++
	Ciliated cells	−

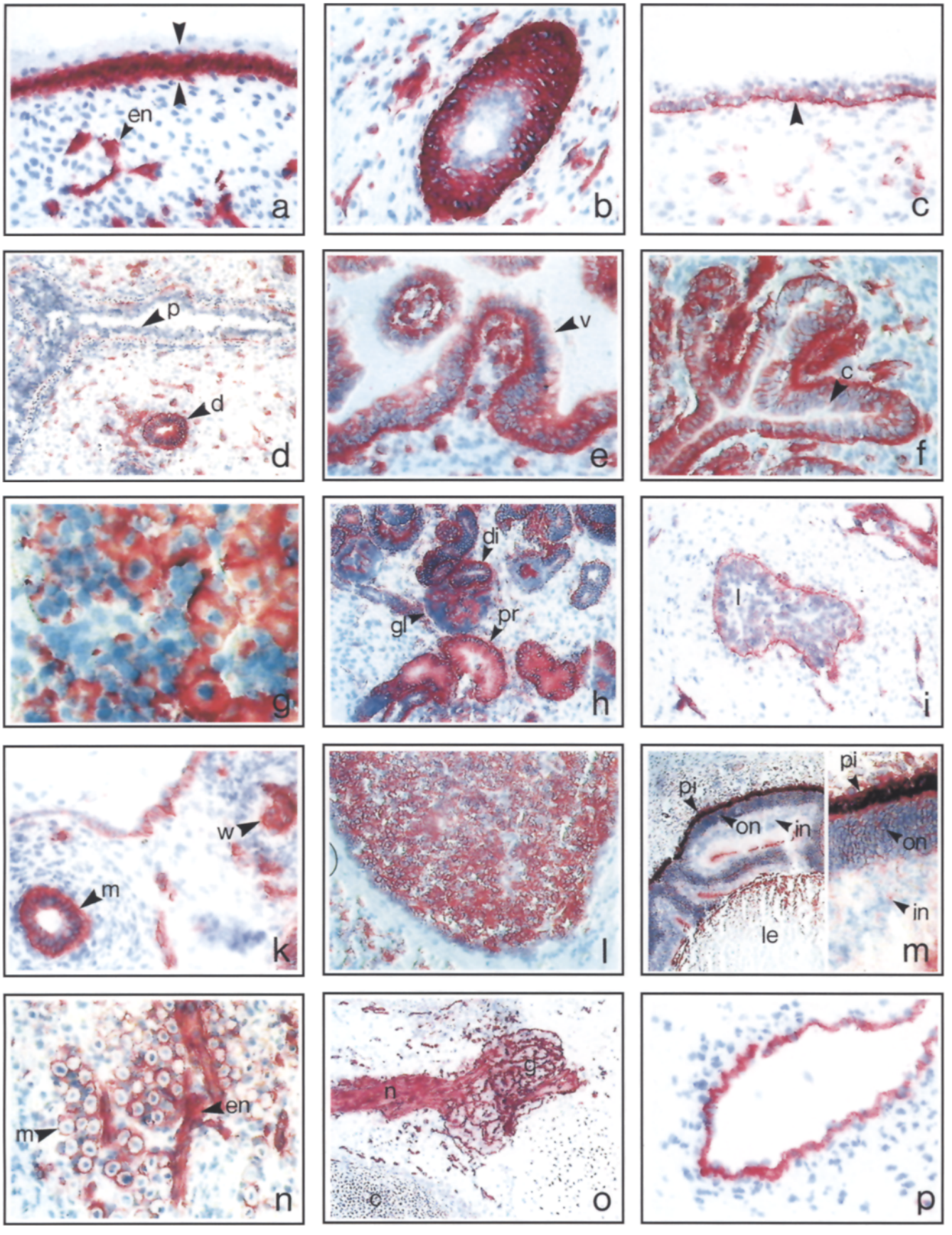
For definition of the categories of immunohistochemical staining see footnote to Table 1

As already found in the embryo, peripheral neurons strongly expressed α6 integrins (Fig. 2d), whereas nerve cell bodies in the cerebellum showed only weak expression and those in the cortex and the medulla of the brain were negative (Fig. 2a–c). A major difference to the embryo was found in the staining pattern of vascular endothelium. While in the embryo all endothelial cells were strongly positive for α6 integrins (Fig. 1), strong staining in the adult was only found on large venules and capillaries (Figs. 2e and 3o); large arteries showed very faint staining (Fig. 3p). In lymphoid tissues we found α6 integrin positive cells in the germinal centres and the T-cell regions of lymph nodes (Fig. 3h, i). The thymus showed the most intensive staining on its vascular endothelial cells and on epithelial cells, both in the cortex and the medulla. Thymocytes had only moderate α6 integrin expression (Fig. 3l, m). In addition, a different α6 expression pattern between the embryonic and the adult organism was also observed in muscle tissues, the embryonic showing strong and non-polarized ex-

Fig. 1a–p Frozen tissue sections of various embryonic human tissues labelled for α6 integrin by the antibody EA-1 using the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique. **a** Skin; *arrowheads* point to the basal and intermediate epithelial layer; *arrowhead* (*en*) points to a blood vessel. **b** Hair follicle. **c** Trachea; the *arrowhead* points to the basal epithelial layer. **d** Lung; *arrowhead* (*p*) points to a differentied proximal tubule; *arrowhead* (*d*) points to a non-differentiated distal tubule. **e** Small intestine; *arrowhead* (*v*) points to the luminal side of a developing villus. **f** Large intestine; *arrowhead* (*c*) points to the luminal side of a crypt. **g** Liver. **h** Kidney; *arrow* (*g*) points to glomerulum; *arrowhead* (*di*) to a distal tubule; and *arrowhead* (*pr*) toa proximal tubule. **i** Ureter showing luminal (*l*) differentiated epithelial cells. **k** Retroperitoneal region, *arrowhead* (*m*) points to Müllerian duct, *arrowhead* (*w*) to the Wolffian duct. **l** Gonads; germ cells are all strongly positive for α6 integrin. **m** Retina showing pigment epithelium (*pi*), outer neuroblast cells (*on*), inner neuroblast cells (*in*), and lens (*le*). **n** Muscle; *arrowhead* (*m*) points to a muscle cell which is positive for α6; *arrowhead* (*en*) points to a blood vessel. **o** Ganglion from the thorax region showing peripheral nerve cell bodies (*g*), peripheral neurons (*n*) and cartilage (*c*). **p** Arteria renalis. **m, o,** × 100; **d, h, i, l,** × 200; **a–c, e–g, k, m, n, p,** × 400

Fig. 2a–p Frozen tissue sections of various normal adult human tissues labelled for α6 integrin by the antibody EA-1 using the APAAP technique. **a** Brain; the *left* part is cortex, the *right* medulla. **b** Higher magnification of brain sections showing nerve cells (*nc*), astrocytes (*as*), vascular endothelium (*en*). **c** Brain; stratum granullosum of the cerebellum showing nerve cells (*nc*), endothelial cells (*en*). **d** Skin; corium, *arrowhead* points to a peripheral nerve. **e** Skin; *arrowheads* point to epidermis (*en* skin endothelium). **f** Skin; *arrowheads* point to hair follicle. **g** Skin showing sweat glands; *arrows* point to ducts (*du*) and to acinus cells (*ac*). **h** Salivary gland; a duct is shown in the centre; *insert* shows acinus cells and intercalated portions marked by an *asterix*. **i** Mammary gland; the *insert* shows surrounding connective tissue. **k** Trachea; *arrowheads* point to respiratory epithelium. **l** Oesophagus; *arrowheds* point to non-keratinized squamous epithelium. **m** Stomach showing sub mucosa (*sm*), mucosa epithelial cells (*mu*). **n** Small intestine showing epithelial cells of the jejunum crypts. **o** Liver; *arrowheads* point to a bile duct, *arrowhead* (*en*) points to a blood vessel. **p** Liver. **a, h, i, n,** × 100; **e–g, h, k–m, o,** × 200; **b–d, p,** × 400

Fig. 3a–p Frozen tissue sections of various normal adult human tissues labeled for α6 integrin by the antibody EA-1 using the APAAP technique. **a** Pancreas showing acinus cells (*ac*) and islet of Langerhans (*il*). **b** Kidney showing cortex, proximal (*pr*) and distal (*di*) tubules, endothelial cells of the glomerulus (*gl*). **c** Kidney; medulla, collecting tubules (*arrowhead*). **d** Urinary bladder; *arrowheads* point to transitional epithelium. **e** Endometrium; *arrowheads* point to the glands, stromal cells (*st*). **f** Prostata gland. **g** Epididymis showing basal cells of ductuli efferentes (*arrowheads*). **h** Peripheral lymph node showing follicular dendritic cell region in germinal center (*gc*). **i** Peripheral lymph node showing T cell region. **k** Peripheral lymph node showing sinus (*si*) region. **l** Thymus showing cortical region (*co*), epithelial cells (*ep*), endothelial cells (*en*). **m** Thymus showing cortical (*co*) and medullary region (*me*), *arrowhead* points to a larger thymic blood vessel. **n** Myometrium showing smooth muscle cells. **o** Large venule. **p** Large artery. **b–d, g, n,** × 100; **a, ,e, h, i, k–m,** × 200; **f,** × 400; **o, p,** × 800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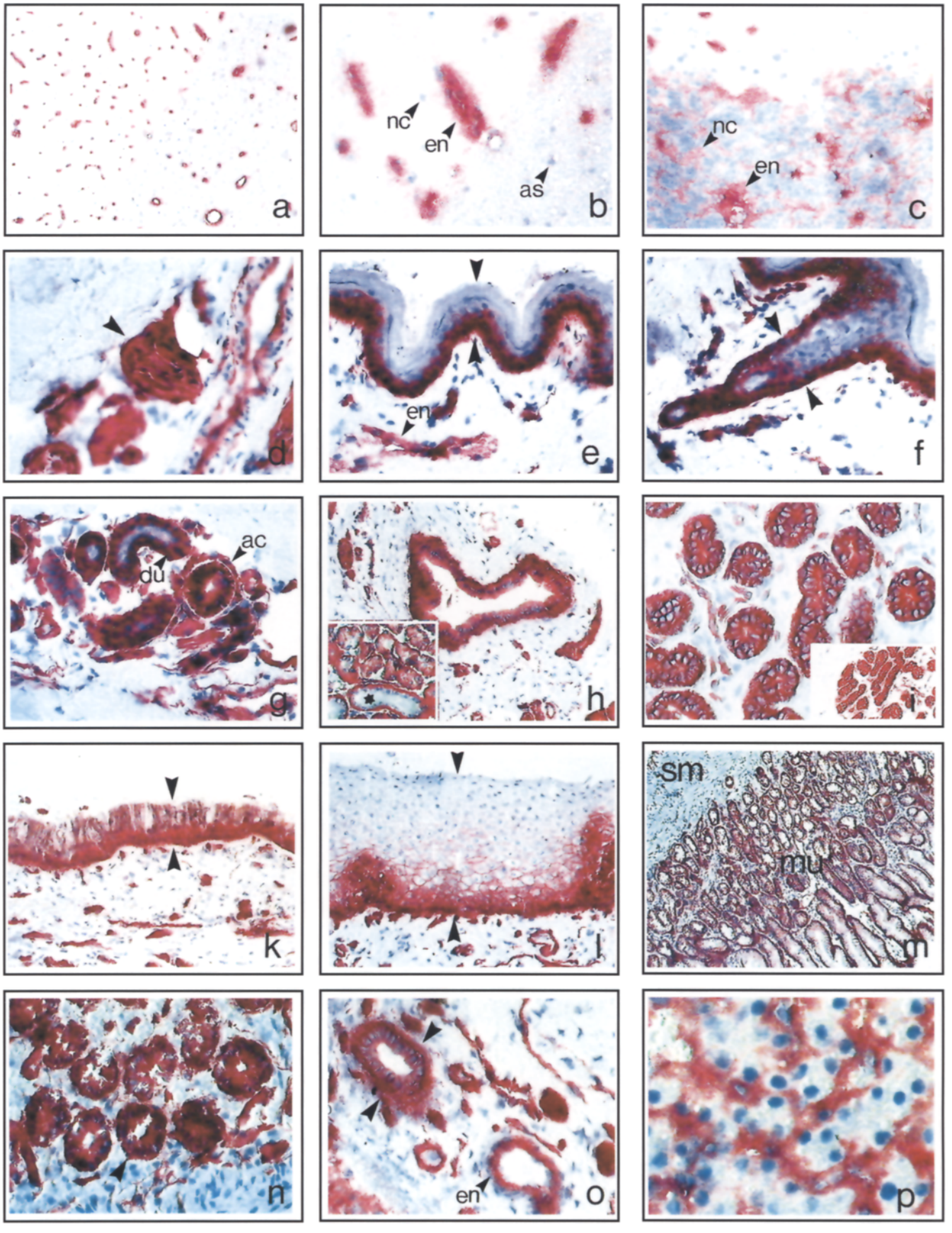

Fig. 2 (legend see page 44)

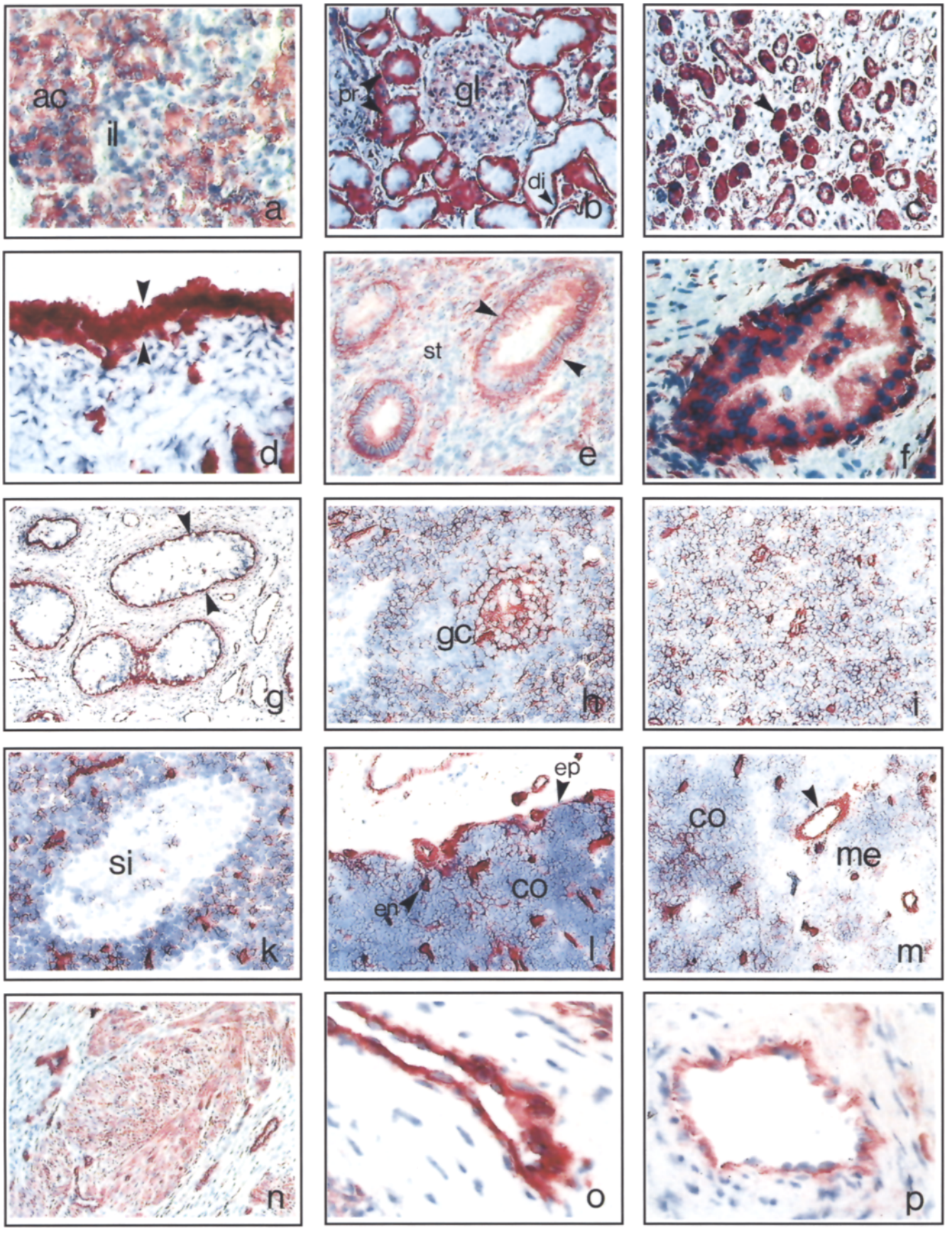

Fig. 3 (legend see page 44)

pression (Fig. 1n) and the adult smooth and striated muscle being negative (Fig. 2m). One exception was the α6 integrin positive smooth muscle of the myometrium (Fig. 3n).

Discussion

In this work we present a catalogue of α6 integrin tissue distribution of the adult and embryonic human body. Immunohistological staining was performed with rat monoclonal antibody, EA-1, which recognizes the α6 chain of human and mouse α6β1 and α6β4 integrins. Semi-quantitative estimation of the expression level was possible in this study because all tissue sections were prepared from freshly snap-frozen specimens and the histochemical reaction conditions were kept constant during the entire study. This makes the present paper of valuable help for clinical studies, since data on α6 integrin expression are fragmentary (Hogervorst 1993; Sonnenberg and Linders 1990).

Most striking was the prominent expression of α6 integrins in epithelia and this general rule holds for adult and embryonic tissues. As already seen for certain epithalia by others, α6 integrin expression was often stronger in epithelial cells located adjacent to basement membranes (DeLuca et al. 1990). This location may be due to one of the α 6 integrin functions, which is its binding ability to molecules of the laminin family (Sonnenberg et al. 1988). Interaction of epithelial cells with laminin may be one factor in maintaining the high proliferation rate of these cells, especially in the skin, the oesophagus and the gut. This would be likely to preclude the possibility that α6 integrins, upon interaction with its ligand laminin, may also transduce signals into the cell. In fact, signalling by α6 integrins has been found to regulate the activity of the chemotactic receptor for elastin-derived peptides (Blood and Zetter 1993). Whether such signals can be transduced by phosphorylation of the cytoplasmic part of the α6 chain is still a matter of debate (Hogervorst 1993; Shaw et al. 1990).

Alpha 6 integrin expression has been found in nerve cells of the developing embryo, but not in the adult organism. This may indicate that α6 integrins are involved in the migration of nerve cells or in axonal outgrowth, probably guided by laminin (Baier and Bonhoeffer 1991). As soon as these processes are completed, nerve cells may downregulate the integrin. By contrast, in the adult nervous system α6 integrins are expressed mainly in Schwann cells and not on neural cell bodies. This finding is particularly interesting, since the α6 integrins may mediate cell-cell contact and possibly interact with a putative cellular ligand rather than with the extracellular matrix component laminin. Mediation of cell-cell contact by α6 integrins may be involved in muscle cell interactions during embryogenesis and it could even play a role in myotube formation, i.e. playing a similar role as that described for α7β1 integrin (Song et al. 1992). This α6 integrin function in organogenesis would no longer be of need in the adult muscle. Accordingly, our data show that adult striated muscle does not express α6 integrins. There was one exception: the adult smooth muscle of the myometrium expressed α6 integrins, which might be explained by the fact that these muscle cells undergo hyperthrophy during pregnancy to a length ten times longer than normal (Bloom and Fawcett 1975).

A further important tissue showing α6 integrin positivity in the embryo and the adult is the vascular endothelium. Most striking was the different expression level in arteries and venules; generally venular endothelium expressed higher levels of α6 integrins. It seems that both types of endothelia use α6 integrins in order to interact with laminin present in the basement membrane. We and others found basolateral α6 integrin expression on most endothelial cells (Kennel et al. 1992). However, in venular vessels polarized expression does not occur and α6 integrins were also found on the luminal side (Ruiz et al. 1993). We recently found that luminal α6 integrins are involved in adhesion and homing of haemopoietic cells (Imhof et al. 1991; Lenter et al. 1993). Thus endothelial cells may use α6 integrin expression in the basolateral region to reinforce their contact to the extracellular matrix and expression at the luminal side for inducing the adhesion of ciculating cells. Preliminary experiments from our laboratory indicate that signal transduction induced by α6 integrin occupancy induces tight adhesion of lymphoid cells to the endothelium, probably followed by their extravasation.

In summary, we have described the distribution of α6 integrins in most human tissues. Further work will be necessary to establish their functional role and regulation in the different tissues during development and adult life. Moreover, understanding of the differential α6 integrin expression during tumour progression will be a central to further pathological study, since normal fibroblasts did not express α6 integrins whereas fibrosarcomas did (H.J. Terpe, unpublished results). This finding is in accordance with in vitro data showing that untransformed fibroblast cell lines do not express α6 integrins, immortalized, non-tumorigenic cell lines do to a moderate extent, and metastatic fibrosarcomas bear high levels of α6 integrin receptors on their cell surface (Lin et al. 1993).

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References

Albelda SM, Buck CA (1990) Integrins and other cell adhesion molecules. FASEB J 4:2868–2880

Baier H, Bonhoeffer F (1991) Axon guidance of gradients of a target-derived component. Science 255:472–475

Blood CH, Zetter BR (1993) Laminin regulates a tumor cell chemotaxis receptor through the laminin-binding integrin subunit alpha 6. Cancer Res 53:2661–2666

Bloom W, Fawcett DW (1975) Histology. WB Saunders Company, Philadelphia, pp 1033

Burdsal CA, Damsky CH, Pedersen RA (1993) The role of E-cadherin and integrins in mesoderm differentiation and migration at the mammalian primitive streak. Development 118:829–844

Cooper HM, Tamura RN, Quaranta V (1991) The major laminin receptor of mouse embryonic stem cells is a novel isoform of the alpha 6 beta 1 integrin. J Cell Biol 115:843–850

Cordell JL, Falini B, Erber WN, Gosh AK, Abdulaziz Z, Macdonald S, Pulford KAF, Stein H, Mason DY (1984) Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). J Histochem Cytochem 32:219–229

DeLuca M, Tamura RN, Kajiji S, Bondanza S, Cancedda R, Rossino P, Marchisio PC, Quaranta V (1990) Polarized integrin alpha 6 beta 4 mediates keratinocytes adhesion to basal lamina. Proc Natl Acad Sci USA 87:6888–6892

Hierck BP, Thorsteinsdóttir S, Niessen CM, Freund E, Iperen LV, Feyen A, Hogervorst F, Poelmann RE, Mummery CL, Sonnenberg A (1993) Variants of the α6β 1 laminin receptor in early murine development: distribution, molecular cloning and chromosomal localization of the mouse integrin α6 subunit. Cell Adhesion Commun 1:33–53

Hogervorst F (1993) Biochemical characterization and tissue distribution of the A and B variants of the integrin alpha 6 subunit. J Cell Biol 121:179–191

Hogervorst F, Kuikman I, van Kessel A, Sonnenberg A (1991) Molecular cloning of the human alpha 6 integrin subunit. Alternative splicing of alpha 6 mRNA and chromosomal localization of the alpha 6 and beta 4 genes. Eur J Biochem 199:425–433

Hynes RO (1992) Integrins: versatility, modulation, and signaling in cell adhesion. Cell 69:11–25

Imhof BA, Ruiz P, Hesse B, Palacios R, Dunon D (1991) EA-1, a novel adhesion molecule involved in the homing of progenitor T lymphocytes to the thymus. J Cell Biol 114:1069–1078

Kennel SJ, Godfrey V, Chang LY, Lankford TK, Foote LJ, Makkinje A (1992) The beta 4 subunit of the integrin family is displayed on a restricted subset of endothelium in mice. J Cell Sci 101:145–150

Lee EC, Lotz MM, Steele GJ, Mercurio AM (1992) The integrin alpha 6 beta 4 is a laminin receptor. J Cell Biol 117:671–678

Lenter M, Uhlig H, Hamann A, Jenö P, Imhof BA, Vestweber D (1993) A monoclonal antibody against an activation epitope on mouse integrin chain β1 blocks adhesion of lymphocytes to the endothelial integrin α6β1. Proc Natl Acad Sci USA 90:51–55

Lin C-S, Zhang K, Kramer R (1993) Alpha 6 integrin is up-regulated in step increments accompanying neoplastic transformation and tumorigenic conversion of human fibroblasts. Cancer Res 53:2950–2953

Quaranta V (1990) Epithelial integrins. Cell Differ Dev 32:361–366

Ruiz P, Dunon D, Sonnenberg A, Imhof BA (1993) Suppression of mouse melanoma metastasis by EA-1, a monoclonal antibody specific for alpha 6 integrins. Cell Adhesion Commun 1:67–81

Shaw LM, Messier JM, Mercurio AM (1990) The activation dependent adhesion of macrophages to laminin involves cytoskeletal anchoring and phosphorylation of the alpha 6 beta 1 integrin. J Cell Biol 110:2167–2174

Song WK, Weiwang W, Foster RF, Bielser DA, Kaufman SJ (1992) H36-alpha 7 is a novel integrin alpha chain that is developmentally regulated during skeletal myogenesis. J Cell Biol 117:643–657

Sonnenberg A, Linders CJT (1990) The alpha 6 beta 1 (VLA-6) and alpha 6 beta 4 protein complexes: tissue distribution and biochemical properties. J Cell Sci 96:207–217

Sonnenberg A, Modderman PW, Hogervorst F (1988) Laminin receptor on platelets in the integrin VLA-6. Nature 336:487–489

Sonnenberg A, Linders CJ, Modderman PW, Damsky CH, Aumailley M, Timpl R (1990) Integrin recognition of different cell-binding fragments of laminin (P1, E3, E8) and evidence that alpha 6 beta 1 but not alpha 6 beta 4 functions as a major receptor for fragment E8. J Cell Biol 110:2145–2155

Sorokin L, Sonnenberg A, Aumailley M, Timpl R, Ekblom P (1990) Recognition of the laminin E8 cell-binding site by an integrin possessing the alpha 6 subunit is essential for epithelial polarization in developing kidney tubules. J Cell Biol 111:1265–1273

Tryggvason K (1993) The laminin family. Curr Opin Cell Biol 5:877–882

Wolf GT, Carey TE, Schmaltz SP, McClathey KD, Poore J, Glaser L, Hayashida SJS, Hsu S (1990) Altered antigen expression predicts outcome in squamous cell carcinoma of the head and neck. J Natl Cancer Inst 82:1566–1572