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REVIEW ARTICLE

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Molecular variants of fibronectin and laminin: structure, physiological occurrence and histopathological aspects

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Abstract This review deals with biological and pathological aspects of various isoforms of the matrix molecules fibronectin and laminin. They are generated by different molecular mechanisms: ED-A$^+$ and ED-B$^+$ fibronectin by alternative splicing of pre mRNA, de novo-glycosylated fibronectin by alternative post-translational *O*-linked glycosylation of the IIICS region, and the laminin isoforms by exchange of single chains of the heterotrimeric molecule. In contrast to the "common" fibronectin, the distribution of ED-B$^+$ and de novo-glycosylated fibronectin is restricted to embryonic tissues; they subsequently reappear in granulation tissue, in fibrosing processes and in tumour stroma. The expression of these so-called oncofetal fibronectins is stimulated by growth factors (TGFβ). The association of the ED-B$^+$ fibronectin with proliferative activity and newly formed vessels identifies this fibronectin variant as a marker of cellular activity in the process of fibrosis and as a suitable agent for the evaluation of tumour angioneogenesis. Initial results suggest a correlation between the amount of ED-B$^+$ and de novo-glycosylated fibronectin in tumour stroma and the behaviour of carcinomas with regard to their invasiveness and propensity for metastatic dissemination. The current nomenclature of the laminin molecule family is presented. The laminin chain constitution of basement membranes switches from embryonic or proliferatively active to adult terminally differentiated tissues [disappearance of the laminin β2 (s) chain] and depends on the tissue type. The discrepancy between the loss of basement membranes (multiple basement membrane defects) in carcinomas and the recently reported increased laminin chain synthesis in these tumours may be explained by abundant laminin chain deposition outside the basement membrane in the carcinoma invasion front, possibly associated with enhanced adhesion of budding tumour cells.

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Introduction

Histopathology is based on the microscopic evaluation of cells and their arrangement within extracellular matrix (tissue structure). It is well accepted that there is a close relationship between cellular phenotype and specialized extracellular matrix formation. Cells synthesize an extracellular matrix according to their state of maturation and function and, matrix components are able to modulate fundamental cellular properties, for example proliferation and differentiation [1, 8, 9].

Tissue modulation processes in embryonal and immature tissues require a supporting and connecting extracellular matrix, which enables sufficient flexibility for structural changes. Specialized and terminally differentiated cells in mature tissues are associated with a more complex extracellular matrix, often organized into a basal lamina [1]. In differentiation, the extracellular matrix molecules fibronectin and laminin have been investigated extensively, and fibronectin has been found to be a nearly ubiquitous cell–cell and cell–matrix adhesion molecule. Vertebrate cells express fibronectin during morulation; it is a precondition for gastrulation and further morphogenetic cell migration during embryogenesis [10]. Fibronectin preferentially supports cellular proliferation, migration and early differentiation [129].

In contrast to this, laminin seems to be more related to advanced cellular differentiation. In various cell lineages laminin is a prerequisite for terminal differentiation and the realization of specialized cell functions. Only in the presence of laminin are rhabdomyoblasts capable of cell fusion and myotube formation [70, 82]. Breast epithelium requires contact with laminin for milk protein synthesis [114]. Unlike fibronectin, laminin is almost exclusively localized in basal laminae and basement membranes as part of a specialized structural matrix organization. Laminin is the quantitatively most im-

portant non-collagenous matrix protein of basement membranes.

In spite of its association with fundamental cellular functions and its presence in nearly all tissues, detection of fibronectin has had no importance for diagnostic pathology until recently. Detection of laminin may have a diagnostic significance owing to its exclusive occurrence in basement membranes, and the immunohistochemical demonstration of laminin can discriminate basal lamina forming from basal lamina-free sarcomas and may visualize basement membrane defects in carcinomas [24, 67, 90].

Studies on the structure of both matrix proteins have revealed isoforms that show a distinct tissue distribution pattern, which is in line with the assumption of different biological functions. This article reviews the present knowledge on fibronectin and laminin isoforms with regard to their structure and their occurrence under physiological and pathologic conditions.

Fibronectin and its molecular variants

Fibronectin is a high-molecular-mass dimer glycoprotein (about 2500 amino acids). As shown by sequence analyses, the molecule consists of three different sequence homologies, designated type I, II and III repeats. In the molecular architecture the repeats form globular domains with affinities for cell surface and other ligands (e.g. heparin, DNA, gelantin, collagen) [128].

Molecular variants of fibronectin are generated by alternative processing of the pre-mRNA (alternative splicing) or by post-translational modifications of the protein itself (de novo glycosylation).

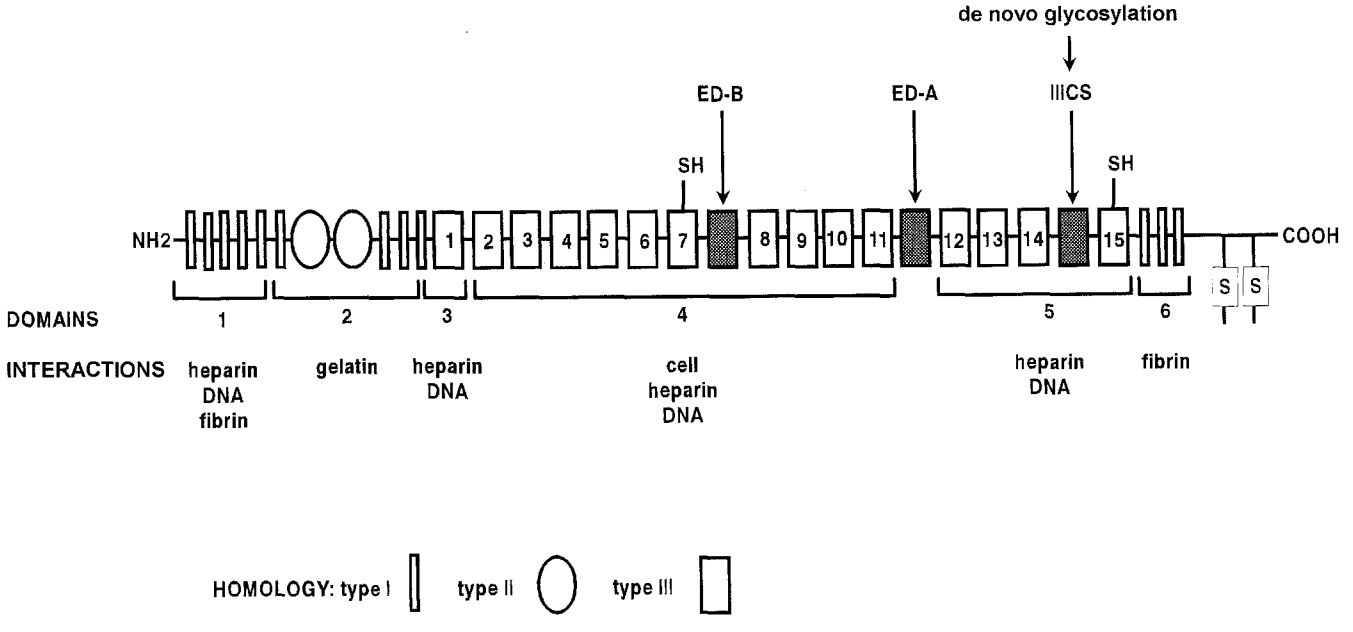
The alternative splicing of the pre-mRNA comprises a facultative elimination of encoding sequences. As a consequence, an alternative mRNA strand results compared with the original sequence. There are three sites of alternative splicing identified within the fibronectin molecule: the ED-A (extra domain A), ED-B (extra domain B) and IIICS (type III homology connecting segment) regions.

Fibronectin occurs in two different forms. The soluble plasma fibronectin is found in blood and other body fluids. An insoluble form, cellular fibronectin, is part of the extracellular matrix [107, 129]. Plasma fibronectin has a lower molecular mass because of the shorter peptide sequence in the C-terminal region (alternative splicing of the IIICS region). This form of alternative splicing is important in hepatocytes, since the liver is the source of plasma fibronectin in the body. Cellular fibronectin is a larger molecule, with an additional domain in the IIICS region. The molecular polymorphism of fibronectin is extensive owing to splicing variants with additional domains, including the domain ED-A and/or the domain ED-B (type III repeats).

Moreover, a further fibronectin isoform has been characterized, defined by a de novo *O*-linked glycosylation in the IIICS domain (Fig. 1) [88]. The enzymatic basis of this alternative glycosylation of the fibronectin protein seems to be a small change in the substrate specificity of the α-Gal-NAc transferase [25, 76].

The regulation of the expression of the different fibronectin isoforms is incompletely understood. Induction of ED-A$^+$ and ED-B$^+$ fibronectin by TGFβ has been demonstrated in cell culture fibroblasts and endothelium [4, 12, 64]. Moreover, there is colocalization of ED-B$^+$ fi-

Fig. 1 Model of the domain structure of a fibronectin subunit. The subunit consist of internal homologies, type I, II and III repeats, and are assembled into disulfide bounded dimers via the pair of cysteine residues at the carboxyterminus. The three sites of alternative splicing (ED-B, ED-B and IIICS), the locus of the *O*-linked de novo-glycosylation and the major macromolecules interacting with the various fibronectin domains are indicated. (Adapted from [20])



bronectin, de novo-glycosylated fibronectin, TGFβ synthesis and TGFβ protein labelling in vivo [7]. A reduced intracellular pH also results in an increased expression of the ED-B-containing fibronectin (Dr. L. Zardi, personal communication).

The biological significance of the alternative splicing of the IIICS region is that it determines the pattern of incorporation of fibronectin into the extracellular matrix.

The ED-A and ED-B regions are also of importance for the function of the molecule. Their localization adjacent to the central cell-binding region suggests a role in cell adhesion and migration [112]. Mardon et al. and Devlin and Harris found that tumorigenic and non-tumorigenic fibroblasts incorporated the ED-B$^+$ fibronectin-splicing variant in their matrix in a different manner [26, 78], suggesting a role in proliferation control. These findings are in keeping with the occurrence of ED-B$^+$ fibronectin in embryonic tissues [36].

Isoforms of laminin

Laminin is a heterotrimeric cross-shaped molecular complex of 850 000 Da. The laminin molecule consists of a large α chain and two different smaller chains, the β and the γ chains, connected by disulfide bridges. Large α-helical segments included in each chain are responsible for the assembly of the single chains to a coiled-coil domain at the base of the long arm of the laminin molecule [29]. The three short arms of the laminin molecule, formed by the N-terminal part of the single chains, consist of EGF-like repeats and globular domains.

The prototype of laminin is EHS laminin, isolated from the murine Engelbreth-Holm-Swarm tumour in 1979. It is made up of the α1 (formerly A), β1 (B1) and γ1 (B2) chains [118]. The sequences of the single chains have been determined [96]. Known proteins, such as merosin, and newly discovered proteins have been recognized as variants of laminin, resulting in a new definition of laminin as a molecule family [119].

The new laminins retain the originally described trimer molecular structure. The laminin isoforms arise from an exchange of single chains. In 1994 a uniform nomenclature of the laminin chains, their genes and of the whole laminin molecules was proposed (Table 1, Fig. 2) [15]. The laminin isoforms can be identified in situ by chain-specific antibodies or by mRNA in situ hybridization with probes applied to the individual chains [56, 85, 106, 117].

Immunohistochemical studies using antibodies to extracellular matrix components of the skeletal muscle showed different staining of synaptic and extrasynaptic basal lamina structures of cross-striated muscle [83, 109, 110]. Later the synaptic laminin, s-Laminin (laminin-3 according to the current nomenclature), was identified by means of molecular cloning. The s chain is a homologue of the laminin β1 (B1) chain [55, 83].

In the basal lamina of skeletal muscle cells and in the basal lamina of murine cardiomyocytes a novel laminin variant, merosin, containing the α2 (M) chain was demonstrated. The α2 (M) chain was classified as a homologue of the laminin α1 (A) chain [28, 71, 101].

In 1991 Rousselle and her group [106] defined an epithelial basement membrane-specific adhesion molecule, which they designated as kalinin and which, as shown later, corresponds to the adhesion molecule nicein [80]. The kalinin/nicein molecule belongs to the laminin fami-

Table 1 Nomenclature of the laminin chains (new and former names) and their genes [15]

New	Former	Gene
α1	A, Ae	*LAMA1*
α2	M, Am	*LAMA2*
α3	200 kDa	*LAMA3*
β1	B1, B1e	*LAMB1*
β2	s, B1s	*LAMB2*
β3	140 kDa	*LAMB3*
γ1	B2, B2e	*LAMC1*
γ2	B2t	*LAMC2*

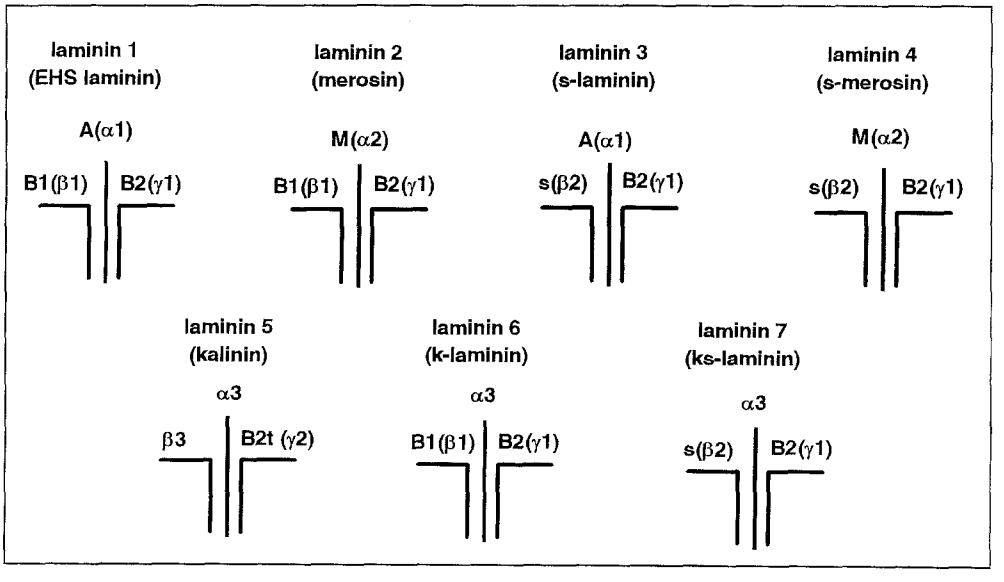


Fig. 2 Laminin chain composition of the seven different laminin isoforms [117]

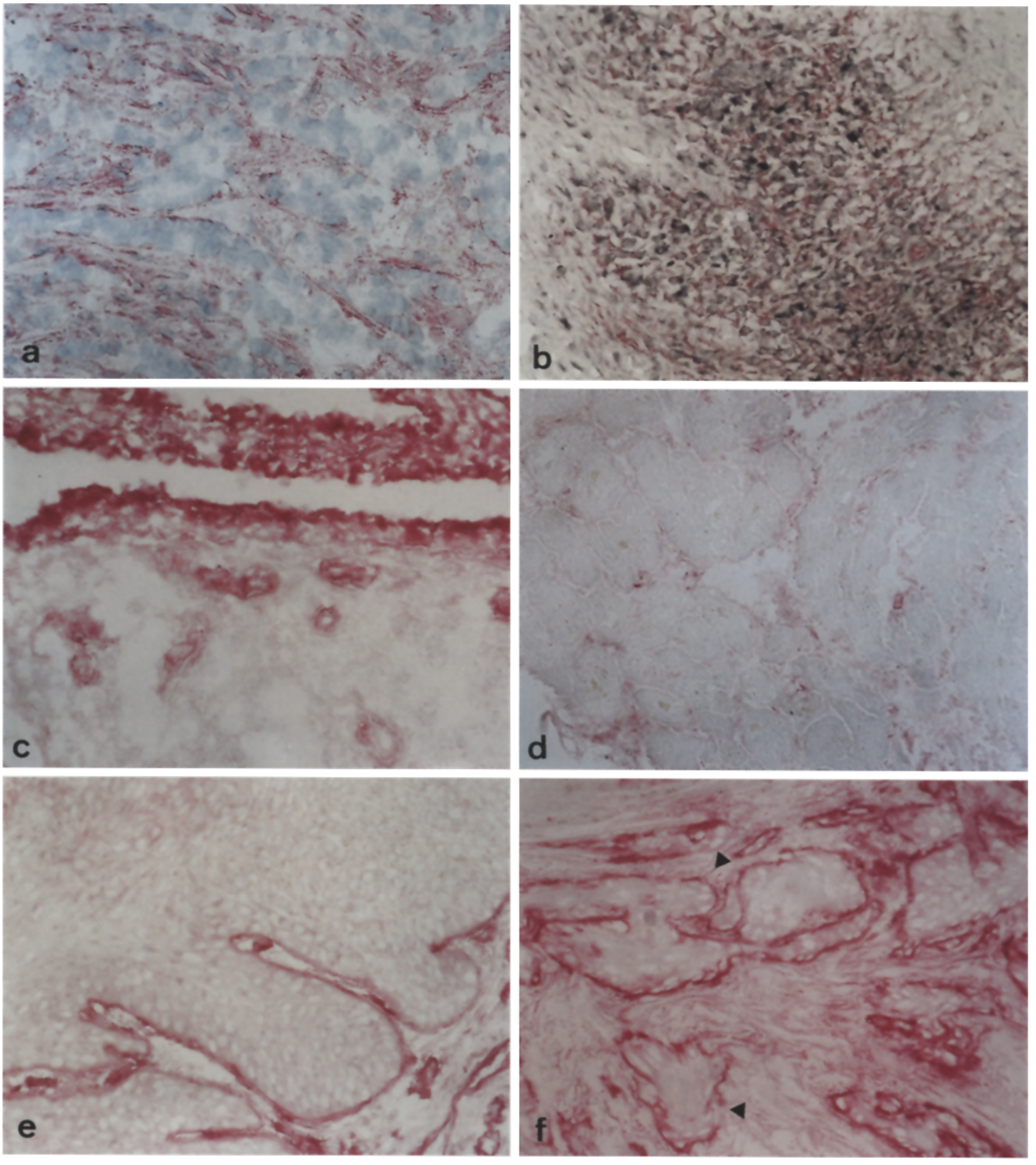


Fig. 3a–f Examples of fibronectin and laminin isoform distribution in human tissues. Immunohistochemical demonstration of ED-B$^+$ fibronectin in the stroma of invasive ductal carcinoma of the breast (**a**), in the lining layer and some newly formed blood vessels of synovial tissue in rheumatoid arthritis (**c**) and in biopsy specimen of a human endomyocardium affected by dilated cardiomyopathy (**d**). Monoclonal antibody BC1, APAAP technique,×150. **b** Colocalization of α-smooth muscle actin (monoclonal antibody 1A4, APAAP technique, red labelling) and ED-B$^+$ fibronectin synthesis (mRNA in situ hybridization, black labelling) in nodular palmar fibromatosis indicates that the myofibroblast is a source of ED-B$^+$ fibronectin. ×75. **e** In contrast to normal adult oral squamous epithelium, the basement membrane of hyperplastic epithelium contains the laminin β2 chain. Monoclonal antibody C4, APAAP technique, ×150. Demonstration of the laminin α3 chain in the invasion front of oral squamous carcinoma. The immunostaining makes the α3 chain visible both in the basement membrane and in the adjacent stroma and reveals focal membrane defects (*arrowhead*). Monoclonal antibody BM165, APAAP technique, ×150

ly and contains a new large A (α3) chain, a new B (β3) [102] and new truncated B2t (t for truncated, γ2) chain with identified sequences [63, 117]. Another molecule of the epithelial basement membranes is epiligrin [19, 125]. Epiligrin, kalinin, and nicein appear to be closely related [120, 127], although complete identity has not been shown[108]. In the new nomenclature of the laminins they are uniformly classified as laminin-5 [117], which has already been accepted as an appropriate term in further publications [105].

Marinkovich et al. [79] separated a further representative of the laminin family from the kalinin molecule: k-laminin (laminin-6 according to the modern nomenclature) with the β1 (B1) and the γ1 (B2) chains known from EHS laminin, and the large α3 chain. Like kalinin, k-laminin is also localized in the anchorage filaments of the dermal–epidermal junction. The α3 chain of laminin-5 (kalinin) is probably not completely identical with the α chain of laminin-6 (k-laminin) and laminin-7 (ks-laminin) [41], as assumed in the nomenclature proposal. Iivanainen et al. [57] presented a new α4 chain, which is similar to the α3 chain. In one of their publications Matsui et al. [86] have already used the designation "α4 chain" for the large chain of laminin-6.

Histopathological aspects of fibronectin variants

Because of the preferential occurrence of the ED-B$^+$ and de novo-glycosylated fibronectin variants in fetal and tumour tissues these fibronectin isoforms are designated oncofetal [87, 98, 130] or embryonic [37] fibronectins. In contrast to "common fibronectin", the demonstration of ED-A$^+$, ED-B$^+$ and de novo-glycosylated fibronectin may have some diagnostic significance in histopathology. The reasons for this are (1) the substantially more severely restricted tissue distribution pattern of these isoforms than of common fibronectin and (2) the availability of specific monoclonal antibodies that label these extra domains or domain-associated molecule configuration changes [11, 17, 76, 87, 131]. ED-B$^+$ and de novo-glycosylated fibronectin are present in large amounts in fetal tissues and are not demonstrable in adult tissues [37, 99], apart from some rare exceptions, for example nontracheal cartilage [132]. A raised level of de novo-glycosylated fibronectin in the cervix or vaginal secretion may serve as predictor of preterm delivery [74, 92].

ED-B$^+$ and de novo-glycosylated fibronectin in granulation tissue, organ fibrosis and fibromatosis

Regular reappearance of the ED-B$^+$ and de novo-glycosylated fibronectin variants is observed in granulation tissue and related tissue alterations [38], including fibrosis (liver cirrhosis [115], dilated cardiomyopathy [40]) and fibromatosis [45]. Fibroblasts and macrophages in healing wounds are able to express ED-A$^+$ and ED-B$^+$ fibronectin [14]. There is some evidence that the regeneration of other tissues, such as peripheral nerves, is accompanied by a re-expression of ED-B$^+$ fibronectin [84].

In normal adult myocardium ED-B$^+$ and de novo-glycosylated fibronectin were not detected. However, in dilated cardiomyopathy, defined histologically by disturbed tissue integrity and interstitial fibrosis, spot-like interstitial deposition of ED-B$^+$ and de novo-glycosylated fibronectin was revealed in human endomyocardial biopsies before the appearance of other microscopic alterations of the myocardium (Fig. 3d). The quantity of interstitial ED-B$^+$ and de novo-glycosylated fibronectin correlates with the decrease in the left ventricular ejection fraction of the heart in this disease [40]. The re-expression of the ED-B$^+$ and de novo-glycosylated fibronectin in the adult heart is not disease specific; it is also found in myocardial hypertrophy in the rat [33, 34].

In experimental proliferative glomerulonephritis a spatial and temporal modulation of the mRNA level of ED-A$^+$ and ED-B$^+$ fibronectin occurs [5]. ED-A$^+$ and ED-B$^+$ fibronectin mRNAs are most abundant within sclerosing crescents (late scar forming phase of the disease) [93]. As shown by immunohistochemistry, in nodular palmar fibromatosis the active fibromatosis nodules (proliferative and early involutional phase according to Luck) alone showed ED-A$^+$, ED-B$^+$, and de novo-glycosylated fibronectin, whereas in the residual phase of fibromatosis none of these fibronectin variants could be demonstrated. From the congruity of the immunostaining results (ED-B$^+$, de novo-glycosylated fibronectin) with the signal of RNA in situ hybridization using fibronectin cDNA it can be concluded that the synthesis of these fibronectin isoforms is restricted to the active foci of fibromatosis. Therefore, it seems justified to regard these fibronectin isoforms as markers of an active disease process in fibromatosis.

In nodular palmar fibromatosis the active fibromatosis nodules consist mainly of myofibroblasts. By means of immunohistochemical double staining an association of the myofibroblast phenotype with an ED-B$^+$ and de novo-glycosylated fibronectin containing extracellular matrix has been demonstrated [69]. Moreover, using double labelling α-smooth muscle actin immunohistochemistry and mRNA in situ hybridization for ED-B$^+$ fibronectin, ED-B$^+$ fibronectin synthesis by the myofibroblast is shown (Fig. 3b).

We are presently seeing the first observations concerning ED-B$^+$-and de novo-glycosylated fibronectin expression in rheumatoid arthritis (Fig. 3c). ED-A$^+$ fibronectin, however, which is not so strictly related to oncofetal tissue modulation processes, is synthesized by the synovial lining fibroblast-like (type B) cells and appears to be expressed in association with activated or transformed states of synovium [53]. In rheumatoid arthritis a high level of oncofetal glycosylated fibronectin can be detected in the synovial fluid, whereas in osteoarthritis only a small increase is observed [18].

ED-B+ and de novo-glycosylated fibronectin in tumour stroma

ED-B+ and de novo-glycosylated fibronectin are regularly detected in the stroma of carcinomas by means of immunohistochemistry (Fig. 3a). In principle, the expression of ED-B+ and de novo-glycosylated fibronectin is also possible in benign tumours, and it has been described in pituitary adenomas, for example [35]. As in fibromatosis, in the stroma of breast carcinoma colocalization of ED-B+ fibronectin and myofibroblasts was found [13]. In cell culture studies, activated stromal fibroblasts and transformed fibroblasts are able to synthesize oncofetal fibronectin, but the extent of epithelial tumour cell production of oncofetal fibronectins is not clear [59, 94].

The quantity of ED-B+ and de novo-glycosylated fibronectin deposition in the stroma of various carcinoma types is different. It can be shown that, regardless of the morphological malignancy grade in invasive ductal carcinomas of the breast [61] and oral squamous cell carcinomas [77], the whole stroma compartment shows a diffuse positivity for ED-B+ and de novo-glycosylated fibronectin. However, in adenocarcinomas of the colon [59] and in renal cell carcinomas [75, 95] ED-B+ and de novo-glycosylated fibronectin depositions were detected mostly in a spot-like manner. In colon carcinomas a correlation was noted between a large amount of stromal oncofetal fibronectin and advanced local stage of disease (Dukes C), liver metastasis and general prognosis [47, 59]. Therefore, there may be correlations between the invasive capacity of colon carcinoma cells and the extent of oncofetal glycosylation of fibronectin. This assumption is supported by findings in the placenta. Frank et al. [39] observed the oncofetal glycosylated domain of fibronectin exclusively in the invasive phenotype of the placental trophoblast. Differences in the depositions of oncofetal fibronectins between different carcinoma types and the correlations with the malignancy grade may mirror the different capability of carcinoma cells to recruit and activate a supportive stroma by means of growth factors [7, 13].

ED-B+ fibronectin – a marker of angioneogenesis

At present, the microvessel density in tumour stroma of carcinomas is a frequently investigated and critically discussed prognostic variable [42, 62, 126]. Whereas ED-B+ fibronectin is never seen in mature vessels, newly formed vessels in normal and tumour tissues are characterized by an abundant ED-B+ fibronectin matrix [16] (Fig. 3c). The association of ED-B+ fibronectin with angiogenesis is demonstrated by study of endometria. There is a strong reaction in the vessels of the functional layer, where the angiogenetic process is present during the proliferative phase, but not in the endometrial basal layer and in the myometrium. The immunohistochemical finding of a ED-B+ fibronectin-positive cytoplasm suggests synthesis by the activated endothelial cells themselves. Because growing and migrating endothelial cells are associated with a fibronectin-rich extracellular matrix [58], a role is assumed for ED-B+ fibronectin in endothelial cell migration during angioneogenesis [20]. Therefore, quantification of the ED-B+ fibronectin-positive vessels should allow conclusions about the capability of tumours to induce a new vasculature (angioneogenesis) [21]. To date, there are no investigations on the question of whether or not quantification of ED-B+ fibronectin-positive vessels in tumour stroma is superior to the evaluation of microvessel density with regard to prognostic significance.

Histopathological aspects of laminin isoforms

In contrast to the preferential occurrence of ED-B+ and de novo-glycosylated fibronectin in relation to cellular immaturity, expression of the different laminin isoforms is dependent on tissue type and tissue maturation, resulting in a differentiation- and maturation-dependent heterogeneity of basement membranes [32, 100]. A complete catalogue of the distribution pattern of the laminin isoforms in adult and embryonic tissues is not yet available. In general, there seems to be a relation of the expression of the α2 (M) chain with cross-striated muscle, the expression of β2 (s) chain with nerve and the expression of α3, β3 and γ2 chain (laminin-5/kalinin) with epithelial basement membranes.

Laminin-5 in gene dermatosis

Against the background of the close association of laminin-5 (kalinin) to the anchorage filaments of the dermoepidermal junction, it is not surprising that in dermatosis characterized by altered adhesion (blistering) in the epithelium–stroma interface of skin and mucous membranes [102] mutations in the genes of laminin-5 can be found (laminin-5 gene dermatosis) [103]. Different mutations in the laminin-5 genes are accompanied by a different severity of clinical symptoms in this group of genetic dermatoses [89, 120]. Examples of prenatal diagnosis of Herlitz junctional epidermiolysis bullosa have been already published [81, 121].

Laminin-2 in muscle and nerve

In congenital muscular dystrophies, a heterogeneous group of autosomal recessive skeletal muscle diseases, mutations in the *LAMA2* gene of the α2 chain of laminin-2 (merosin) have been found, which cause an immunohistochemically detectable laminin-2 (merosin) deficiency [48–50, 52]. In the corresponding mouse model with laminin-2 deficiency dysmyelinization also occurs [3, 30]. Laminin-2 may be more responsible for the cellular integrity of skeletal muscle than for skeletal muscle cell development, because in laminin-2-deficient mouse

muscle differentiation in the embryo does not seem to be seriously affected [44]. The immediate application of the recent findings in the case of merosin allows the molecular classification of muscular dystrophies, and this includes the possibility of prenatal diagnosis [31].

In peripheral nerves the perineurial basal lamina shows preferential laminin-3 (s-laminin) and no laminin-2 (merosin), whereas the endoneurial basal lamina contains laminin-2 and no laminin-3 [111].

Smooth muscle cells and myofibroblasts produce a β2 (s) chain-positive matrix [43], as confirmed in cell culture investigations [124].

Laminin chain distribution in kidney

In arteries and in the glomerular basement membrane, laminin-3 (s-laminin) is present but no laminin-2 (merosin) [111]. Laminin-2 (merosin) is found in the glomerular mesangial matrix [54]. Reports on the composition of the tubular basement membranes are not completely consistent. Virtanen et al. [122] did not find immunoreactivity for the α3 and β3 chain of laminin-5 (kalinin), but in the collecting tubules of embryonic and adult mice laminin-5 can be visualized by means of an in situ hybridization technique [2, 41]. The results agree with our own findings (demonstration of kalinin using the monoclonal antibody BM 165 [106]) in collecting tubules of human adult kidney. In contrast to the adult tubule system, the wide distribution of the β2 (s) chain in embryonic tubules is undisputed [56, 122]. The laminin chain heterogeneity of the tubular basement membranes related to tubule segmentation and maturation corresponds to a segment-related heterogeneity of integrin type receptors [66, 116]. The variations of laminin matrix and integrins in normal tissues should therefore be kept in mind when the integrin pattern of the pertinent tumours is interpreted.

Laminin chain distribution in gastric mucosa

In gastric mucosa a correlation has been shown between the laminin chain content of epithelial basement membranes, integrins and the proliferation and differentiation of the glandular epithelium. The chains of laminin-1 are ubiquitous throughout the epithelial basement membranes. The basement membranes of glands and the lower parts of the gastric pits contain the laminin α2 chain, whereas the laminin α3 chain immunoreactivity is confined to basement membranes underneath the surface epithelium and upper parts of the pits [123].

Laminin chains in tumours

In principle, the differentiation-associated laminin chain pattern of normal tissues is retained by their corresponding tumours. For example, laminin-2 (merosin) is seen as a characteristic feature of Schwann cells and Schwann cell tumours [73]. Furthermore, it is found in association with intermediate trophoblast cells of the placenta as well as in choriocarcinoma [72]. Laminin-5 is a feature of the basement membrane of the squamous epithelium and squamous cell carcinoma [104].

The diminution of cellular differentiation in malignant tumours can be accompanied by a re-expression of embryonal properties and proteins such as α-fetoprotein or carcinoembryonal antigen. In this context, in contrast to adult tissues, there is a wide distribution of the β2 (s) chain in embryonic basement membranes. In embryonic tissues the β2 chain seems to replace the β1 chain functionally [56]. In proliferating benign and carcinomatous lesions of the breast [51], in hyperplastic oral squamous epithelium (Fig. 3e) and in activated fibro/myofibroblasts of nodular palmar fibromatosis [69] a re-expression of the β2 chain can be demonstrated regularly in line with a proliferation and differentiation-associated developmental switch [91]. Simultaneously, there is a decrease in α1 and α2 chain expression [127]. Remarkably, carcinoma cell lines synthesize the β2 (s) chain independently of their differentiation [127], which may be interpreted as the reappearance of an embryonic pattern in culture.

Structural irregularities of basement membranes in malignant tumours are well defined [23]. Basement membrane defects with a coincident loss of integrin chains are characteristic for at least some carcinoma types [22, 27, 68]. The quantity of the basement membrane defects correlates with the invasive and metastatic potential of oral squamous cell carcinoma [46]. However, reports on the association of an increased expression of the α2 (M) chain in malignant tumours with raised metastatic ability [60] or on the existence of a preferential and increased γ2 chain expression of laminin-5 (kalinin) in budding carcinoma cells of the invasion front are perplexing with regard to the aforementioned focal loss of basement membrane [104, 105]. Perhaps the increased synthesis of single laminin chains indicates tumour-associated disorganization accompanied by incomplete molecule assembly and/or an irregular deposition in the extracellular matrix. Indeed, in the invasion front of oral squamous cell carcinoma basement membrane defects can be detected immunohistochemically in association with deposition of laminin chains both on the surfaces of budding cells and in the neighbouring stroma (Fig. 3f). The presentation of single laminin chains on tumour cell surfaces and the loss of structural basement membrane organization may lead to retention of the cellular adhesion and support tumour cell invasion. There is further evidence that a structurally altered basement membrane is able to modulate the adhesive and invasive properties of tumour cells, perhaps by virtue of its altered laminin isoforms. On electron microscopy the loss of hemidesmosomes is a characteristic feature of invasive breast cancer cells [6]. Laminin-5 is a component of the anchoring filaments that are the connecting elements between hemidesmosome and basement membrane [97, 106]. Indeed, in breast carcinoma a strong diminution of the α3 chain of laminin 5 (antibody BM165) has been demonstrated [51].

Concerning the regulation of the laminin isoforms or single chain expression very few data are available. Without doubt, cytokines are involved: in squamous epithelium cell culture TGFβ enhances the expression of the α3, β3 and γ2 chains, whereas TNFα reduces the synthesis of the α3 chain without any influence on the other chains [65]. Ultimately, the ideas concerning the different expression of laminin chains are provisional, since it is unlikely that all laminin chains have been identified [113].

Prospects for further investigation and application of fibronectin and laminin isoforms for histopathological diagnosis

The association of ED-B$^+$ and de novo-glycosylated fibronectin with activated and proliferating fibro/myofibroblasts qualifies these proteins as markers of an active disease process in nonneoplastic and neoplastic fibroplasia. Simultaneously, in organ fibrosis the amount of oncofetal fibronectins may reflect the extent of disturbance of parenchymal stromal cells and may correlate with functional restriction. From the examples discussed it is clear that the expression of ED-A$^+$, ED-B$^+$ and de novo-glycosylated fibronectin is not disease specific but it is strictly correlated with the extent of the tissue modulation process, and in particular with connective tissue formation (fibroplasia).

ED-B$^+$ and de novo-glycosylated fibronectin are induced by growth factors. Therefore, the amount of oncofetal fibronectins in the tumour stroma may indicate the ability of the tumour cells to recruit a supporting stroma. Because of changed adhesive properties, the oncofetal fibronectins should be associated with invasion and metastasis. An examination of a possible prognostic value of these fibronectin variants is required.

The association of ED-B$^+$ fibronectin with newly formed vessels enables an appraisal of true angioneogenesis. It might be expected that neo-angiogenesis has a higher predictive value for tumour behaviour than microvessel density. Therefore, the screening of this fibronectin variant could be a valuable tool in the determination of a prognosis (Table 2).

The differentiation-dependent laminin isoform constitution of basement membranes discloses two different diagnostic applications. First, at least in skin, muscle and nerve, distinct laminin isoforms are a prerequisite for differentiation and/or tissue integrity. The immunohistochemical or molecular biological demonstration of missed or altered laminin proteins allows the definitive classification of blistering genetic dermatoses and skeletal muscle dystrophies. Second, the retention of a distinct laminin isoform pattern in normal tissue in the corresponding tumour may serve as a diagnostic aid in tumour typing.

Moreover, as demonstrated for the renal tubulus system or the gastric mucosa, a topographical and perhaps functional laminin isoform heterogeneity of the basement membrane exists. It might be supposed that disease processes have an influence on the arrangement of laminin isoforms in the basement membrane. The histopathological evaluation of these basement membrane alterations should be the object of further investigations.

Apart from the differentiation-dependent modulation of laminin isoform content in basement membranes, there are maturation-associated changes [97], which may have diagnostic implications. There is a developmental switch in the laminin isoform content of the basement membranes from embryonic or proliferatively active to adult terminally differentiated tissues. Because of the

Table 2 Potential applications of fibronectin and laminin isoforms in diagnostic histopathology

	Isoforms of fibronectin and laminin	Histopathological relevance	References
Fibronectin	ED-A$^+$ fibronectin	Marker of an inflammatory active disease in rheumatoid arthritis	[18, 53]
	ED-B$^+$ and/or de novo-glycosylated fibronectin	Marker of an active fibroplasia in fibromatosis, liver cirrhosis, dilated cardiomyopathy and proliferative glomerulonephritis	[40, 69, 93, 115]
	ED-B$^+$ and/or de novo-glycosylated fibronectin	Prognostic marker in some carcinoma entities (colon and renal carcinoma)	[59, 95]
	ED-B$^+$ fibronectin	Marker of angioneogenesis in tumour and nontumour diseases	[20, 21]
	De novo-glycosylated fibronectin	Predictor of preterm birth	[74, 92]
Laminin	Changes in organotypic laminin chain distribution	Disease marker indicating severity of tissue alterations in inflammatory and degenerative diseases	[91, 123]
	Laminin-5	Classification of blistering gene dermatosis	[81, 89, 120, 121]
	Laminin-2	Classification of congenital muscular dystrophies	[31, 48–50]
	Differentiation-dependent laminin chain distribution	Diagnostic aid in tumour typing	[72, 73]
	Focal loss of laminin chains, re-expression of the β2-chain, raised expression of the γ2-chain	Assessment of tumour malignancy	[51, 60, 104, 105, 127]

widespread distribution of the laminin β2 (s) chain during embryogenesis, the laminin β2 (s) chain can reflect the state of tissue maturation. Immature tissue has a β2 (s) chain, and mature tissue usually has none. The demonstration of the laminin β2 (s) chain in degeneratively or inflammatory damaged adult tissue permits the evaluation of the extent of the cell and tissue disturbance.

With reference to the evaluation of tumour tissue disorganization and malignancy grade three facts should be pointed out.

First, a carcinoma-associated embryonal basement membrane and a embryonal stroma milieu occur, with their distinct compositions. The tumour cells and stromal cells (myofibroblasts) are able to re-express the laminin β2 (s) chain. Additionally, the myofibroblasts synthesize oncofetal fibronectin variants, resulting in an embryonic matrix milieu in the tumour stroma, with the effect of supporting the invasion and migration of tumour cells.

Second, several carcinoma types show structural changes of laminin isoform distribution. For instance, laminin-5 is no longer demonstrable in association with hemidesmosome structures or is totally lost.

Third, besides the loss of laminin chains in the basement membrane, an increased laminin chain synthesis by budding cells of the tumour invasion front is seen. The deposition of these laminin chains outside the basement membrane may represent a molecular pathway and promote tumour cell migration and invasion.

Quantification of these phenomena should contribute to malignancy grading and may have a predictive value (Table 2).

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References

1. Adams JC, Watt FM (1993) Regulation of development and differentiation by the extracellular matrix. Development 117: 1183–1198
2. Aberdam D, Aguzzi A, Baudoin C, Galliano M-F, Ortonne J-P, Meneguzzi G (1994) Developmental expression of nicein adhesion protein (Laminin-5) subunits suggests multiple morphogenic roles. Cell Adhes Commun 2:115–129
3. Arahata K, Hayashi YK, Koga R, Goto K, Lee JH, Miyagoe Y, Ishii H, Tsukahara T, Takeda S, Woo M, Nonaka I, Matsuzaki T, Sugita H (1993) Laminin in animal models for muscular dystrophy. Defect of laminin M in skeletal and cardiac muscles and peripheral nerve of the homozygous dystrophic dy/dy mice. Proc Jpn Acad [B] 69:259–264
4. Balza E, Borsi L, Allemanni G, Zardi L (1988) Transforming growth factor β regulates the levels of different fibronectin isoforms in normal human cultured fibroblasts. FEBS Lett 228:42–44
5. Barnes JL, Torres ES, Mitchell RJ, Peters JH (1995) Expression of alternatively spliced fibronectin variants during remodelling in proliferative glomerulonephritis. Am J Pathol 147:1361–1371
6. Bergstraesser LM, Srinivasan G, Jones JCR, Stahl S, Weitzman SA (1995) Expression of hemidesmosomes and component proteins is lost by invasive breast cancer cells. Am J Pathol 147:1823–1839
7. Berndt A, Kosmehl H, Mandel U, Gabler U, Luo X, Celeda D, Zardi L, Katenkamp D (1995) TGFβ and bFGF synthesis and localization in Dupuytren's disease (nodular palmar fibromatosis) relative to cellular activity, myofibroblast phenotype and oncofetal variants of fibronectin. Histochem J 27: 1041–1020
8. Bissell MJ, Ram TG (1989) Regulation of functional cytodifferentiation and histogenesis in mammary epithelial cells. Role of extracellular matrix. Environ Health Perspect 80: 61–70
9. Bissell MJ, Hall HG, Parry G (1982) How does the extracellular matrix direct gene expression? J Theor Biol 99:31–68
10. Bocaut JC, Darribere T, Boulekbache H, Thiery JP (1984) Prevention of gastrulation but not neurulation by antibodies to fibronectin in amphibian embryos. Nature 307:364–367
11. Borsi L, Carnemolla B, Castellani P, Rosellini C, Vecchio D, Allemanni G, Chang SE, Taylor-Papadimitriou J, Pande H, Zardi L (1987) Monoclonal antibodies in the analysis of fibronectin isoforms generated by alternative splicing of mRNA precursors in normal and transformed human cells. J Cell Biol 104:595–600
12. Borsi L, Castellani P, Risso AM, Leprini A, Zardi L (1990) Transforming growth factor-β regulates the splicing pattern of fibronectin messenger RNA precursor. FEBS Lett 261: 175–178
13. Brouty-Boyé D, Magnien V (1994) Myofibroblast and concurrent ED-B fibronectin phenotype in human stromal cells cultured from non-malignant and malignant breast tissue. Eur J Cancer 30A:66–73
14. Brown LF, Dubin D, Lavigne Liz, Logan B, Dvorak HF, Van De Water L (1993) Macrophages and fibroblasts express embryonic fibronectins during cutaneous wound healing. Am J Pathol 142:793–801
15. Burgeson RE, Chiquet M, Deutzmann R, Ekblom P, Engel J, Kleinmann H, Martin GR, Meneguzzi G, Paulsson M, Sanes J, Timpl R, Tryggvason K, Yamada Y, Yurchenco PD (1994) A new nomenclature for the laminins. Matrix Biol 14: 209–211
16. Carnemolla B, Balza E, Siri A, Zardi L, Nicotra MR, Bigotti A, Natali PG (1989) A tumour-associated fibronectin isoform generated by alternative splicing of messenger-RNA precursors. J Cell Biol 108:1139–1148
17. Carnemolla B, Leprini A, Allemanni G, Saginati M, Zardi L (1992) The inclusion of the type III repeat ED-B in the fibronectin molecule generates conformational modifications that unmask a cryptic sequence. J Biol Chem 267:24689–24692
18. Carsons S, Clausen H, Wolf J (1994) Expression of a developmentally regulated epitope on fibronectins from the synovial fluid of patients with rheumatoid disease. J Rheumatol 21:1888–1891
19. Carter WG, Ryan MC, Gahr PJ (1991) Epiligrin, a new cell adhesion ligand for the integrin α3β1 in epithelial basement membranes. Cell 65:599–610
20. Castellani P, Viale G, Dorcaratto A, Nicolo G, Kaczmarek J, Querze G, Zardi L (1994) The fibronectin isoform containing the ED-B oncofetal domain: a marker of angiogenesis. Int J Cancer 59:612–618
21. Castellani P, Viale G, Dorcaratto A, Querze G, Allemanni G, Zardi L, Siri A (1995) Specific expression of the ED-B containing fibronectin isoform by endothelial cells of growing tumour vessels. Antibody, Immunoconjugates and Radiopharmaceuticals 8:325–332
22. Charpin C, Andrac L, Devictor B, Habib MC, Vacheret H, Xerri L, Lavaut MN, Toga M (1989) Type IV collagen im-

munostaining and computerized image analysis (SAMBA) in breast and endometrial disorders. Histopathology 14:47–60
23. Damjanov I (1990) Heterogeneity of basement membranes in normal and pathologically altered tissues. Virchows Arch [A] 416:185–188
24. D´Ardenne AJ (1989) Use of basement membrane markers in tumour diagnosis. J Clin Pathol 42: 449–457
25. David L, Mandel U, Clausen H, Sobrinho-Simoes M (1993) Immunohistochemical expression of oncofetal fibronectin in benign and malignant lesions of the stomach. Eur J Cancer 29:2070–2071
26. Devlin J, Harris H (1994) Selective incorporation of fibronectin isoforms into the extracellular matrix of hybrids between human fibrosarcoma cells and normal human fibroblasts. Exp Cell Res 214:429–432
27. Downer CS, Watt FM, Speight PM (1993) Loss of α6β4 integrin subunits coincidences with loss of basement membrane components in oral squamous cell carcinomas. J Pathol (Lond) 171:183–190
28. Ehrig K, Leivo I, Argraves WS, Ruoslahti E, Engvall E (1990) Merosin, a tissue specific basement membrane protein, is a laminin-like protein. Proc Natl Acad Sci USA 87: 3264–3268
29. Engel J (1993) Structure and function of laminin. In: Rohrbach DH, Timpl R (eds) Molecular and cellular aspects of basement membranes. Academic Press, San Diego, pp 147–176
30. Engvall E (1994) Cell adhesion in muscle. Braz J Med Biol Res 27:2213–2227
31. Engvall E (1995) Structure and function of basement membranes. Int J Dev Biol 39:781–787
32. Engvall E, Earwicker D, Haaparanta T, Ruoslahti E, Sanes JR (1990) Distribution and isolation of four laminin variants; tissue restricted distribution of heterotrimers assembled from five different subunits. Cell Regul 1:731–740
33. Farhadian F, Barrieux A, Lortet S, Marotte F, Olievero P, Rappaport L, Samuel J-L (1994) Differential splicing of fibronectin pre-messenger ribonucleic acid during cardiac ontogeny and development of hypertrophy in the rat. Lab Invest 71:552–559
34. Farhadian F, Contard F, Corbier A, Barrieux A, Rappaport L, Samuel J-L (1995) Fibronectin expression during physiological and pathological cardiac growth. J Mol Cell Cardiol 27: 981–990
35. Farnoud MR, Farhadian F, Samuel JL, Derome P, Peillon F, Li JY (1995) Fibronectin isoforms are differentially expressed in normal and adenomatous human anterior pituitaries. Int J Cancer 61:27–34
36. ffrench-Constant C (1995) Alternative splicing of fibronectin – many different proteins but few functions. Exp Cell Res 221:261–271
37. ffrench-Constant C, Hynes RO (1989) Alternative splicing of fibronectin is temporally and spatially regulated in chicken embryo. Development 106:375–388
38. ffrench-Constant C, Van De Water L, Dvorak HF, Hynes RO (1989) Reappearance of embryonic pattern of fibronectin splicing during wound healing in the adult rat. J Cell Biol 109:903–914
39. Frank H-G, Huppertz B, Kertschanska S, Blachard D, Roelcke D, Kaufmann P (1995) Anti-adhesive glycosylation of fibronectin-like molecules in human placental matrix-type fibrinoid. Histochem Cell Biol 104:317–329
40. Gabler U, Berndt A, Kosmehl H, Mandel U, Zardi L, Müller S, Stelzner A, Katenkamp D (1996) Matrix remodelling in dilated cardiomyopathy entails the occurrence of oncofetal fibronectin molecular variants. Heart 75: 358–362
41. Galliano M-F, Aberdam D, Aguzzi A, Ortonne J-P, Meneguzzi G (1995) Cloning and complete primary structure of the mouse laminin α3 chain. Distinct expression pattern of the laminin α3A and α3B chain isoforms. J Biol Chem 270: 21820–21826
42. Gasparini G (1995) Angiogenesis in preneoplastic and neoplastic lesions. Cancer J 8:91–93
43. Glukhova M, Koteliansky V, Fondacci C, Marotte F, Rappaport L (1993) Laminin variants and integrin receptors in developing and adult human smooth muscle. Dev Biol 267: 437–447
44. Gullberg D, Ekblom P (1995) Extracellular matrix and its receptors during development. Int J Dev Biol 39:845–854
45. Halliday NL, Rayan GM, Zardi L, Tomasek JJ (1994) Distribution of ED-A and ED-B fibronectin isoforms in Dupuytren's disease. J Hand Surg [Am] 19:428–434
46. Harada T, Shinohara M, Nakamura S, Oka M (1994) An immunohistochemical study of the extracellular matrix in oral squamous cell carcinoma and its association with invasive and metastatic potential. Virchows Arch 424:257–266
47. Hauptmann S, Zardi L, Siri A, Carnemolla B, Borsi L, Castellucci M (1995) Extracellular matrix proteins in colorectal carcinomas: expression of tenascin and fibronectin isoforms. Lab Invest 73:172–182
48. Hayashi YK, Engvall E, Arikawa-Hirasawa E, Goto K, Koga R, Nonaka I, Sugita H, Arahata K (1993) Abnormal localization of laminin subunits in muscular dystrophies. J Neurol Sci 119:53–64
49. Hayashi YK, Koga R, Tsukahara T, Ishii H, Matsuishi T, Yamashita Y, Nonaka I, Arahata K (1995) Deficiency of laminin α2-chain mRNA in muscle in a patient with merosin-negative congenital muscular dystrophy. Muscle Nerve 18: 1027–1030
50. Helbling-Leclerc A, Zhang X, Topaloglu H, Cruaud C, Tesson F, Weissenbach J, Tomé FMS, Schwartz K, Fardeau M, Tryggvason K, Guicheney P (1995) Mutations in the laminin α2-chain gene (LAMA2) cause merosin-deficient congenital muscular dystrophy. Nat Genet 11:216–218
51. Henning K, Kosmehl H, Berndt A, Rousselle P, Gabler U, Katenkamp D (1995) Differential expression of laminin chains in benign and malignant lesions of the breast. Pathol Res Pract 191:175
52. Hillaire D, Leclerc A, Fauré S, Topaloglu H, Chiannilkulchai N, Guicheney P, Grinas L, Legos P, Philpot J, Evangelista T, Routon M-C, Mayer M, Pellissier J-F, Estournet B, Barois A, Hentati F, Feingold N, Beckmann JS, Dubowitz V, Tomé FMS, Fardeau M (1994) Localization of merosin-negative congenital muscular dystrophy to chromosome 6q2 homozygosity mapping. Hum Mol Genet 3:1657–1661
53. Hino K, Shiozawa S, Kuroki Y, Ishikawa H, Shiozawa K, Sekiguchi K, Hirano H, Sakashita E, Miyashita K, Chihara K (1995) EDA-containing fibronectin is synthesized from rheumatoid synovial fibroblast like cells. Arthritis Rheum 38: 678–683
54. Hori H, Kanamori T, Mizuta T, Yamaguchi N, Liu Y, Nagai Y (1994) Human laminin M chain: epitope analysis of its monoclonal antibodies by immunoscreening of cDNA clones and tissue expression. J Biochem 116:1212–1219
55. Hunter DD, Shah V, Merlie JP, Sanes JR (1989) A laminin like adhesive protein concentrated in the synaptic cleft of the neuromuscular junction. Nature 338:229–233
56. Iivanainen A, Vuolteenaho R, Saino K, Eddy R, Shows TB, Sariola H, Tryggvason K (1994) The human laminin β2 chain (s-laminin): structure, expression in fetal tissues and chromosomal assigment of the LAMB2 gene. Matrix Biol 14:489–497
57. Iivanainen A, Sainio K, Sariola H, Tryggvason K (1995) Primary structure and expression of a novel human laminin α4 chain. FEBS Lett 365:183–188
58. Ingber DE, Folkman J (1989) Mechanochemical switching between growth and differentiation during fibroblast growth factor – stimulated angiogenesis in vitro: role of extracellular matrix. J Cell Biol 109:317–330
59. Inufusa H, Nakamura M, Adachi T, Nakatani Y, Shindo K, Yasutomi M, Matsuura H (1995) Localization of oncofetal and normal fibronectin in colorectal cancer. Correlation with histologic grade, liver metastasis and prognosis. Cancer 75: 2802–2808

60. Jenq W, Wu S-J, Kefalides NA (1994) Expression of the α2-subunit of laminin correlates with increased cell adhesion and metastatic propensity. Differentiation 58:29–36
61. Kaczmarek J, Castellani P, Nicolo G, Spina B, Allemanni G, Zardi L (1994) Distribution of oncofetal fibronectin isoforms in normal, hyperplastic and neoplastic human breast tissues. Int J Cancer 58:11–16
62. Kainz C, Speiser P, Wanner C, Obermair A, Tempfer C, Sliutz G, Reinthaller A, Breitenecker G (1995) Prognostic value of tumour microvessel density in cancer of the uterine cervix stage IB to IIB. Anticancer Res 15:1549–1552
63. Kallunki P, Sainio K, Eddy R, Byers M, Kallunki T, Sariola H, Beck K, Hirvonen H, Shows TB, Tryggvason K (1992) A truncated laminin chain homologous to the B2 chain: structure, spatial expression, and chromosomal assignment. J Cell Biol 119:679–693
64. Kocher O, Kennedy SP, Madri JA (1990) Alternative splicing of endothelial cell fibronectin mRNA in the IIICS region. Am J Pathol 137:1509–1524
65. Korang K, Christiano AM, Uitto J, Mauviel A (1995) Differential cytokine modulation of the genes LAMA3, LAMB3, and LAMC2, encoding the constitutive polypeptides, α3, β3, and γ2, of human laminin 5 in epidermal keratinocytes. FEBS Lett 368:556–558
66. Korhonen M, Sariola H, Gould VE, Kangas L, Virtanen I (1994) Integrins and laminins in human renal carcinoma cells and tumours grown in nude mice. Cancer Res 54:4532–4538
67. Kosmehl H, Langbein L, Katenkamp D, Vogel W, Berndt A (1993) Laminin matrix formation and S-100 protein and/or desmin-positive cells in malignant fibrous histiocytoma (MFH). Histol Histopathol 8:425–428
68. Kosmehl H, Berndt A, Katenkamp D, Hyckel P, Stiller KJ, Gabler U, Langbein L, Reh T (1995) Integrin receptors and their relationship to cellular proliferation and differentiation of oral squamous cell carcinoma. A quantitative immunohistochemical study. J Oral Pathol Med 24:343–348
69. Kosmehl H, Berndt A, Katenkamp D, Mandel U, Bohle RM, Gabler U, Celeda D (1995) Differential expression of fibronectin splice variants, oncofetal glycosylated fibronectin and laminin isoforms in nodular palmarfibromatosis (morbus Dupuytren). Pathol Res Pract 191:1105–1113
70. Langbein L, Kosmehl H, Katenkamp D, Neupert G, Stiller KJ (1990) Experimetally induced murine rhabdomyosarcomas – correlations between cellular contacts, matrix formation and cellular differentiation. Differentiation 44:185–196
71. Leivo I, Engvall E (1988) Merosin, a protein specific for basement membranes of Schwann cells, striated muscle, and trophoblast, is expressed late in nerve and muscle development. Proc Natl Acad Sci USA 85:1544–1548
72. Leivo I, Laurila P, Walström T, Engvall E (1989) Expression of merosin,a tissue-specific basement membrane protein, in the intermediate trophoblast cells of choriocarcinoma and placenta. Lab Invest 60:783–790
73. Leivo I, Engvall E, Laurila P, Miettinen M (1989) Distribution of merosin, a laminin-related tissue-specific basement membrane protein, in human Schwann cell neoplasms. Lab Invest 61:426–432
74. Lockwood CJ, Wein R, Lapinsky R, Casal D, Berkowitz G, Alvarez M, Berkowitz RL (1993) The presence of cervical and vaginal fibronectin predicts preterm delivery in an inner-city obstetric population. Am J Obstet Gynecol 169:789–804
75. Lohi J, Tani T, Laitinen L, Kangas L, Lehto V-P, Virtanen I (1995) Tenascin and fibronectin isoforms in human renal cell carcinomas, renal cell carcinoma cell lines and in xenografts in nude mice. Int J Cancer 63:442–449
76. Mandel U, Therkildsen MH, Reibel J, Sweeney B, Matsuura H, Hakomori S-I, Dabelsteen E, Clausen H (1992) Cancer-associated changes in glycosylation of fibronectin. Immunohistological localization of oncofetal fibronectin defined by monoclonal antibodies. APMIS 100:817–826
77. Mandel U, Gaggero B, Reibel J, Therkildsen MH, Dabelsteen E, Clausen H (1994) Oncofetal fibronectins in oral carcinomas: correlation of two different types. APMIS 102: 695–702
78. Mardon HJ, Grant RP, Grant KE, Harris H (1993) Fibronectin splice variants are differentially incorporated into the extracellular matrix of tumorigenic and non-tumorigenic hybrids between normal fibroblasts and sarcoma cells. J Cell Sci 104:783–792
79. Marinkovich PM, Lunstrum GP, Keene DR, Burgeson RE (1992) The dermal-epidermal junction of the human skin contains a novel laminin variant. J Cell Biol 119:695–703
80. Marinkovich MP, Verrando P, Keene DR, Meneguzzi G, Lunstrum GP, Ortonne JP, Burgeson RE (1993) The basement membrane proteins kalinin and nicein are structurally and immunologically homologous. Lab Invest 69:295–299
81. Marinkovich MP, Meneguzzi G, Burgeson RE, Blanchet-Bardons C, Holbrook KA, Smith LT, Christiano AM, Ortonne J-P (1995) Prenatal diagnosis of Herlitz junctional epidermolysis bullosa by amniocentesis. Prenat Diagn 15: 1027–1034
82. Mark K von der, Öcalan M (1989) Antagonistic effects of laminin and fibronectin expression on the myogenic phenotype. Differentiation 40:150–157
83. Martin PT, Ettinger AJ, Sanes JR (1995) A synaptic localization domain in the synaptic cleft protein laminin β2 (s-laminin). Science 269:413–416
84. Mathews GA, ffrench-Constant C (1995) Embryonic fibronectins are up-regulated following peripheral nerve injury in rats. J Neurobiol 26:171–188
85. Matsui C, Nelson CF, Hernandez GT, Herron S, Bauer EA, Hoeffler WK (1995) γ2 chain of laminin-5 is recognized by monoclonal antibody GB3. J Invest Dermatol 105:648–652
86. Matsui C, Wang CK, Nelson CF, Bauer EA, Hoeffler WK (1995) The assembly of laminin-5 subunits. J Biol Chem 270:23496–23503
87. Matsuura H, Hakamori S-I (1985) The oncofetal domain of fibronectin defined by monoclonal antibody FDC-6: its presence in fibronectins from fetal and tumour tissues and its absence in those from normal adult tissues and plasma. Proc Natl Acad Sci USA 82:6517–6521
88. Matsuura H, Greene T, Hakomori S-I (1989) An α-N-acetylgalactosaminylation at the threonine residue of a defined peptide sequence creates the oncofetal peptide epitope in human fibronectin. J Biol Chem 264:10472–10476
89. McGrath JA, Pulkkinen L, Christiano AM, Leigh IM, Eady RAJ, Uitto J (1995) Altered laminin 5 expression due to mutations in the gene encoding the β3 chain (LAMB3) in generalized atrophic benign epidermolysis bullosa. J Invest Dermatol 104:467–474
90. Miettinen M (1983) Immunohistochemical demonstration of laminin, the major glycoprotein of basement membranes, as an aid in the diagnosis of soft tissue tumours. Am J Clin Pathol 79:306–311
91. Miner JH, Sanes JR (1994) Collagen IV α3, α4 and α5 chains in rodent basal laminae: sequence, distribution, associations with laminins, and developmental switches. J Cell Biol 127:879–887
92. Nageotte MP, Casal D, Senyei A (1994) Fetal fibronectin in patients at increased risk for premature birth. Am J Obstet Gynecol 170:20–25
93. Nickeleit V, Zagachin L, Nishikawa K, Peters JH, Hynes RO, Colvin RB (1995) Embryonic fibronectin isoforms are synthesized in crescents in experimental autoimmune glomerulonephritis. Am J Pathol 147:965–978
94. Nicolo G, Salvi S, Oliveri G, Borsi L, Castellani P, Zardi L (1990) Expression of tenascin and ED-B containing oncofetal fibronectin isoform in human cancer. Cell Differ Dev 32:401–408
95. Nötzold U, Kosmehl H, Berndt A, Zardi L, Mandel U, Graupner H, Janitzky V, Katenkamp D (1996) Oncofetal fibronectins in renal cell carcinoma. Correlations to proliferative activity and nuclear malignancy grade. Pathol Res Pract 192: 345

96. Olsen D, Nagayoshi T, Fazio M, Peltonen L, Jaakkola S, Sanborn D, Sasaki T, Kuivaniemi H, Chu M-L, Deutzmann R, Timpl R, Uitto J (1989) Human laminin: cloning and sequence analysis of cDNAs encoding A, B1 and B2 chains, and expression of the corresponding genes in human skin and cultured cells. Lab Invest 60:772–782
97. Orian-Rousseau V, Aberdam D, Fontao L, Chevalier L, Meneguzzi G, Kedinger M, Simon-Assmann P (1996) Developmental expression of laminin-5 and HD1 in the intestine: epithelial to mesenchymal shift for the laminin γ2 chain subunit deposition. Dev Dynamics 206:12–23
98. Oyama F, Hirohashi S, Shimosato Y, Titani K, Sikiguchi K (1989) Deregulation of alternative splicing of fibronectin pre-mRNA in malignant human liver tumours. J Biol Chem 264:10331–10334
99. Oyama F, Hirohashi S, Shimosato Y, Titani K, Sikiguchi K (1990) Oncodevelopmental regulation of alternative splicing of fibronectin pre-messenger RNA in human lung tissues. Cancer Res 50:1075–1078
100. Paulsson M (1993) Laminin and collagen IV variants and heterogeneity in basement membrane composition. In: Rohrbach DH, Timpl R (eds) Molecular and cellular aspects of basement membranes. Academic Press, San Diego, pp 177–187
101. Paulsson M, Saladin K (1989) Mouse heart laminin. Purification of the native protein and structural comparison with Engelbreth-Holm-Swarm tumour laminin. J Biol Chem 264:18726–18732
102. Pulkkinen L, Christiano AM, Gerecke D, Wagman DW, Burgeson RE, Pittelkow MR, Uitto J (1994) A homozygous nonsense mutation in the β3 chain gene of laminin 5 (LAMB3) in Herlitz junctional epidermolysis bullosa. Genomics 24:357–360
103. Pulkkinen L, McGrath JA, Christiano AM, Uitto J (1995) Detection of sequence variants in the gene encoding the β3 chain of laminin 5 (LAMB3). Hum Mutat 6:77–84
104. Pyke C, Romer J, Kallunki K, Lund LR, Ralfkiaer E, Dano K, Tryggvasson K (1994) The γ2 chain of kalinin/laminin 5 is preferentially expressed in invading malignant cells in human cancers. Am J Pathol 145:782–791
105. Pyke C, Salo S, Ralfkiaer E, Romer J, Dano K, Tryggvasson K (1995) Laminin-5 is a marker of invading cancer cells in some human carcinomas and is coexpressed with the receptor for urokinase plasminogen activator in budding cancer cells in colon adenocarcinomas. Cancer Res 55:4132–4139
106. Rousselle P, Lunstrum GP, Keene DR, Burgeson RE (1991) Kalinin: an epithelium-specific basement membrane adhesion molecule that is a component of anchoring filaments. J Cell Biol 114:567–576
107. Ruoslahti E, Engvall E, Heyman EG (1981) Fibronectin. Current concepts of its structure and function. Collagen Res 1:95–128
108. Ryan MC, Tizard R, VanDevanter D, Carter WG (1994) Cloning of the LamA3 gene encoding the α3 chain of the adhesive ligand epiligrin. J Biol Chem 269:22779–22787
109. Sanes JR, Chiu AY (1983) The basal lamina of the neuromuscular junction. Cold Spring Harbor Symp Quant Biol 48:667–678
110. Sanes JR, Hall ZW (1979) Antibodies that bind specifically to synaptic sites on muscle fibre basal lamina. J Cell Biol 83:357–370
111. Sanes JR, Engvall E, Butkowski R, Hunter DD (1990) Molecular heterogeneity of basal laminae: isoforms of laminin and collagen IV at the neuromuscular junction and elsewhere. J Cell Biol 111:1685–1695
112. Schwarzbauer JE (1991) Alternative splicing of fibronectin: three variants, three functions. Bioessays 13:527–533
113. Sorokin L, Girg W, Göpfert T, Hallmann R, Deutzmann R (1994) Expression of a novel 400-kDa laminin chain by mouse and bovine endothelial cells. Eur J Biochem 223:603–610
114. Streuli CH, Bailey N, Bissell MJ (1991) Control of mammary epithelial differentiation: basement membrane induces tissue specific gene expression in the absence of cell-cell interaction and morphological polarity. J Cell Biol 115: 1385–1395
115. Tavian D, De Petro G, Colombi M, Portolani N, Giulini SM, Gardella R, Barlati S (1994) RT-PCR detection of fibronectin EDA+ and EDB+ mRNA isoforms: molecular marcers for hepatocellular carcinoma. Int J Cancer 56:820–825
116. Terpe H-J, Tajrobehkar K, Günther U, Altmannsberger M (1993) Expression of cell adhesion molecules α-2, α-5 and α-6 integrin, E-cadherin, N-CAM and CD 44 in renal cell carcinomas. Virchows Arch [A] 422:219–224
117. Timpl R, Brown JC (1994) The laminins. Matrix Biol 14:275–281
118. Timpl R, Rohde H, Gehron Robey P, Rennard SI, Foidart JM, Martin GR (1979) Laminin – a glycoprotein from basement membranes. J Biol Chem 254:9933–9937
119. Tryggvason K (1993) The laminin family. Curr Opin Cell Biol 5:877–882
120. Uitto J, Pulkkinen L, Christiano AM (1994) Molecular basis of the dystrophic and junctional forms of epidermiolysis bullosa: mutations in the type VII collagen and kalinin (Laminin 5) genes. J Invest Dermatol 103:39S-46S
121. Vailly J, Pulkkinen L, Miquel C, Christiano AM, Gerecke D, Burgeson RE, Uitto J, Ortonne J-P, Meneguzzi G (1995) Identification of a homozygous one-basepair deletion in exon 14 of the LAMB3 gene in a patient with Herlitz junctional epidermolysis bullosa and prenatal diagnosis in a family at risk for recurrence. J Invest Dermatol 104:462–466
122. Virtanen I, Laitinen L, Korhonen M (1995) Differential expression of laminin polypeptides in developing and adult human kidney. J Histochem Cytochem 43:621–628
123. Virtanen I, Tanni T, Bäck N, Häppölä O, Laitinen L, Kiviluoto T, Salo J, Burgeson RE, Lehto V-P, Kivilaakso E (1995) Differential expression of laminin chains and their integrin receptors in human gastric mucosa. Am J Pathol 147:1123–1132
124. Walker-Caprioglio HM, Hunter DD, McGuire PG, Little SA, McGuffee LJ (1995) Composition in situ and in vitro of vascular smooth muscle laminin in the rat. Cell Tissue Res 281:187–196
125. Wayner EA, Gil SG, Murphy GF, Wilke MS, Carter WG (1993) Epiligrin, a component of epithelial basement membranes, is an adhesive ligand for α3β1 positive T lymphocytes. J Cell Biol 121:1141–1152
126. Weidner N, Carrol PR, Flax J, Blumenfeld W, Folkman J (1993) Tumour angiogenesis correlates with metastasis in invasive prostate carcinoma. Am J Pathol 143:401–409
127. Wewer U, Wayner EA, Hoffstrom BG, Lan F, Mayer-Nielsen B, Engvall E, Albrechtsen R (1994) Selective assembly of laminin variants by human carcinoma cells. Lab Invest 71:719–730
128. Yamada KM (1991) Fibronectin and other cell interactive glycoproteins. In: Hay ED (eds) Cell biology of extracellular matrix. Plenum Press, New York, pp 111–146
129. Yamada KM, Akiyama SK, Hasegawa T, Hasegawa E, Humphries MJ, Kennedy DW, Nagata K, Urushihara H, Olden K, Chan WT (1985) Recent advances in research on fibronectin and other cell attachment proteins. J Cell Biochem 28:79–97
130. Zardi L, Carnemolla B, Siri A, Petersen TE, Paolella G, Sebastio G, Baralle FE (1987) Transformed human cells produce a new fibronectin isoform by preferential alternative splicing of a previously unobserved exon. EMBO J 6:2337–2342
131. Zhang D, Burton-Wurster N, Lust G (1994) Antibody specific for extra domain B of fibronectin demonstrates elevated levels of both etra domain B(+) and B(–) fibronectin in osteoarthritic canine cartilage. Matrix Biol 14:623–633
132. Zhang D, Burton-Wurster N, Lust G (1995) Alternative splicing of ED-A and ED-B sequences of fibronectin pre-mRNA differs in chondrocytes from different cartilaginous tissues and can be modulated by biological factors. J Biol Chem 270:1817–1822