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Editorial

Perlecan: the multidomain heparan sulphate proteoglycan of basement membrane and extracellular matrix

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Introduction

The last few years have seen proteoglycans rapidly assuming a central importance in many diverse areas of biology. In addition to their well-known biophysical attributes, proteoglycans are now known to function as cell surface receptors, ligands and antagonists for growth factors and cytokines, and extracellular signals for cellular growth, migration and differentiation (Wight et al. 1992). A universal rigid classification system for proteoglycans would be difficult to devise, since the only common factor grouping these molecules together is the covalent attachment of one or more glycosaminoglycan chains, sulphated linear polysaccharides, with precisely defined repeating disaccharide units. The protein cores of these molecules can be unique, or fall into broad family groupings, and the same core can even carry different glycosaminoglycans under varying circumstances. However, the proteoglycans are most commonly ordered under the major type of glycosaminoglycan bound, and further subdivided into the location of the molecule within the cell, at the plasma membrane or in the pericellular space or extracellular matrix (Gallagher 1989).

The focus of this brief synopsis will be the large heparan sulphate proteoglycan of basement membranes and extracellular matrix, also known as perlecan for its "beaded" pearl-like appearance on rotary shadowed electron micrographs of isolated molecules (Pausson et al. 1987; Laurie et al. 1988). Heparan sulphate proteoglycans from basement membranes have been the subject of extensive investigations both from human tissues and the murine Engelbreth-Holm-Swarm (EHS) tumour, which has facilitated a vast increase in our knowledge of many basement membrane constituents. Estimates of proteoglycan protein core size have varied and gradually increased over the years. From first estimates of 10–20 kDa, improved tissue dissociation and extraction methods and a greater appreciation of the activities of proteases gave core sizes as large as 400 kDa (for selected references, see Heremans et al. 1989). Recently cloned cDNAs for the human molecule predict a Mr for the perlecan protein with no glycosylation or glycation of 467 kDa (Kallunki and Tryggvason 1992; Murdoch et al. 1992), one of the largest polypeptides in the human body. It now seems likely that the large ($\geq$ 400 kDa) heparan sulphate proteoglycans found in basement membranes and in the pericellular matrix of such cells as colon carcinoma cells (Iozzo 1984) and lung fibroblasts (Heremans et al. 1989) all represent the same proteoglycan. Some workers contend that the smaller heparan sulphate proteoglycans isolated from some tissue sources represent a distinct gene product (Paulsson et al. 1987; Kato et al. 1987) whereas others have evidence for proteolytic processing and a precursor/product relationship (Klein et al. 1988). Intriguingly, specific antibodies, raised against the large perlecan protein core were able to immunolocalize this heparan sulphate proteoglycan to basement membranes from which cores of such size were still experimentally unextractable. The presence of perlecan has been demonstrated in this manner in virtually all vascular basement membranes, and those at epithelial/mesenchymal interfaces (Fig. 1). Also these probes revealed that this intrinsic basement membrane component was not restricted to this location, being found in stromal elements of connective tissues and sinusoidal regions of organs such as liver (Couchman and Ljubimov 1989) where no classically described basement membrane exists (Martinez-Hernandez and Amenta 1983).

Molecular structure of perlecan: a chimeric molecule with diverse structural domains

Examination of the predicted primary structure of perlecan reveals that this extremely large and complex molecule can be described as an assembly of five distinct structural domains. Computer-assisted analysis indicates

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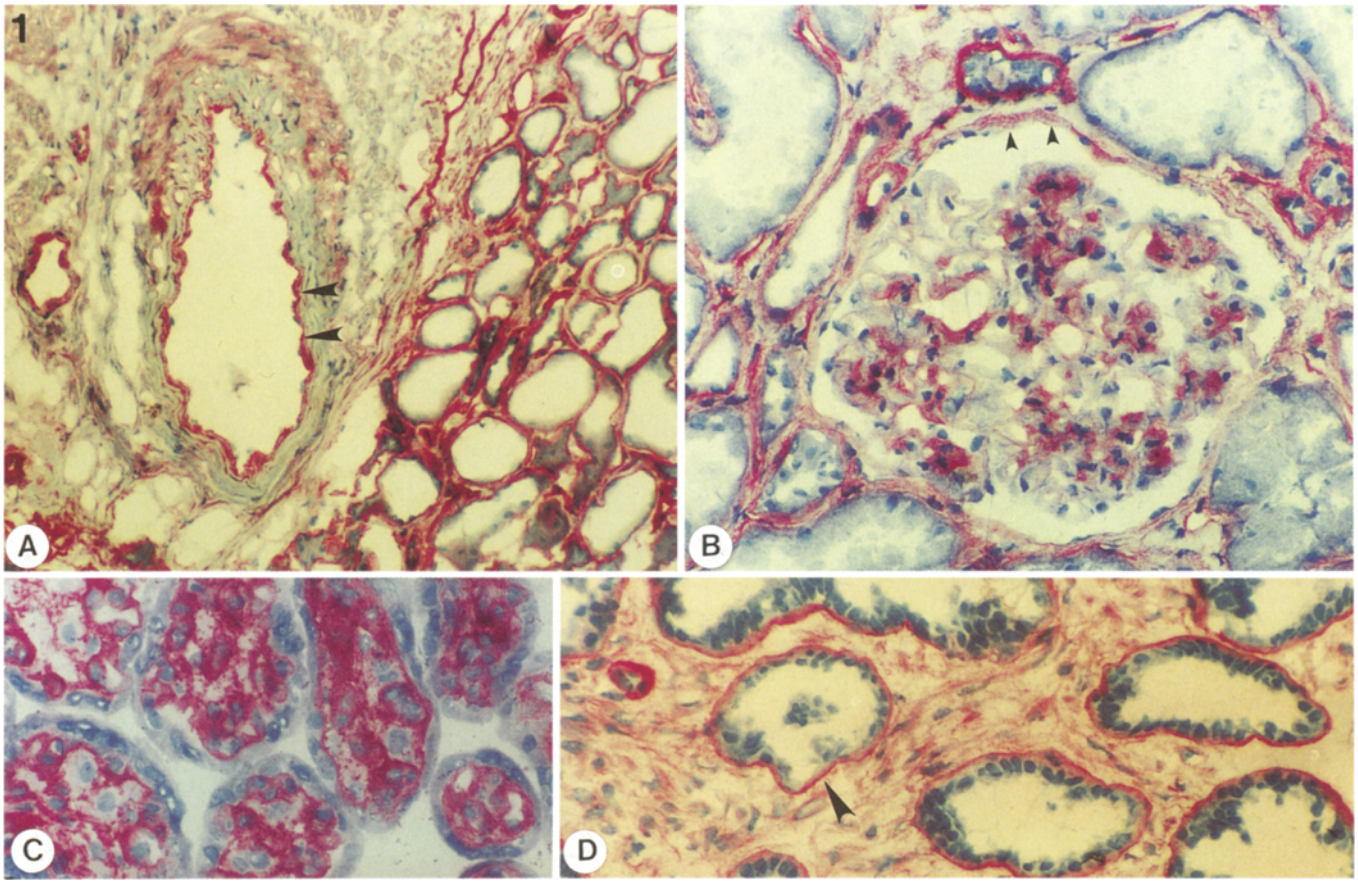

that these portions of the protein core are related to highly disparate molecules of the cell surface and extracellular matrix (Fig. 2). Domain I appears to be unique to perlecan, the only related structure being in the highly similar mouse homologue (Noonan et al. 1991). This domain is expected to be a site of heparan sulphate glycosaminoglycan attachment, based on the presence of a cluster of three potential glycosaminoglycan attachment sites, and the appearance on rotary shadowed electron micrographs of purified EHS perlecan of a cluster of heparan sulphate chains at one end of the molecule (Paulsson et al. 1987; Laurie et al. 1988). Domain II is a cysteine-rich domain with quite striking homology to the low-density lipoprotein (LDL) receptor (Yamamoto et al. 1984). The second repeat in perlecan contains the pentapeptide DGSDE, conserved in all the repeats in the LDL receptor itself, and thought to mediate the binding of LDL to the receptor. A proline-rich region of unknown function is found between the first and second repeats in perlecan. Domain III has similarities to the region of laminin chains, particularly the A chain, that form the short arms of the laminin multimer (Haaparanta et al. 1991). Four cysteine-rich subdomains are interspersed with cysteine-free globular regions most similar to the laminin sub-domain IV. Domains IV and IIa of perlecan are composed of multiple internal repeats homologous to those found in the neural cell adhesion molecule N-CAM (Cunningham et al. 1987), making perlecan a member of the immunoglobulin gene superfamily. Domain IV contains the only major structural difference so far reported between the human and murine perlecan proteins. With 21 repeats, the human molecule is 7 larger than the mouse and is the mammalian protein containing the largest assembly of IgG-like repeats. Interestingly, there is evidence for the mouse cDNAs containing additional repeats in this portion of the structure, indicating possible alternative splicing of mRNA (Noonan and Hassell 1993). However, there is no clear evidence to date to support similar processing in the human species. Domain V contains seven discrete subdomains: four cysteine-rich domains similar to those described in epidermal growth factor (EGF) (Appella et al. 1988) and three globular subdomains with homology to the carboxyl terminal G domains of laminin A and merosin chains. A combination of EGF and G domain repeats is also found in the neurexins: neuronal cell surface proteins (Ushkaryov et al. 1992).

Protein modules of perlecan: implications for function

Duplication of modular structures to form complex multidomain proteins is a common theme in macromolecules of the extracellular matrix. These diverse structures derive from either primordial gene duplication or from exon shuffling. The question remains as to whether a particular domain performs the same or similar functions, regardless of the molecule in which it is found.

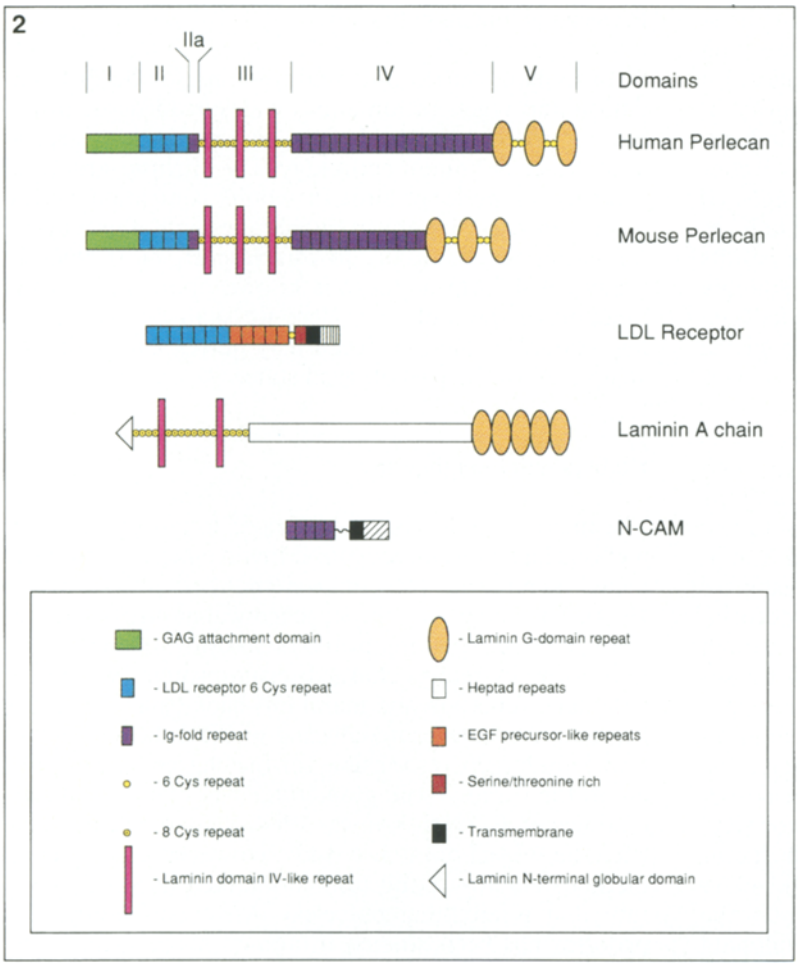

Fig. 1. Immunoenzymatic localization of perlecan in human kidney (**A, B**), placenta (**C**) and ovarian tumour (**D**). Notice the presence of perlecan epitopes along the endothelial basement membrane (*arrowheads*, **A**) and in the basement membrane region of renal tubules (**A**) and glomerular capillaries (**B**). The Bowman capsule (*arrowheads*, **B**) is also positive, as well the villi of full-term placenta (**C**) and the basement membrane of a follicular adenoma of the ovary (*arrowhead*, **D**). Frozen sections were reacted with the HS42 monoclonal antibody (Isemura et al. 1988) which recognizes the core protein of human perlecan (Murdoch et al. 1992). The HS42 antibody was diluted 1:1000 and immunoreactive material was visualized by rabbit anti-mouse bridging antibodies and alkaline phosphatase/anti-alkaline phosphatase antibodies

Fig. 2. Schematic representation of the multidomain nature of human perlecan and related proteins. A key to some of the repetitive structures (modules) is given in the bottom panel. For additional details, see the text

Nomenclature can be potentially misleading since domains tend to be named after the molecule in which they were first recognized, and even small changes in sequence and/or conformation may alter or completely remove any particular activity. One must therefore exercise some caution in ascribing similar functions to modules with even quite high homology at the amino acid level. As sequence data rapidly accumulate, there is an increasing void between these and experimentally verified functions. However, the perlecan core is such a large protein and its sequence is so conserved across species that it would seem an extravagant waste of resources to produce without a functional necessity for such size. Aside from this teleological reasoning, the mosaical domain structure of perlecan is immediately suggestive of many functions without considering the possibility of multiple forms generated by alternative mRNA splicing or post-translational modifications. So, do each of the domains of perlecan have a specific function? Are these functions similar to those of the families to which they are structurally related?

There are a growing number of identified roles for perlecan, aside from anchoring the highly polyanionic heparan sulphate chains in the extracellular matrix, and the homologies seen at the amino acid level have suggested some more, initially less obvious avenues of investigation. The protein core binds fibronectin (Isemura et al. 1987; Heremans et al. 1990) possibly via domain III, and other components of the stromal extracellular matrix such as type I collagen (Koda and Bernfield 1984). Binding studies with basement membrane components are sparse, however in at least one cell line, perlecan appears to associate with laminin at a very early stage of synthesis and before secretion from the cell (Frenette et al. 1989). More work remains to be done on the matrix interactions of this complex multimodular structure and its involvement in basement membrane assembly.

A number of cell types interact with the perlecan core. Hepatocytes may bind through potential cell surface receptors of 26–38 and 80 kDa (Clément et al. 1989; Clément and Yamada 1990), and endothelial cells adhere to murine perlecan via $\beta 1$ and $\beta 3$ integrins in a largely RGD-dependent manner (Hayashi et al. 1992). Interestingly, since the RGD triplet sequence is not present in human perlecan, this interaction in humans must depend on an alternative mechanism. In relation to this point, the homology of domains III and V of perlecan to regions of the laminin molecule does not extend to most of the many peptide sequences associated with the cell binding properties of laminin. One exception in domain V is the presence of two Leu-Arg-Glu (LRE) sequences,

potential mediators of motor neuron attachment in s-laminin (Hunter et al. 1989). One of the most interesting aspects of perlecan biology is its possible implication in the orderly development and functional integrity of skeletal muscle. A recent study by Moerman and co-workers (Rogalski et al. 1993) has demonstrated a conserved species of perlecan in the worm *Caenorhabditis elegans*. A null mutation of this gene is lethal and induces a disruption of the skeletal architecture. These results suggest the possibility that perlecan may be seemingly involved in mammalian control of skeletal muscle function since this proteoglycan is widely distributed in the basement membranes of skeletal and cardiac myocytes (Murdoch et al. 1992).

No contemporary discussion of proteoglycans can be complete without reference to their ability to bind growth factors and cytokines. The discovery of these interactions has moved proteoglycans towards centre stage in many biological systems (Ruoslahti and Yamaguchi 1991). Unlike some proteoglycans, for example the small extracellular matrix proteoglycan decorin, which can bind transforming growth factor-β through its core protein (Yamaguchi et al. 1990), the major association of heparan sulphate proteoglycans with these potent modulators of cell proliferation and differentiation is through the heparan sulphate glycosaminoglycans. Heparan sulphate has been shown to bind interleukin-3, granulocyte-macrophage colony stimulating factor (Roberts et al. 1988) and basic fibroblast growth factor (FGF) (Vigny et al. 1988; Bashkin et al. 1989), all in active or potentially active forms. More recently the binding site for bFGF in a cell surface heparan sulphate proteoglycan has been identified as a specific carbohydrate sequence within the heparan sulphate chains (Turnbull et al. 1992). It is not known whether extracellular matrix heparan sulphate proteoglycans such as perlecan bind bFGF through this same oligosaccharide. This is obviously a highly important aspect of heparan sulphate function with respect to growth and differentiation and in disease states. It has been shown that in order for bFGF to bind to its high affinity receptor and potentiate effects within the cell, it must first bind to a cell surface heparan sulphate proteoglycan or free heparan sulphate chains (Yayon et al. 1991). The simplest explanation offered for this phenomenon is that binding of the growth factor to the glycosaminoglycan may alter the conformation of the bFGF such that it can then bind to the receptor. Basic FGF bound to heparan sulphate chains and sequestered in the extracellular matrix is protected from proteolytic degradation (Saksela et al. 1988) and thus may represent a pool or "bank" of growth factors stored in the local microenvironment for later use. This might possibly be achieved by displacement from the matrix by the selective temporal and spatial expression of cell surface heparan sulphate proteoglycans during morphogenesis and development (e.g. syndecan) (Bernfield and Sanderson 1990), or by degradation with proteases or endoglycosidases, releasing the bFGF in a heparan sulphate-bound active form.

Perlecan in human pathology

With so many varied potential roles in growth, differentiation and regeneration and a widespread tissue distribution, perlecan seems likely to be a molecule of crucial importance to human pathology. The intrinsic structural diversity of perlecan protein would contribute to the heterogeneity of basement membranes and offer a target for pathology to occur. In this respect, more lines of inquiry will form as more is known about the normal function of the molecule. Here we shall discuss only a few examples where perlecan may participate in the development of the pathological state.

Diabetic microangiopathy

The microvascular complications of long-term diabetes mellitus have a well-recognized involvement of the basement membrane (Martinez-Hernandez and Amenta 1983). The underlying physicochemical nature of the matrix is altered, resulting in the breakdown of the barrier that normally retains charged serum proteins within the vascular circulation and maintains haemostasis. Because heparan sulphate proteoglycans in the basement membrane are largely responsible for the charge barrier present in the vascular and glomerular basement membrane (Kanwar et al. 1980; Kanwar 1984) these molecules have been the subject of intense study. However, experimental methods employed and results obtained have left a picture that is anything but clear. Most commonly, this problem has been studied in animal models, following spontaneous or chemical induction of the diabetic state. Investigators have examined a number of basement membrane parameters in whole kidneys, isolated glomeruli and even in EHS tumour grown in diabetic mice. Findings from studies on perlecan synthesis include evidence for a decrease in sulphate incorporation and an overall reduction in proteoglycan synthesis (Rohrbach et al. 1992, 1983) and conversely no change in perlecan mRNA levels in diabetic animals (Ledbetter et al. 1990). As suggested above, the experimental methods used by different workers can make direct comparison of results of somewhat dubious value. However, a generally agreed effect appears to be that of a net reduction in overall charge on the affected basement membrane.

Alzheimer's disease

Interest in the participation of basement membrane components and perlecan in particular in the pathogenesis of Alzheimer's disease has been sparked in recent years by the discovery of this molecule in the vascular and neuritic plaque deposits characteristic of this condition (reviewed in Snow and Wight 1989) and other amyloidoses (Snow et al. 1990). It is not clear whether this high affinity association is secondary to the formation of plaques, however in vitro binding has been shown to induce structural changes in the Alzheimer amyloid β-protein to a form found in amyloid deposits. Quantita-

tive analysis of perlecan/amyloid precursor protein binding data has shown high affinity interactions with dissociation constants in the order of 1–10 nM, a value similar to that observed for fibronectin (Narindrasorasak et al. 1991). These data suggest that a specific binding of perlecan protein core to the amyloid precursor may be involved in the nucleation stages of Alzheimer's disease type amyloidogenesis. Work continues in animal models to define the roles played by this and other basement membrane components during development of in vivo amyloid formation.

Tumour progression

The relationship within a tissue between the cells and their surrounding matrix is a complex and dynamic state of flux. Synthesis and degradation of matrix and division of cells are maintained in balance at least in part by a combination of growth factors, extracellular matrix macromolecules, proteases, protease inhibitors and their associated activities. Neoplastic cells are presented with a number of barriers to tumour progression and need to migrate through the extracellular matrix and across a vascular basement membrane in order to metastasize. Alterations in the local extracellular matrix may facilitate this progression, for example in colon carcinoma. Normal colon synthesizes heparan sulphate proteoglycans (Iozzo and Wight 1982) known to bind and activate protease inhibitors (Pejler et al. 1987) and some dermatan sulphate proteoglycan. Colon carcinoma tissue, conversely, synthesizes proteoglycans containing largely chondroitin sulphate, thus changing the nature and properties of the local extracellular matrix. Alterations in the tumour matrix either directly, or by influence on surrounding stromal cells (tumour desmoplasia) may result in a matrix permissive for tumour progression. The release of glycosaminoglycan/growth factor complexes as a result of degradative enzymes secreted by actively metastasizing cells may also play some role in the establishment of a blood supply to an actively growing tumour by the local stimulation of vascularization. For more thorough reviews of the complex interactions of neoplastic cells with their surrounding stroma and the regulation of protease and growth factor activities by the matrix, see Iozzo (1985) and Flaumenhaft and Rifkin (1991), respectively.

Future prospects

Our knowledge and understanding of perlecan structure and function have accelerated rapidly in the last five years. Much more remains to be discovered. For example, no diseases resulting from an alteration in the perlecan gene have been recognized at this time. This may be because such mutations would always prove lethal to the developing embryo. However, genetic changes affecting perlecan function could also reside in the genes responsible for the many post-translational modifications applied to the maturing proteoglycan. An experimental genetic approach using transgenic animals and transfected cell lines should prove to be extremely useful in deciphering the potentially multiple roles of perlecan in normal growth, development and disease. As with many other proteins, future perlecan research will exploit the mass of raw data generated by the power of recombinant DNA techniques. The challenging but exciting prospect is that of integrating new discoveries into a coherent picture of function in cooperation with cell surface molecules and other residents of the extracellular matrix.

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