

### Life Without Perlecan Has its Problems

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**P**ERLECAN is a large heparan sulfate proteoglycan with a wide tissue distribution and multiple potential functions (see Iozzo, 1998). The glycosaminoglycan chains, located in the NH<sub>2</sub>-terminal domain of the core protein, bind basic FGF-2 and have been shown to promote the mitogenic and angiogenic activities of FGF-2. They also interact with the basement membrane components, laminin-1 and collagen IV, and are thought to represent a barrier to the passage of cationic macromolecules across glomerular basement membranes in the kidney. The 400–450-kD core protein, composed of several protein modules arranged in five distinct domains, binds to a variety of small and large molecules, including FGF-7, fibronectin, heparin, laminin-1, PDGF-BB, and integrins (Fig. 1). The physiological significance of such interactions is illustrated by the demonstration that lowering of perlecan levels by stable expression of antisense constructs in colon carcinoma cells depresses their FGF-7-dependent growth, and reduces tumor growth and tumor-induced angiogenesis in nude mice (Sharma et al., 1998). Also, perlecan is a strong inducer of FGF-2-dependent neovascularization (Aviezer et al., 1994). These same interactions of perlecan may well explain the finding that perlecan produced by vascular endothelial cells can inhibit proliferation and binding of FGF-2 in smooth muscle cells (Forsten et al., 1997).

Perlecan is frequently described as a member of a small group of basement membrane proteoglycans, the other members being agrin and bamacan (Iozzo, 1998). While there is no doubt that perlecan is an important molecule within basement membranes, recent data also suggest that it may have critical functions in other extracellular matrices (Handler et al., 1997). Particularly interesting is the expression in developing and mature cartilage, raising the possibility that it has a structural or signaling function in cartilage development and endochondral ossification.

All these studies suggest that perlecan may not only look like the pearls on a string structure suggested by its rotary shadowing EM image, but in fact may be a real pearl among extracellular matrix components: a multifunctional macromolecule for many reasons and seasons. That this may indeed be so, is now indicated by the consequences of knocking out the perlecan gene in mice.

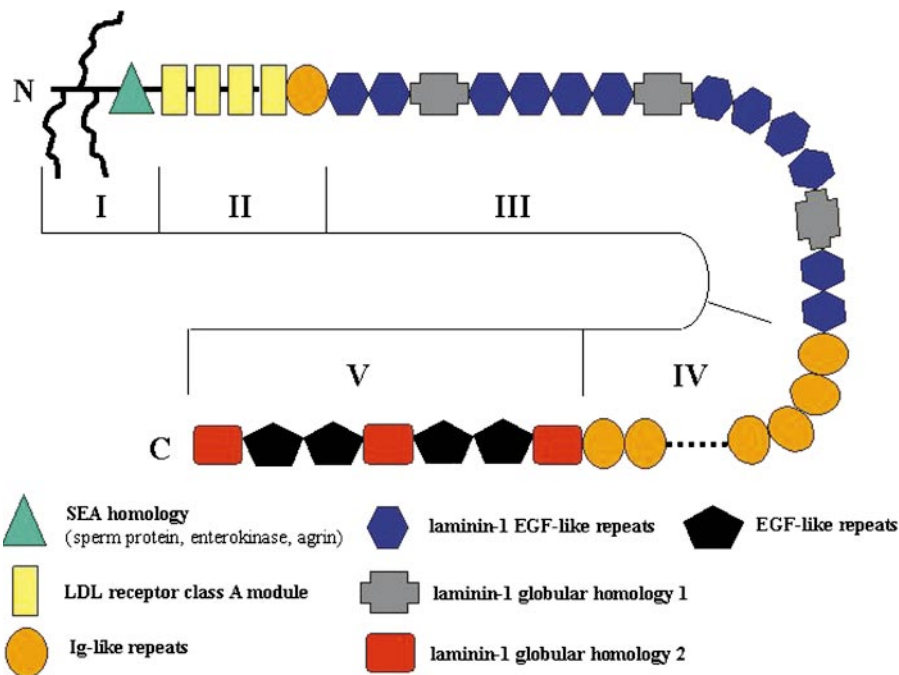
Costell et al. (1999) describe what happens when perlecan expression is completely abolished. The results are surprising, both for the severity of the abnormalities and the specific sites that are affected. In homozygous knockout embryos, no abnormalities are observed before embryonic day 10 (E10)<sup>1</sup>, but between E10 and E12, most of the embryos die with evidence of bleeding into the pericardial sac. A few animals survive, but die around birth with severe defects in the brain and in the skeleton. The lack of any defects before E10 is surprising because perlecan is first expressed in two-cell embryos and increases on the external surface of trophoblast cells of blastocysts. Thus, it has been thought to play a role in the initial attachment of the embryo to the uterine wall (Smith et al., 1997). The absolutely normal Mendelian ratio of wild-type, heterozygous, and homozygous embryos at E9.5 rules out a limiting role for perlecan during implantation. Also, the normal appearance of most basement membranes in homozygous embryos suggests that perlecan does not have a critical role in the assembly of basement membranes (Timpl and Brown, 1996). Perhaps the proteoglycan agrin, which shares homology with perlecan, can substitute for the absence of perlecan. Another possibility is that collagen XVIII, also a heparan sulfate proteoglycan component of most basement membranes, can substitute for the loss of perlecan function (Muragaki et al., 1995; Halfter et al., 1998).

The demise of homozygous knockouts by cardiac arrest between E10 and E12 is associated with loss of normal basement membrane structure around myocardial cells and appearance of holes in the myocardium. Since basement membrane structures were seen in the myocardium of all embryos at E9.5, Costell et al. (1999) conclude that basement membranes can form in the absence of perlecan, but perlecan is essential for maintenance of the mechanical and functional integrity of basement membranes in the heart, particularly at a time when the intraventricular blood pressure is thought to increase significantly. The interpretation is supported by the abnormalities caused by mutations in the perlecan homologue *unc-52* in *Caenorhabditis elegans*. Depending on the nature of the mutation, *unc-52* larvae are either paralyzed and fail to develop beyond the twofold stage or are paralyzed as adults with disrupted myofibril-membrane attachments in body wall muscle cells (Rogalski et al., 1993).

Costell et al. (1999) also invoke a loss of mechanical

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1. Abbreviation used in this paper: E, embryonic day.



**Figure 1.** Structure of perlecan (see Iozzo, 1998). Several distinct protein modules are organized in five domains (I-V). Domain I contains the glycosaminoglycan side chains (indicated by wavy lines). Note that in domain IV only six of the 21 Ig-like repeats are shown.

function to explain the abnormalities seen in embryos that survive the cardiac crisis period: loss of basement membrane structure at the surface of the developing brain with neuroepithelial cells migrating into the surrounding mesenchyme. This results in lack of calvarial bone formation and exencephaly in some embryos and neuronal ectopias in all embryos. The defects are thought to be caused by decreased mechanical strength of basement membranes and localized rupture in areas that are particularly sensitive to the pressure within the brain vesicles. While a mechanical basement membrane abnormality seems a reasonable explanation, another possibility that needs to be considered in future studies is that the absence of perlecan results in increased proteolytic degradation of basement membrane components during the remodeling of basement membranes that takes place as the brain grows. Perlecan may protect proteins against proteolytic attack and may represent an antiproteolytic shield around protease-sensitive components (such as nidogen) in basement membranes (Gupta-Bansal et al., 1995).

Surprisingly, all homozygous embryos surviving to birth also show severe skeletal defects with short axial and limb bones, cleft palate, and striking abnormalities in the growth plates of long bones. Although perlecan is expressed in developing cartilage and can promote differentiation of chondrocytes in culture (French et al., 1999) such an essential role for perlecan in cartilage growth and endochondral ossification is quite unexpected. In normal growth plates, chondrocytes are arranged into zones of resting, proliferating, and mature (hypertrophic) cells, and the cellular activities within each zone and the transitions between them are regulated by key signaling molecules. In perlecan knockouts, the proliferating zone is disorganized, the hypertrophic zone shows signs of increased levels of synthesis of several matrix molecules, and is frequently separated from the proliferative zone. Ossification below

the growth plate appears to extend as much radially as it does along the longitudinal axis of long bones.

The authors suggest that perlecan protects the extracellular matrix of cartilage by inactivating matrix proteases or masking/protecting proteins against proteolytic degradation (Costell et al., 1999). A role for perlecan in modulating important signaling pathways that regulate chondrocyte proliferation and hypertrophy is also a strong possibility and needs to be considered in future studies. FGFR3 is a negative regulator of chondrocyte proliferation and hypertrophy, and perlecan may modulate its activity by sequestering FGF ligands (Deng et al., 1996). Furthermore, Indian hedgehog is produced by cells defining the transition between proliferating and hypertrophic chondrocytes, and it controls the expression of parathyroid hormone-related peptide (PTHrP), a negative regulator of hypertrophy, through a feedback loop that involves Patched-expressing cells in the surrounding perichondrium (Vortkamp et al., 1996). Identification of a gene (*tout-velu*) in *Drosophila* that promotes the diffusion of hedgehog over several cell diameters as a homologue of a human glycosyl transferase, raises the very interesting possibility that perlecan may play a role in diffusion of Indian hedgehog in the growth plates of long bones (Bellaiche et al., 1998; Lind et al., 1998). The glycosyl transferase homologue of *tout-velu* (EXT1) is involved in the synthesis of heparan sulfate proteoglycans; mutations in EXT1 cause the formation of multiple cartilage-capped bone tumors around growth plates (exostoses) in affected individuals (Ahn et al., 1995). Heparan sulfate proteoglycans may, therefore, be critical for normal diffusion of sonic or Indian hedgehog.

Further studies of these and other possibilities would benefit from the generation of conditional knockouts to circumvent the bottleneck of embryonic lethality. Such conditional knockouts would also be extremely useful in

analyses of the role of perlecan in promoting the growth and invasion of tumors, its role in glomerular filtration in the kidney, and its angiogenesis promoting activities. With a variety of cell-specific promoters available, both for vascular endothelial cells and proliferating and hypertrophic chondrocytes, generation of conditional perlecan mutants should be relatively straightforward. We look forward to further exciting news from the perlecan front.

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