Chapter 6
Small Leucine-Rich Proteoglycans

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Abstract The small leucine-rich proteoglycans (SLRPs) comprise an expanding family of proteoglycans and glycoproteins that now encompass five distinct groups including three canonical and two noncanonical classes based on shared structural and functional parameters. SLRPs are tissue organizers by orienting and ordering various collagenous matrices during ontogeny, wound repair, and cancer and interact with a number of surface receptors and growth factors, thereby regulating cell behavior. The focus of this chapter is on novel conceptual and functional advances in our understanding of SLRP biology with special emphasis on genetic diseases, cancer growth, fibrosis, osteoporosis, and other biological processes where these proteoglycans play a central role.

6.1 Introduction

Small leucine-rich proteoglycans (SLRPs) (Iozzo 1999) are present within the extracellular matrix of all tissues and within the thin membranes that envelop all the major parenchymal organs such as pericardium, pleura, periosteum, perimesium, and adventitia of blood vessels. This strategic location suggests that SLRPs are involved in the control of organ shape and size (Iozzo 1998). The characteristic hallmark of all the SLRPs is their intrinsic ability to interact with other proteins. Foremost among these interactions are those with collagens, growth factors, and various plasma membrane receptors. Various SLRPs interact with fibrils of collagen

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type I, II, III, V, and XI, forming a "surface coat". Indeed, the eponym "decorin" is based on its ability to decorate fibrillar (banded) collagen in a periodic fashion. The surface coat formed by various SLRPs is a sort of biological processor that regulates the physiology of collagenous matrices in a tissue-specific manner. This coat plays two fundamental roles: (1) it regulates proper fibril assembly, which occurs through lateral association of collagen molecules, and (2) it protects collagen fibrils from cleavage by collagenases by acting as a steric barrier limiting the access of the collagenases to their cleavage sites. This biological activity is governed by SLRP dual activities evoked by the glycosaminoglycan or protein core moieties. Some of the SLRPs contain stretches of amino acids that can be sulfated such as the polytyrosine sulfate in fibromodulin or the polyaspartate region of asporin. The region containing either the sulfated glycosaminoglycan(s) or the charged amino acid residues is consistently located at the N terminus, outside the leucine-rich repeats (LRRs). First, we will review recent advances in the biology of SLRPs with special emphasis on the molecular interactions and mechanisms of action of SLRP signaling and as causative agents of genetic diseases. Then, we will critically assess the involvement of SLRPs in various pathologies, including inflammation, fibrosis, bone diseases, cancer, and angiogenesis.

6.2   Structure, Evolutionary Conservation, and Specificity of Function

SLRPs are grouped into five classes based mainly on evolutionary conservation, homology at both the protein and genomic level, and chromosomal organization (Fig.6.1a) (Schaefer and Iozzo 2008). In total, there are 18 genes that encode SLRPs spread over seven chromosomes. Until recently, SLRPs were grouped into only three canonical classes (Iozzo and Murdoch 1996; Iozzo 1997). However, two new noncanonical classes have been recently introduced (Schaefer and Iozzo 2008): class IV, which includes chondroadherin, nyctalopin, and tsukushi, and class V with podocan and podocan-like protein 1. Regardless of the classification used, SLRPs share common functionality. For example, decorin, biglycan, asporin, and podocan bind to type I collagen, while decorin, biglycan, and lumican inhibit cell growth and various SLRPs interact with TGF-β and bone morphogenetic protein (BMP).

   The typical, easily recognizable, structural features of SLRPs include a variable number of LRRs in the central portion of the protein (Fig. 6.1b). LRRs are units of ~24 amino acids characterized by a conserved pattern of hydrophobic residues. Each LRR folds into a secondary structure comprising a short parallel β-sheet, a turn, and a more variable region. Essentially, the LRRs form a curved, solenoid structure where specific protein interactions are mediated through the side chains of variable residues protruding from the short parallel β-strands that form the inner (concave) surface of the solenoid, a sort of 3D coil. The LRRs are preceded by a cysteine-rich region at the N terminus, comprising four cysteine residues with a variable number of intervening amino acids, which defines the various classes (Fig. 6.1b). A C-terminal
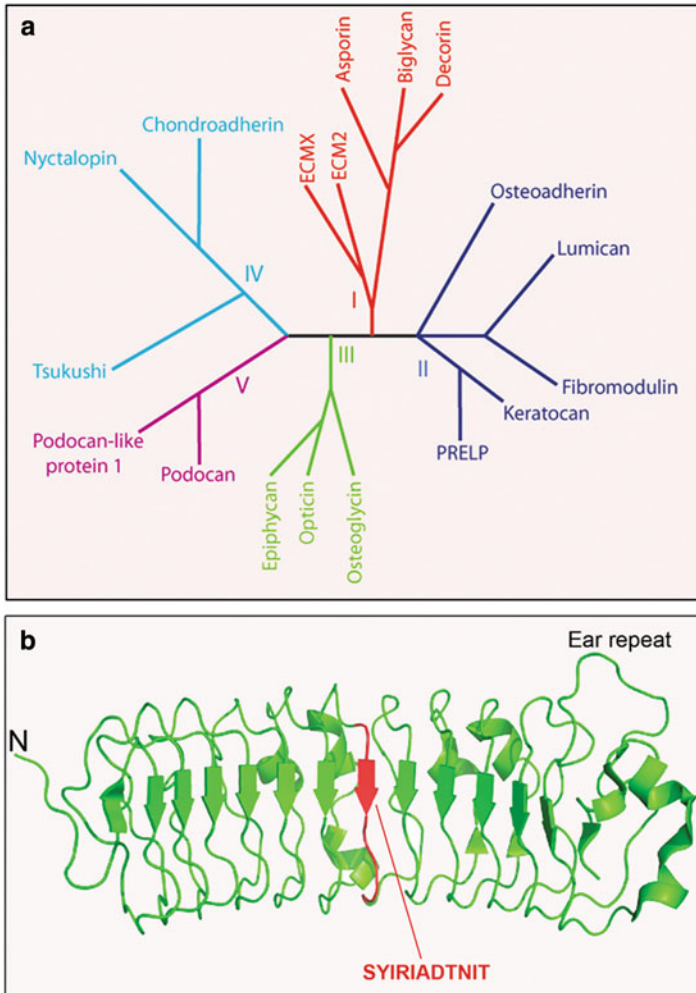
Fig. 6.1 Phylogenetic tree of small leucine-rich proteoglycans (SLRPs) and structure of the prototype decorin. (**a**) Dendogram of the five SLRP classes, *color-coded*. Various human sequences were first aligned with CLUSTALW and then an unrooted dendogram was generated using Biology Workbench. (**b**) *Ribbon diagram* of the crystal structure of monomeric bovine decorin rendered with Pymol2 (PDB accession number 1XKU). *Arrows* indicate β-strands. The area highlighted in *red* corresponds to the sequence (SYIRIADTNIT) involved in the binding to collagen type I. The ear repeat in the terminal leucine-rich repeat cysteine capping motif is also indicated (Park et al. 2008)

capping motif encompasses two terminal LRRs and includes the so-called ear repeat (Fig. 6.1b), which is present in the canonical SLRPs (classes I–III) but absent in the other two noncanonical classes (Park et al. 2008).

Decorin protein core, a Zn^{2+} metalloprotein (Yang et al. 1999; Dugan et al. 2003), is biologically active as a monomer in solution (Goldoni et al. 2004) and

binds noncovalently to an intraperiod site on the surface of collagen fibrils every D period, approximately every 67 nm (Scott 1988). Specifically, decorin protein core binds near the C terminus of collagen α1(I) in isolated procollagen molecules close to an intermolecular cross-linking site (Keene et al. 2000). The glycosaminoglycan chains can also be involved in collagen interaction (Rühland et al. 2007; Henninger et al. 2006; Raspanti et al. 2008). Indeed, an interesting feature of decorin (and biglycan) is that the glycosaminoglycan-binding region is located near the N terminus. This feature provides a degree of mobility for the dermatan sulfate chain, which can align orthogonally or parallel to the major axis of the collagen fibril. This leads to two major properties: (1) it maintains interfibrillar space in the corneal collagen, thereby providing transparency (Scott 1988), and (2) in tendon and skin, and perhaps in other connective tissues, it guarantees the mechanical coupling of fibrils and could distribute the mechanical stress throughout the whole tissue (Vesentini et al. 2005; Reed and Iozzo 2002). The estimated binding force of ~12 $\times$ 10^3 nN of decorin core to collagen fibrils is greater than that exerted by the binding of the dermatan sulfate chain for the collagen fibrils. This suggests that overloads or other forms of mechanical stress are likely to damage the collagen mechanical integrity by disrupting the glycosaminoglycan/collagen interaction rather than decorin/collagen interaction (Vesentini et al. 2005). This is interesting insofar as genetic disruption of dermatan sulfate epimerase 1, the enzyme required for the modification of glucuronic acid to iduronic acid (responsible for the genera- tion of dermatan from chondroitin sulfate chains), causes a mild skin fragility phenotype reminiscent of the decorin-null mice (Maccarana et al. 2009). Thus, the absence of dermatan sulfate could disrupt the proper interaction of decorin with collagen fibrils during development as also suggested by studies involving 3D collagenous matrices, which support a negative role for the glycosaminoglycan chain of decorin on collagen fibril diameter at early stages of fibril assembly (Rühland et al. 2007).

Asporin binds to collagen with an affinity in the low nanomolar range as decorin does (Kalamajski et al. 2009). Notably, asporin and decorin bind on the same region in fibrillar collagen insofar as they can effectively compete with each other at equi- molar concentrations, whereas biglycan does not. However, the collagen-binding domains in these two class I SLRPs differ, being LRR$_7$ and LRR$_{10-12}$ in decorin and asporin, respectively (Kalamajski et al. 2009). Another example of diversified functional activity is provided by two members of class II SLRPs, fibromodulin and lumican, both of which utilize LRR$_{5-7}$ to bind collagen (Kalamajski and Oldberg 2009, 2010). However, during development of tendons, both lumican and fibromo- dulin regulate the initial assembly of collagen protomers, but only fibromodulin facilitates growth steps leading to mature fibrils (Ezura et al. 2000). Similarly, there is strong genetic evidence for the coordinated control of collagen fibrillogenesis by decorin and biglycan during development (Zhang et al. 2009), and for regulating acquisition of biomechanical properties during tendon development (Robinson et al. 2005; Zhang et al. 2006).

Another level of intricacy is provided by the potential SLRP substitution with glycosaminoglycan side chains of various types. For example, canonical class I

members contain chondroitin or dermatan sulfate side chains with the exception of asporin, ECM2, and ECMX. All class II members contain polylactosamine or keratan sulfate chains in their LRRs and sulfated tyrosine residues in the N-terminal ends. Class III members contain chondroitin/dermatan sulfate (epiphycan), keratan sulfate (osteoglycin), or no glycosaminoglycan (opticin) chain. Noncanonical class IV and V members do not contain any glycosaminoglycan chain with the exception of chondroadherin, which is substituted with keratan sulfate. The attachment of the chondroitin and dermatan sulfate chains is tissue specific. For instance, in bone, the chains on both decorin and biglycan are primarily chondroitin sulfate, while in skin they are primarily dermatan sulfate. Thus, the presence or absence of specific glycosaminoglycans, together with changes in degree of sulfation or epimerization (chondroitin versus dermatan sulfate, for example), endows this class of proteoglycans with an additional layer of structural complexity.

Overall, there is specificity of binding that presumably dictates specificity of "function" among various SLRPs, in spite of their highly conserved structure. The differential binding of various combinations of SLRPs, together with differential temporal expression of SLRPs binding to collagen via the same LRRs, may indeed shape collagenous matrices into "stromal compartments" (Kalamajski and Oldberd 2010) that characterize specialization of tissues and organs.

6.3   Lessons from Gene Targeting Studies

Key information has been gathered regarding the function and tissue expression pattern of SLRPs from the available knockout mice and it has become clear that these mice can represent valuable in vivo models for various diseases such as skin fragility, osteoporosis, and muscular dystrophy (Ameye and Young 2002). The first SLRP-encoding gene to be targeted was decorin (Danielson et al. 1997), which shows a complex genomic organization and transcriptional control (Santra et al. 1994; Iozzo and Danielson 1999) as well as a widespread tissue distribution (Danielson et al. 1993; Scholzen et al. 1994). The phenotype of the decorin-deficient mice provides strong genetic evidence, in a defined animal model, for the essential role of SLRPs in regulating collagen fibrillogenesis, which was until then mostly based on cell-free experimental systems (Vogel et al. 1984). These mice present with abnormal collagen fibril morphology in the skin and tail tendon (Table 6.1). Presumably, collagen fibrils lacking decorin might be less stable due to abnormal posttranslational modifications such as cross-linking (Keene et al. 2000) or enhanced susceptibility to collagenases (Geng et al. 2006). The most obvious phenotype of the decorin-null mice, explainable with the high decorin expression in the dermis, is skin fragility resulting from thinner dermis and reduced tensile strength, a mechanical impairment directly linked to the abnormal collagen network. This phenotype mimics some of the cutaneous defects observed in the human Ehlers–Danlos syndrome, also known as *Cutis hyperelastica*, characterized by skin hyperextensibility and tissue fragility. Ultrastructural analysis of dermal collagen

Table 6.1 Pathological consequences of targeted ablation of various SLRP genes in mice

Targeted gene	Molecular pathology	Phenotype	References
Decorin	Abnormal collagen fibril structure in dermis and tendon	Skin fragility	Danielson et al. (1997)
	Disruption of enteric cell maturation	Intestinal tumor formation	Bi et al. (2008)
Biglycan	Reduced growth rate and decreased bone mass	Progressive osteoporosis	Xu et al. (1998)
	Structural abnormalities of collagen fibrils in aortic media	Spontaneous aortic dissection and rupture	Heegaard et al. (2007)
Lumican	Abnormal collagen fibril architecture in cornea and dermis	Skin fragility and corneal opacity	Chakravarti et al. (1998)
Fibromodulin	Abnormal collagen fibril structure in tendon	No overt phenotype	Svensson et al. (1999)
Keratocan	Abnormal collagen fibril structure in the corneal stroma	Altered cornea shape and reduced visual acuity	Liu et al. (2003)
Biglycan/Decorin	Abnormal collagen fibril formation in bone, tendon and dermis	Mimics the progeroid variant of human Ehlers–Danlos syndrome	Corsi et al. (2002)
	Hypomineralization of frontal and parietal craniofacial bones	Impaired posterior frontal sutural fusion	Wadhwa et al. (2007)
	Impaired amelogenesis: massive deposition of enamel and hypomineralization of dentin	Abnormal tooth development	Goldberg et al. (2005)
Biglycan/fibromodulin	Structural and mechanical abnormalities in collagen fibrils in tendon affected by exercise	Progressive gait impairment, ectopic tendon ossification and premature osteoarthritis; Impaired tendon function	Ameye et al. (2002); Kilts et al. (2009)
Decorin/p53	Cooperative action of germ-line mutation permissive for tumorigenesis	Rapid development of T cell lymphomas	Iozzo et al. (1999a)
Decorin/dentin sialophosphoprotein	Lack of decorin rescues the abnormal dentin mineralization caused by deficiency of dentin sialophosphoprotein	Restoration of predentin structure	Haruyama et al. (2009)
Lumican/fibromodulin	Abnormal collagen maturation and architecture in tendons	Joint laxity and impaired tendon function	Jepsen et al. (2002)
Epiphycan/biglycan	Abnormal collagen fibrils in sclera, increased ocular axial length, thin sclera and retinal detachment	Mimics high myopia	Chakravarti et al. (2003); Nuka et al. (2010)
	Damage and erosion of articular cartilage; Increased osteophyte formation within joint and ossification of tendons	Premature onset of osteoarthritis	

fibrils in decorin-null mice displays irregular outlines and size variability with uncontrolled lateral fusion (Fig. 6.2a, b). The periodicity of collagen fibers is maintained in the decorin-null mice, likely because of compensatory occupation of the *d* band by other SLRPs (Fig. 6.2c, d), suggesting that the concerted action of multiple SLRPs might determine the final structure and function of collagenous matrices. Accordingly, the decorin-null mouse has a mild phenotype and has become one of the most utilized animal models to investigate the role of this SLRP under various experimental challenges (Brown et al. 2001; Schaefer et al. 2002;
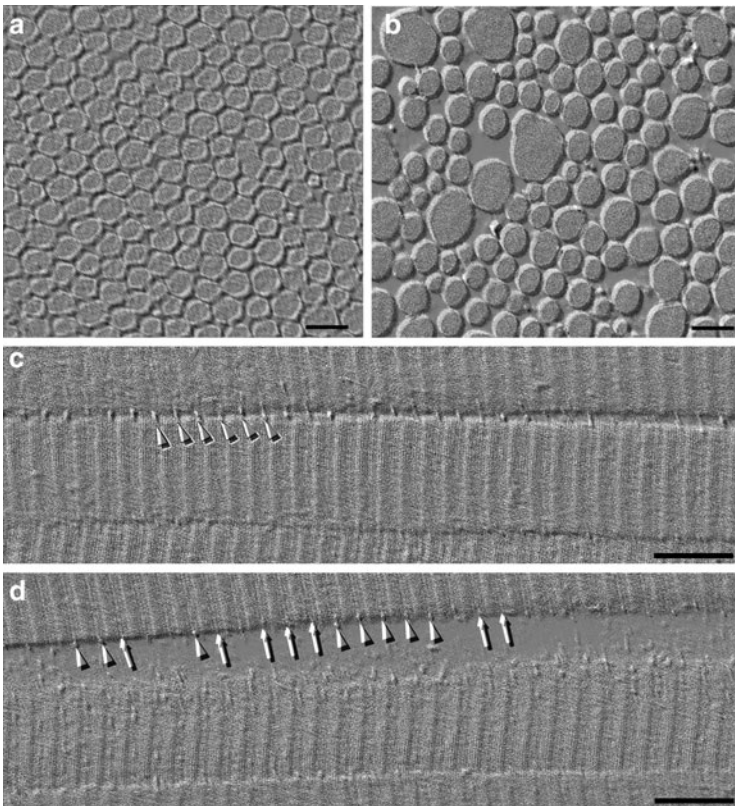

Fig. 6.2 Abnormal collagen fibrillogenesis in the absence of decorin. (**a**, **b**) Cross sections of wild-type (**a**) and decorin-deficient (**b**) dermal collagen fibers. Note the presence of larger and irregular fibrils in the decorin-deficient animals. *Scale bars* ~120 nm. (**c**, **d**) Longitudinal sections of tendon collagens from wild-type (**c**) and decorin-deficient animals (**d**) following staining with *cuprolinic blue*. Notice the presence of proteoglycan granules (*arrowheads*) in nearly all the *d* bands of banded collagen in wild-type tendons (**d**). In contrast, decorin-deficient animals show areas lacking proteoglycan granules (*arrows*), albeit the cross-banding of collagen is relatively well maintained (**d**). All the electron micrographs were modified (*embossed*) using Adobe Photoshop CS2 to enhance visualization of fibril architecture. *Scale bars* ~200 nm

Häkkinen et al. 2000; Weis et al. 2005; Elliott et al. 2003; Liang et al. 2004; Fust et al. 2005; Williams et al. 2007; Merline et al. 2009) (Table 6.1).

Notably, knockdown of zebrafish decorin causes a severe phenotype, presumably because of lack of compensation by other SLRPs, which is characterized by abnormal convergent extension, craniofacial abnormalities, and cyclopia (Zoeller et al. 2009). These features are similar to several zebrafish mutants affecting the noncanonical Wnt signaling pathway, suggesting that decorin might play a role in this pathway.

The targeted deletion of the biglycan gene, which codes for a proteoglycan with a widespread tissue distribution and a pronounced expression in bone (Bianco et al. 1990; Wegrowski et al. 1995), reveals a central role for this SLRP in regulating postnatal skeletal growth (Xu et al. 1998). Bones grow more slowly and are ultimately shorter and the bone mass is reduced compared with wild-type mice due to a significant decline in osteoblast number and progressive depletion of the bone marrow stromal cells (Xu et al. 1998). For this reason biglycan-null mice represent a good model to study osteoporosis (Table 6.1). These mutant mice also display broader metadentin and altered dentin mineralization leading to enamel structural defects. Although decorin deficiency results in changes in collagen fibril size and organization in the bone, it does not affect bone mass and growth like in the case of biglycan deficiency, pointing at non-overlapping and specific functions that have evolved for these two highly homologous class I SLRPs in vivo.

The complete lack of decorin in mouse cornea can be compensated by biglycan and the lack of both decorin and biglycan results in a more severe phenotype, supporting the idea that these two SLRPs have some overlapping functions (Zhang et al. 2009). Indeed, decorin, biglycan, and lumican play an interactive role in regulating collagen fibrillogenesis in the mouse endometrium, a biological process linked to the stage of pregnancy (Sanches et al. 2010).

Fibromodulin-deficient mice develop structural and mechanical collagen alterations in their tendons (Svensson et al. 1999), which could explain the observed joint laxity (Jepsen et al. 2002) and increased incidence of osteoarthritis (Gill et al. 2002) in these mutant animals. Fibromodulin-deficient mice also have impaired collagen fibrillogenesis in predentin, which could be the basis for altered dentin mineralization directly and indirectly for defects in enamel formation arising from abnormal epithelial (enamel)–mesenchymal (dentin) interaction (Goldberg et al. 2006).

Lumican is highly expressed in the cornea and lumican-null mice develop progressive corneal opacification, indicating that lumican is not essential for a correct embryonic corneal development but plays an important role during postnatal life (Chakravarti 2003). Collagen fibrils in the posterior area of the cornea are thicker and loosely packed and consequently the light is poorly reflected. In addition to the corneal phenotype, these mice display a skin phenotype similar to the decorin-null mice. Notably, transplant of human stem cells isolated from corneal stroma into the corneas of lumican-null mice is capable of restoring corneal transparency (Du et al. 2009). This is an exciting translational study and supports

the idea of the "immune privilege" of adult stem cells and the ability to regenerate tissue in a fashion analogous to organogenesis and noticeably different from normal wound healing. Stem cell-based therapy might become an effective treatment of human corneal diseases in the near future.

Keratocan is another major component of the cornea and the keratocan-deficient mouse displays a cornea-specific phenotype where the corneal stroma is thinner, due to minor collagen fibrillogenesis alterations, and the cornea–iris angle is narrower (Liu et al. 2003). Interestingly, in the keratocan-null cornea, the expression of the other SLRPs, decorin, lumican, and fibromodulin is not affected. Due to the altered corneal shape, vision acuity is reduced, a feature evident in the human mutation of the keratocan gene (see below).

6.4   Human Genetic Diseases Caused by Mutations in SLRP Genes

There are very few human genetic diseases linked to specific mutations of SLRP genes. With the exception of asporin, in which a D14 allele (14 aspartate residues) has been linked to an increased susceptibility to osteoarthritis predominantly in Asian patients (Kizawa et al. 2005), all the SLRP-linked genetic defects cause ocular defects (Table 6.2). Mutations in the lumican and keratocan genes lead to high myopia and cornea plana, respectively (Wang et al. 2006; Majava et al. 2007). The lack of keratocan results in a flattened curvature of the cornea that leads to hypermetropia, astigmatism, and poor acuity. The case of decorin is particularly interesting because the decorin-null mice do not display any corneal abnormalities, whereas mutations in the human decorin gene cause a rare form of congenital stromal dystrophy of the cornea (Bredrup et al. 2005b; Rødahl et al. 2006). Specifically, a single base pair deletion in exon 10 leads to a loss of the terminal 33 amino acid residues, including the "ear repeat." This truncated decorin would act in a dominant-negative fashion and disrupt the collagen-regulating activity of the intact decorin (Bredrup et al. 2005a).

Mutations of human nyctalopin, a GPI-anchored SLRP expressed in the retina (O'Connor et al. 2005), cause X-linked congenital stationary blindness (Bech-Hansen et al. 2000; Pusch et al. 2000). This suggests that nyctalopin might be directly involved in establishing or maintaining functional contacts between rod photoreceptor cells and postsynaptic neurons, involved in the transmission of visual information.

It has been suggested that the expression of decorin and biglycan is altered in various forms of muscular dystrophies (Brandan et al. 2008). Specifically, both SLRPs are upregulated in skeletal muscle biopsies of Duchenne muscular dystrophy patients and the source was identified in the muscle fibroblasts (Fadic et al. 2006). The increased synthesis suggests a response of the muscle to the dystrophic damage and the fibrotic process. Interestingly, the biglycan-null mice develop a

Table 6.2 Human diseases linked to mutations in SLRP-encoding genes

Gene	Type of mutation	Type of inheritance and affected chromosome (s)	Molecular pathology	Clinical phenotype and references
Decorin	Frameshift mutation generating a C-terminal truncated decorin protein core	Autosomal dominant chromosome 12	Corneal opacities caused by deposition of white fluffy material in the corneal stroma	Congenital stromal dystrophy of the cornea (Bredrup et al. 2005a)
Lumican, fibromodulin, PRELP, and opticin	Intronic variations, nonsynonymous and synonymous changes, SNPs in promoter	Autosomal dominant chromosomes 1 and 2	Corneal detachment and choroidal neovascularization	High myopia (Majava et al. 2007; Wang et al. 2006; Chen et al. 2009).
Keratocan	Missense and frameshift mutations generating a single amino acid substitution or a C-terminal truncated keratocan	Autosomal recessive chromosome 12	Corneal radius of curvature larger than normal producing high hypermetropia with astigmatism and poor acuity	Cornea plana (CNA2) (Pellegata et al. 2000)
Nyctalopin	Intragenic deletions, missense mutations, nonsense mutations, and in-frame insertions	X-linked X chromosome	Disruption of developing retinal interconnections between rod photoreceptors and postsynaptic neurons	Congenital stationary night blindness, with associated myopia, hyperopia, nystagmus, and reduced visual acuity (Bech-Hansen et al. 2000; Pusch et al. 2000)

mild muscular dystrophy explained by lack of the complex between biglycan and α-dystroglycan, suggesting a key role for biglycan in maintaining the structure of the muscle extracellular matrix (Rafii et al. 2006). The regulation and sarcolemmal localization of other critical muscle components including dystrobrevin, syntrophin, and nNOS are also altered in biglycan-null mice and the mild dystrophic phenotype could be "rescued" by injecting biglycan into skeletal muscles (Mercado et al. 2006).

The involvement of SLRPs in muscular dystrophy certainly needs more investigation also considering the small number of patients tested in the available studies. This field of research, once expanded, certainly deepens our knowledge regarding the role of decorin and other SLRPs in inflammation, diabetes, fibrosis, and metabolic pathways.

6.5 Interaction with Growth Factors

It has become clear that SLRPs are signaling molecules in addition to playing structural functions in the extracellular matrix. Through binding to growth factors and receptors on the cell surface, they can regulate the complex intracellular signaling cascade and determine cell fate (Iozzo and Schaefer 2010). The protein core and specifically the LRR motifs have been demonstrated to retain the biological function, but certainly more needs to be investigated regarding the possible role of the glycosaminoglycan side chains during signaling. The high-affinity interaction between decorin and various TGF-β isoforms was discovered two decades ago and explains the antifibrotic effects of decorin in damaged tissues (Yamaguchi et al. 1990). The association is disrupted by matrix metalloproteinases that cleave decorin and cause the release of TGF-β. Decorin/TGF-β interactions are quite complex and lead to a variety of outcomes such as controlling growth and survival of normal and neoplastic cells (Ständer et al. 1998, 1999), regulating matrix organization and mechanical characteristics of 3D matrices (Ferdous et al. 2007, 2008, 2010; Seidler et al. 2005), blocking fibrosis in various animal models (Iozzo 1999), and preventing intimal thickening (Fischer et al. 2001). In addition, decorin and biglycan interact with tumor necrosis factor-α (TNF-α) (Tufvesson and Westergren-Thorsson 2002).

In the muscle, decorin and the TGF-β signaling pathways cooperate in regulating myoblast proliferation and differentiation. Specifically, decorin inhibits the expression of myogenin, a muscle-specific transcription factor that promotes myoblasts differentiation. Decorin has also been reported to modulate myoblasts proliferation in vitro through binding of myostatin (Miura et al. 2006), a member of the TGF-β family of growth factors (Kishioka et al. 2008). Decorin sequesters myostatin in the extracellular matrix and, as a consequence, favors myogenic cell proliferation and differentiation, as proved by increased expression of p21[WAF1], a cyclin-dependent kinase inhibitor, negative regulator of cell cycle progression, MyoD, and myogenin. Notably, decorin interacts with the insulin-like growth factor I (IGFI) as well as its

receptor (Schönherr et al. 2005; Schaefer et al. 2007; Merline et al. 2009) and can bind to platelet-derived growth factor (PDGF) BB via its protein core (Nili et al. 2003) or its dermatan sulfate side chain (Kozma et al. 2009). Decorin overexpression can indeed block PDGF-evoked activation of PDGF receptor and smooth muscle cell growth, thus providing a potential mechanism for the decorin-mediated inhibition of intimal hyperplasia following balloon angioplasty (Nili et al. 2003).

Decorin and biglycan might have different roles during skeletal muscle formation and repair (Brandan et al. 2008). Biglycan expression levels decrease during development and are normally very low in the adult muscle unless muscle damage has occurred. They both sequester TGF-β but, in addition to this, decorin binds to the cell surface receptor low-density lipoprotein receptor-related protein 1 affecting muscle signaling through activation of phosphoinositide 3-kinase and indirect enhancement of the Smad pathway downstream of the TGF-β receptor (Brandan et al. 2006). Overall, decorin and biglycan favor bone formation by sequestering TGF-β.

BMPs are growth factors involved in bone and cartilage formation, also part of the TGF-β superfamily. Both decorin and biglycan have been shown to interact with some members of this family. Specifically, decorin regulates BMP2 signaling during the conversion of myoblasts to osteoblasts (Gutierrez et al. 2006). In *Xenopus,* biglycan binds BMP4 and regulates BMP4 signaling through modulation of the antagonist Chordin (Moreno et al. 2005). Another SLRP, tsukushi, inhibits BMP activity (Ohta et al. 2004, 2006; Kuriyama et al. 2005). In the mouse, lack of biglycan leads to reduced BMP4 binding to osteoblasts, indicating that this SLRP modulates BMP4-evoked signaling to control osteoblast differentiation (Chen et al. 2004). Asporin binds BMP2 and negatively regulates BMP2-induced cytodifferentiation of periodontal ligament cells by preventing binding of BMP2 to its receptor (Yamada et al. 2007). Notably, the binding for BMP2 was mapped to asporin LRR_5, the LRR that binds collagen in the homologous decorin, and some of the BMP2 regulatory activity of asporin could be blocked by a peptide encompassing asporin LRR_5 (Tomoeda et al. 2008).

Additional studies need to be performed to resolve the multiplicity of activities, some specific and some overlapping, of SLRP/growth factor interactions and to help rationalize SLRP complexity.

6.6 Signaling Through Multiple Receptors

6.6.1 EGFR and Met

An emerging body of data indicates that decorin and perhaps other SLRPs play a physiological role in negatively regulating cell proliferation primarily by attenuating receptor tyrosine kinase (RTK) such as members of the ErbB family of RTKs. Decorin binds to a region on the EGFR extracellular domain overlapping with the EGF-binding domain (Santra et al. 2002). Upon binding, the receptor dimerizes and
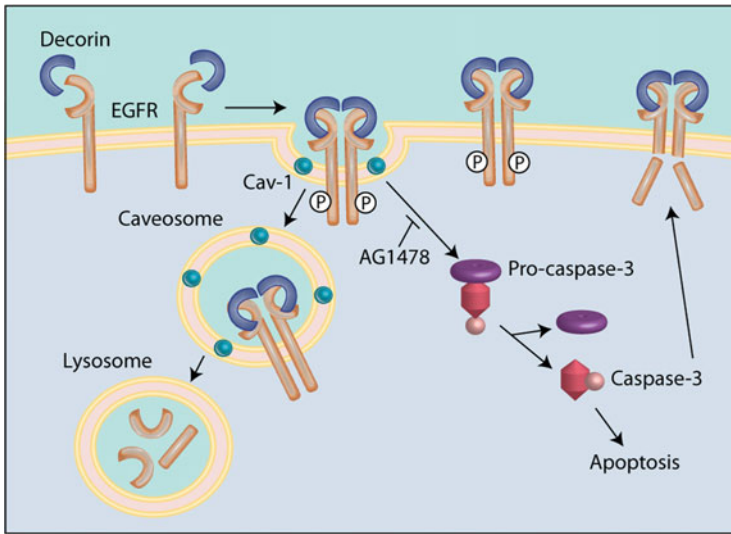
Fig. 6.3 Diagram depicting the mechanism of action of decorin in inhibiting the EGFR signaling pathway. Decorin binds to the receptor and induces caveolin-mediated (Cav-1) internalization and degradation in the lysosomes. An additional mechanism to shut down this pathway is activation of the caspase-3 cascade following transient EGFR phosphorylation, and consequent cleavage of the EGFR kinase domain. AG1478 is a tyrphostin that specifically blocks the activity of EGFR tyrosine kinase and its downstream signaling

is removed from the cell surface through caveolin-mediated endocytosis (Zhu et al. 2005) (Fig. 6.3). Following internalization, EGFR is downregulated by degradation in the lysosome. In contrast, EGF triggers EGFR internalization via clathrin-coated pits, an event that has been associated with signaling and recycling of the receptor to the cell surface. It is very intriguing that EGF, the natural ligand of EGFR, induces the same changes, specifically, dimerization and degradation, but leads to the opposite outcome in terms of signaling and biological effects. Decorin also transiently activates the EGFR and mobilizes intracellular Ca^{2+} stores (Patel et al. 1998), but induces cell growth suppression by evoking the expression of $p21^{WAF1}$ (De Luca et al. 1996). By affecting the EGFR, decorin can inhibit other members of the ErbB family of receptor tyrosine kinases, such as ErbB2 (Santra et al. 2000), which heterodimerizes with EGFR. The consequence of decorin interaction is a prolonged suppression of cellular signaling required for cell survival and proliferation, making decorin a natural "pan-RTK" inhibitor. This is in agreement with the fact that decorin inhibits the proliferation and migration of human trophoblasts via different RTKs (Iacob et al. 2008). In addition, decorin induces apoptosis in carcinoma cells via activation of caspase-3, an event downstream of EGFR signaling (Seidler et al. 2006; Goldoni and Iozzo 2008).

The Met receptor has been found to be directly affected by decorin (Goldoni et al. 2009). Decorin binds to the Met receptor and triggers specific signaling that
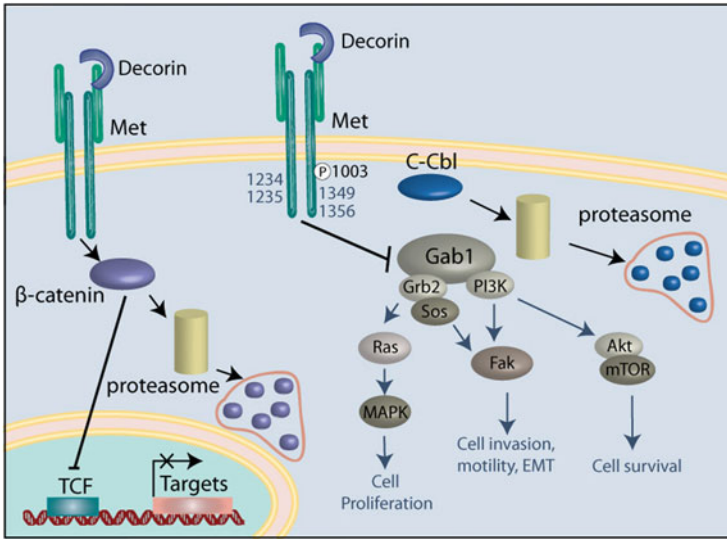
Fig. 6.4 Diagram depicting the mechanism of action of decorin in inhibiting the Met receptor signaling pathway. Following decorin binding, Met-Tyr^{1003} is activated, the ubiquitin ligase c-Cbl is recruited and the receptor is sent for degradation into the proteasome. Note that, at the same time, decorin inhibits Met-Tyr^{1349} impeding the major signaling events downstream of Met that would lead to cell survival, proliferation, and invasion. β-Catenin is also degraded along with the Met receptor, depriving cells of a transcription factor essential for cell cycle progression

leads to its downregulation by both shedding of the extracellular domain and internalization/degradation (Fig. 6.4). In the same study, downregulation of Met following decorin treatment has been linked to degradation of β-catenin, a transcription factor essential for cell cycle progression. Considering that both EGFR and Met are often deregulated in various forms of cancer and that coexpression (and/or co-amplification) of these two receptors drives the tumorigenesis process, a deep understanding of decorin's mechanism of action could lead to novel therapeutic approaches against malignancies.

Considering the opposite biological effects achieved by decorin vis-à-vis EGF/TGF-α and HGF following binding to the same receptors, we envision a scenario where stromal cells, the producers of decorin, a natural EGFR and Met antagonist, could potentially counteract the growth-promoting and prosurvival activities of RTKs within a growing neoplasm. The importance of this biological interplay in a pathological condition such as cancer has been reasonably explored, whereas the role of decorin in physiological tissue homeostasis has not. The most common route by which cell fate is regulated is by signaling through different receptors and ligands and by positive or negative feedbacks originating from inside the cell following the extracellular stimuli. Decorin represents a novel example of cell cycle regulator which, by a unique mode of binding to the EGF and Met receptors, triggers specific downstream signaling that differs from the one evoked by EGF

and HGF. This type of regulation adds an additional layer of complexity to the known canonical pathways by which cells respond to extracellular cues.

6.6.2 Type 1 Insulin-Like Growth Factor Receptor and Toll-Like Receptors

Decorin is also involved in the insulin-like growth factor receptor (IGF-IR) pathway. This interaction has been studied in endothelial cells (Schönherr et al. 2005) where it could represent a major player in the regulation of physiological and pathological angiogenesis (Schönherr et al. 2004, 2005). In the kidney, decorin regulates the deposition of fibrillin-1 by triggering specific signaling in renal fibroblasts through the IGF-IR (Schaefer et al. 2007).

Biglycan is an endogenous ligand of Toll-like receptor 2 (TLR2) and TLR4 in macrophages and stimulates the expression of TNF-α and macrophage inflammatory protein-2 via activation of p38 and NF-κB (Schaefer et al. 2005). This activity is highly proinflammatory (Schaefer 2010) explaining why biglycan-null mice present an advantage in LPS-induced sepsis. The molecular mechanisms linking biglycan action to TLRs require the formation of a receptor cluster with P2x (Babelova et al. 2009). This cooperative receptor clustering triggers the NALP3 inflammasome expression which, in turn, activates caspase-1 and IL-1β release. Biglycan, along with decorin, can also function as an anti-inflammatory protein by binding to and blocking the complement protein C1q, thereby inhibiting activation of the complement cascade and proinflammatory cytokine production at the tissue level (Groeneveld et al. 2005).

Lumican is also involved in innate immune response by affecting TLR4 signaling pathway. Lumican-deficient macrophages show impaired response to LPS resulting in lower production of TNF-α and IL-6 (Wu et al. 2007). A possible mechanism of action involves CD14 and the presentation of LPS to TLR4 through this cell surface molecule. Lumican produced by vascular endothelial cells binds to the surface of extravasating leukocytes via β2-containing integrins and promotes leukocyte migration during inflammation (Lee et al. 2009). In addition to its role in inflammation, lumican has been shown to inhibit proliferation of stromal keratocytes in the cornea through activation of p21^{WAF1} and p53 and to induce apoptosis through enhancing Fas–Fas ligand signaling (Vij et al. 2004).

Biglycan may work through other receptors for immune responses including selectin/CD44 where it can selectively recruit peripheral blood CD16(−) natural killer cells into human endometrium (Kitaya and Yasuo 2009). Other factors may also modulate the LPS-induced inflammation. Both keratocan and lumican regulate neutrophil infiltration and corneal clarity in LPS-induced keratitis by direct interaction with CXCL1 (Carlson et al. 2007). Biglycan, decorin, fibromodulin, and lumican can all bind to C1q and differentially activate the classical complement pathway, thereby having implications in chronic inflammatory processes.

6.7 Skeletal Connective Tissues

6.7.1 Bone Remodeling and Osteoporosis

Biglycan is predominantly expressed in bone and its genetic ablation results in reduced skeletal growth and bone mass leading to generalized osteopenia (Xu et al. 1998). The mice have less trabecular bone volume and reduced cortical thickness, both important for bone strength and integrity. The important role for biglycan in osteogenesis was confirmed by the fact that its absence following marrow ablation directly impedes bone formation (Chen et al. 2003). Notably, the biglycan gene resides on the X chromosome and patients with Turner syndrome (45,X), a disease characterized by short stature and early-onset osteoporosis, display low levels of biglycan expression. This raises the possibility that bone metabolism in biglycan-deficient mice might be gender dependent. In contrast to male mice, the bone tissue of female mice is less affected, suggesting a gender difference in biglycan skeletal function.

The effects of biglycan deficiency on bone can be linked to collagen fibril abnormalities. These mutant fibrils display an irregular profile, a broader-size range, and reduced packing (Corsi et al. 2002). Interestingly, decorin deficiency also affects the collagen fibril size and shape in bone but in an opposite way: decorin-null mice have smaller average fibril diameter and size range in bone compared to wild-type animals, whereas in the dermis and tendon the fibrils are larger. Thus, it is not surprising that the skeletal phenotypes of the biglycan- and decorin-deficient mice differ from one another and that mice lacking decorin do not feature the marked osteopenia of the biglycan-deficient mice. Double-deficient mice display an almost complete loss of fibril basic geometry, with very few fibrils possessing a predominantly circular cross-sectional profile. The vast majority of the fibrils have a "serrated" fibril morphology observed in many human disorders, including Ehlers–Danlos syndrome. Mice lacking both biglycan and decorin are grossly osteopenic and this abnormality is much more severe (Fig. 6.5) and appears at an earlier age as compared to biglycan-null mice (Bi et al. 2005). Thus, decorin deficiency synergizes with biglycan deficiency in controlling bone mass, although the effects of the individual SLRP deficiencies in bone are quite distinct.

In addition to biglycan and decorin, the SLRP asporin may also play a role in regulating collagen fibril structure in bone (Kalamajski et al. 2009). Asporin competes with decorin, but not biglycan, for binding to collagen where the poly-aspartate in asporin directly regulates collagen mineralization by its collagen- and calcium-binding properties (Heinegård 2009).

While biglycan appears to regulate collagen fibril formation, it is unclear how this could impact the geometric and mechanical properties of mature bone. When bones from biglycan-deficient mice were tested for mechanical strength they had decreased failure load (to bend) and yield energy (to break) at 6 months (Corsi et al. 2002), with biglycan-deficient tibia being the most affected (Wallace et al. 2006).

Fig. 6.5 Quantitative backscattered electron imaging of the distal femur from 2-month mice of various genotypes as indicated at the *bottom*. Trabecular bone mass is markedly reduced in double-null animals. A reduced mineral content (as indicated by the *pseudocolor scale*, in which higher values are at the *top*) is observed both in the biglycan-null and in the biglycan/decorin double-deficient animals as compared to the wild-type mice. Reproduced and slightly modified from Corsi et al (2002) with permission of the American Society for Bone and Mineral Research

Biglycan and decorin are highly expressed in the craniofacial bones and cranial sutures of mice and have overlapping yet distinct patterns of expression in the fusing suture and the dura mater (Wadhwa et al. 2007). Mice singly deficient in either biglycan or decorin have no suture formation defects, whereas double-deficient mice have open sutures and severe hypomineralization of both frontal and parietal bones, thus confirming that these two SLRPs have some synergistic effects in regulating craniofacial morphology.

Biglycan-deficient bones exhibit reduced osteoblasts and lower bone formation rates, suggesting that the osteogenic progenitor cells might be affected. Indeed, both the quality and normal activity of bone marrow stromal cells from biglycan-deficient mice are reduced. Specifically, these cells have reduced clonogenic response to TGF-β, produce less type I collagen mRNA and collagen protein, and have enhanced apoptotic rate. Interestingly, when both biglycan and decorin are depleted, the growth factor interplay is also affected but in the opposite way. Specifically, the matrix made by the double-null mice is unable to retain TGF-β and subsequently to maintain proper sequestration (Bi et al. 2005). The excess TGF-β then directly binds to its receptors and over-activates its signaling transduction pathway leading to apoptosis. This results in decreased numbers of osteoprogenitor cells and subsequently reduced bone formation. The class II SLRP PRELP also impairs osteoclastogenesis, thus preventing osteopenia, via its glycosaminoglycan-binding domain which acts as a cell type-specific inhibitor of NF-κB, an established transcriptional inducer of osteoclast-specific gene expression (Rucci et al. 2009). Recently, biglycan and fibromodulin have been found to play key roles

in maintaining cartilage integrity and in regulating chondrogenesis and extracellular matrix turnover during the development of temporomandibular osteoarthritis (Embree et al. 2010).

In addition to TGF-β, BMPs also play important roles in regulating osteoblast differentiation and bone formation. The absence of biglycan causes less BMP4 binding to osteoblasts (Chen et al. 2004), resulting in a reduced BMP4-stimulated expression of the osteoblast-specific transcription factor Cbfa1 and ultimately causing a defect in the differentiation process. Interestingly, decorin accumulates to higher levels in biglycan-deficient cell cultures, and thus the distribution ratio of other matrix proteins is changed as a consequence of missing a single protein. This compensation causes additional changes in the extracellular matrix further influencing growth factor activity.

6.7.2 Nonmineralized Musculoskeletal Tissues

Besides controlling bone growth during aging, SLRPs also play important roles in the assembly of normal tendons as well as in the maintenance of articular cartilage. Collagen fibrils in tendons from mice deficient in biglycan and/or fibromodulin are structurally and mechanically altered resulting in unstable joints (Ameye et al. 2002). As a result, these mice develop gait impairment, ectopic ossification in tendon, and severe premature osteoarthritis. At 3 months, both single- and double-deficient mice display torn cruciate ligaments and ectopic ossification in their quadriceps tendon, menisci, and cruciate and patellar ligaments (Kilts et al. 2009). The phenotype is least severe in fibromodulin-null, intermediate in biglycan-null, and the most severe in the double-deficient mice. These problems worsen with age in all three mouse strains and result in the development of large supernumerary sesmoid bones (i.e., bones formed within tendons in regions that wrap around bony prominences). Moderate exercise decreases ectopic ossification in double-deficient mice compared with unchallenged mice, whereas rigorous forced use of the joints further increases ectopic ossification and osteoarthritis. Loss of decorin affects the patellar tendon causing an increase in modulus and stress relaxation, but with little effect on the flexor digitorum longus tendons (Robinson et al. 2005). Conversely, biglycan loss does not significantly affect the patellar tendons, but causes a reduction in both the maximum stress and modulus of the flexor digitorum longus tendon. Thus, biglycan, decorin, and fibromodulin all play critical roles in regulating the structure and function of tendons.

The presence of ectopic ossification in tendons of mice deficient in both biglycan and fibromodulin suggests that tendons could have stem cells that form bone rather than tendon in pathological situations. Human and mouse tendons indeed harbor a unique cell population, termed tendon stem/progenitor cells, which has universal stem cell characteristics such as clonogenicity, multipotency, and self-renewal capacity (Bi et al. 2007). Isolated tendon stem/progenitor cells can regenerate tendon-like tissues after extended expansion in vitro and transplantation in vivo.

As these cells reside within a niche that is surrounded predominantly by extracellular matrix proteins, the latter likely plays a major role in organizing this specialized stem cell niche. Depletion of biglycan and fibromodulin affects the differentiation of tendon stem/progenitor cells by modulating BMP signaling and thereby impairs tendon formation in vivo.

Biglycan- or fibromodulin-null mice exhibit a mild form of knee osteoarthritis, whereas mice doubly deficient in these SLRPs develop severe and premature osteoarthritis in the knee (Ameye et al. 2002). Osteoarthritis appears within the first 3 months of life, and by 6 months the joint is almost completely destroyed. Specifically, the biglycan/fibromodulin-deficient knees display a progressive degeneration of the articular cartilage from early fibrillation to complete erosion, subchondral sclerosis, osteophytes, and bone cysts. Several cellular events are altered during the progression of osteoarthritis, including abnormal expression of aggrecan and type II collagen. Mice deficient in either lumican or lumican and fibromodulin also develop premature knee osteoarthritis, but this occurs more slowly than the mice doubly deficient in biglycan and fibromodulin (Jepsen et al. 2002). Collectively, these data indicate that SLRPs play critical roles in regulating the formation and function of the skeleton, but have unique roles depending on the tissue context.

6.8   Cardiovascular Homeostasis and Diseases

Biglycan is an important regulator of elastogenesis insofar as ectopic expression of a mutant form of biglycan lacking the two glycosaminoglycan-binding sites in vascular smooth muscle cells induces tropoelastin gene expression and increases deposits of cross-linked elastin in vivo (Hwang et al. 2008). The molecular mechanism of this process is not clear. However, it is known that biglycan protein core binds to tropoelastin and elastic fiber microfibrils where it forms a ternary complex with tropoelastin and microfibrillar-associated glyprotein-1 (MAGP-1) (Reinboth et al. 2002), and enhanced biglycan expression coincides with the elastogenic phase of elastic fiber formation during development of nuchal ligament (Reinboth et al. 2000). Thus, it is likely that biglycan could promote physiological interactions among the major components of the elastic fiber assembly (Hwang et al. 2008). This concept is supported by the evidence that biglycan evokes expression of fibrillin-1, a key constituent of microfibrils that form the scaffold on which tropoelastin builds up (Schaefer et al. 2004).

Other studies link biglycan to the development of atherosclerosis by acting as sink for LDL particles. Biglycan confers LDL-binding properties likely mediated by "hyperelongated" glycosaminoglycan side chains (Little et al. 2008), and its accumulation in early human atherosclerosis precedes the inflammatory response (Nakashima et al. 2007), providing strong evidence for a key role for this SLRP in atherogenesis. The inflammatory marker serum amyloid A (SAA) increases TGF-β and, subsequently, biglycan in aorta tissue (Wilson et al. 2008). SAA is elevated in

obesity and cardiovascular disease and colocalizes with biglycan and ApoB in the vascular wall (King et al. 2009). Biglycan binds to LDL, accumulates in the subendothelial matrix, and is displaced by endostatin. This proteolytic fragment of collagen XVIII is depleted in vessels with atherosclerosis and thereby unable to inhibit biglycan retention in the disease process (Zeng et al. 2005). Notably, long-term treatment of the pro-atherogenic ApoE-deficient mice with telmisartan, an antagonist of the angiotensin II type 1 receptor, reduces biglycan levels in the atherosclerotic plaques and inhibits atherosclerosis independently of its antihypertensive effects (Nagy et al. 2010). Thus, targeting biglycan could become a therapeutic modality against atherogenesis.

As a regulator of collagen fibrillogenesis, biglycan could play a structural role in maintaining vascular integrity. About half of biglycan-deficient male mice die suddenly within the first 3 months of life due to spontaneous aortic dissection and rupture (Heegaard et al. 2007). This is further supported by the association of low biglycan expression in aneurysms of human abdominal aorta (Theocharis and Karamanos 2002). Thus, biglycan is an essential structural component of the aortic wall and contributes to blood vessel homeostasis. Moreover, these animal studies suggest the possibility of a human disease linked to genetic mutations of biglycan (loss of function) that might lead to aortic dissection in humans.

Decorin has also been involved in regulating elastic fiber formation insofar as it binds to both elastic microfibrils and MAGP-1 (Reinboth et al. 2002; Trask et al. 2000). However, the role for decorin in elastogenesis is less clear. Decorin has been implicated in remodeling of experimentally induced myocardial infarction in mice (Weis et al. 2005). In the decorin-null animals, the infarcted areas were larger than controls as were the right ventricular remote hypertrophy and left ventricular dilatation. Moreover, echocardiography revealed depressed left ventricular systolic function, suggesting that decorin is required for proper fibrotic evolution of myocardial infarction (Weis et al. 2005). In agreement with these studies, adenovirus-mediated gene delivery of decorin in postinfarcted hearts mitigates cardiac remodeling and dysfunction (Li et al. 2009), whereas decorin gene delivery inhibits cardiac fibrosis in spontaneously hypertensive rats (Yan et al. 2009). In a similar vein, biglycan expression increases after myocardial infarction. Mice deficient in biglycan repair poorly after experimentally induced myocardial infarction and have reduced tensile strength, and overall impaired cardiac hemodynamic function (Westermann et al. 2008). A gain-of-function approach showed that the overexpression of biglycan in heart tissue increases numerous genes critical for cardiac remodeling including genes associated with cardiac protection and Ca^{2+} signaling, providing further evidence that it could regulate remodeling after myocardial infarction.

6.9 Cancer and Metastasis

One of the earliest indications that decorin affects cell growth emerged from studies using normal cells: decorin gene expression is greatly enhanced after normal diploid fibroblasts reach confluence and cease to proliferate (Mauviel et al. 1995).

In general, malignant cells do not express decorin and respond to recombinant decorin, with the exception of an osteosarcoma cell line which is resistant to decorin treatment (Zafiropoulos et al. 2008). The first evidence linking decorin to cancer development came from a study utilizing decorin/p53 double knockout mice (Iozzo et al. 1999a). Mutations in the tumor suppressor p53 are found in over half of all human cancers, and mice that lack p53 develop a spectrum of sarcomas, lymphomas, and, less frequently, adenocarcinomas. Remarkably, mice lacking both decorin and p53 genes show a faster rate of tumor development and succumb almost uniformly to a very aggressive form of thymic lymphomas within 6 months. These results indicate that decorin absence is permissive for lymphoma tumorigenesis and suggest that there might be a functional synergism between a secreted extracellular "tumor repressor" (decorin) and an intracellular "tumor suppressor" (p53) (Iozzo et al. 1999a). The second line of evidence arose from an extended analysis of the decorin-null mice. About 30% of these mice develop spontaneous intestinal tumors and a high-risk diet (high fat, low calcium and vitamin D) accelerates this process (Bi et al. 2008). A plausible molecular explanation includes the finding that the intestinal epithelium of the decorin-null mice shows a down-regulation of $p21^{WAF1}$ and $p27^{kip1}$, two cyclin-dependent kinase inhibitors, and a concurrent upregulation of β-catenin, a key transcription factor that promotes cell cycle progression. Earlier studies have shown that decorin gene expression is enhanced in the stroma of colon cancer via hypomethylation of its promoter regions (Adany et al. 1990; Adany and Iozzo 1990), suggesting that decorin might be a natural RTK inhibitor. Collectively, this body of literature provides strong direct evidence for a role of decorin as a tumor repressor gene further stressing the role of the tumor microenvironment in cancer progression (Iozzo and Cohen 1993; Iozzo 1995; Friedl 2010).

An anti-oncogenic role for decorin has been documented in various experimental settings including breast cancer cells (Santra et al. 2000), ovarian carcinoma cells (Nash et al. 1999), syngeneic rat gliomas (Biglari et al. 2004), and squamous and colon carcinoma xenografts (Reed et al. 2002; Tralhão et al. 2003; Seidler et al. 2006). Moreover, attenuated decorin expression is associated with poor prognosis in invasive breast cancer (Troup et al. 2003), in aggressive soft tissue tumors (Matsumine et al. 2007), and during mammary gland carcinogenesis in TA2 mice with spontaneous breast cancer (Gu et al. 2010). Collectively, these studies support earlier observations that either ectopic expression of decorin or treatment of various cancer cells of diverse histogenetic backgrounds with exogenous decorin inhibits their growth (Santra et al. 1995). A possible mechanism of action is via a transient activation of the EGFR (Moscatello et al. 1998; Iozzo et al. 1999b), followed by downregulation of the EGFR itself (Csordás et al. 2000; Zhu et al. 2005), and the concomitant induction of $p21^{WAF1}$ (De Luca et al. 1996), which causes the cells to arrest in the G1 phase of the cell cycle (Santra et al. 1997).

Adenovirus-mediated or systemic delivery of decorin prevents metastases in an orthotopic breast carcinoma xenograft model (Reed et al. 2005; Goldoni et al. 2008). Finally, systemic delivery of decorin retards the growth of prostate cancer in a mouse model of prostate carcinogenesis where the tumor suppressor PTEN

gene is conditionally deleted in the prostate (Hu et al. 2009). In this study, decorin counteracted not only EGFR but also androgen receptor activity in human prostate cancer cells. An important effect of decorin on cancer cells is the induction of apoptosis via activation of caspase-3 (Seidler et al. 2006; Goldoni et al. 2008). Overexpression of decorin in normal cells also induces apoptosis (Wu et al. 2008). Ultimately, decorin inhibits metastasis formation in breast carcinoma and osteosarcoma tumor models (Goldoni et al. 2008; Araki et al. 2009; Shintani et al. 2008).

Lumican has also been involved in growth control (Nikitovic et al. 2008a, b). It inhibits melanoma progression (Vuillermoz et al. 2004) and anchorage-independent tumor cell growth (Li et al. 2004) and a peptide derived from lumican LRR$_9$, named "lumcorin," inhibits melanoma cell migration (Zeltz et al. 2009). Low levels of decorin and lumican correlate with a worse prognosis in lymph node-negative invasive breast carcinomas (Troup et al. 2003). Moreover, lumican inhibits melanoma migration by affecting focal adhesion complexes (Brézillon et al. 2009). Thus, targeting constituents of the stable stroma rather than targeting the adaptable cancer cells could be an intelligent therapeutic modality toward solid tumors where tumor stroma is a predominant part of the malignant neoplasm.

6.10   Angiogenesis

The involvement of decorin in angiogenesis and particularly tumor angiogenesis is somewhat controversial. In some experimental settings, decorin seems to be pro-angiogenic (Järveläinen et al. 1992; Nelimarkka et al. 1997, 2001; Schönherr et al. 2001, 2004), whereas in other experimental settings, decorin is anti-angiogenic (de Lange et al. 2001; Grant et al. 2002; Kinsella et al. 2000; Järveläinen et al. 2006). The latter effect occurs via two potential mechanisms: by interfering with thrombospondin-1 (de Lange et al. 2001) and/or by suppressing the endogenous tumor cell production of VEGF (Grant et al. 2002). Another possibility is that decorin inhibition of endothelial cell migration is caused by a decorin-evoked stabilization of pericellular fibrillar matrix, suggesting that balanced decorin expression is important for fine-tuning angiogenesis (Kinsella et al. 2000). In an animal model of angiogenesis, it has been shown that genetic deficiency of decorin markedly increases fibrovascular invasion and enhances the formation of blood vessels in sponge implants (Järveläinen et al. 2006). Recently, peptides derived from the LRR$_5$ of decorin were shown to be the main mediator of decorin anti-angiogenic activity (Sulochana et al. 2005). Moreover, cleavage of decorin by MT1-MMP favors corneal angiogenesis, further indicating an angiostatic role of decorin (Mimura et al. 2008). All these reports point to a key role for decorin in vasculogenesis and angiogenesis. However, the molecular mechanism of action is unclear. As a general rule, decorin seems to be pro-angiogenic in "normal" endothelial cells (i.e., during development and wound healing), but is anti-angiogenic during pathological angiogenesis such as tumor angiogenesis. This concept has been further supported by a recent study comparing the expression of decorin in malignant and

benign vascular tumors (sarcomas versus hemangiomas). Notably, decorin expression was essentially undetectable in the sarcomas whereas there was significant decorin expression in the hemangiomas and surrounding perivascular stroma (Salomäki et al. 2008). Thus, a potential effect of decorin on the tumor microenvironment resides in its ability to modulate angiogenesis.

We hypothesize that all these effects, at time discordant, could be reconciled by a single unifying hypothesis: decorin effect on RTKs such as ErbB and Met receptors is the main mechanism of action. We base this hypothesis on several important pieces of evidence. First, both EGFR and Met are pro-angiogenic and prosurvival receptors, which are enriched in the tumor vasculature (van Cruijsen et al. 2006). Activation of the EGFR pathway increases the production of VEGF, whereas neutralizing antibodies against EGFR and ErbB2 downregulate VEGF production by tumor cells (Petit et al. 1997). Simultaneous blockade of EGFR and VEGFR pathways results in a cooperative antitumor effect (Sini et al. 2005). Activation of the HGF/Met signaling pathway is potently pro-angiogenic by stimulating endothelial cell motility and growth (Bussolino et al. 1992), promoting VEGF secretion (Grant et al. 1993; Zhang et al. 2003; Saucier et al. 2004), or inhibiting the secretion of thrombospondin (Zhang et al. 2003; Saucier et al. 2004), a powerful anti-angiogenic compound. Activation of the HGF/Met axis is also pro-lymphangiogenic (Kajiya et al. 2005; Cao et al. 2006), a quality that could contribute to the metastatic and aggressive behavior of Met-overexpressing tumor cells (Birchmeier et al. 2003). Moreover, tumor angiogenesis is induced by sustained Akt signaling (Phung et al. 2006), a downstream effector of both EGFR and Met signaling pathways.

6.11 Conclusions and Perspectives

When the first two SLRPs were cloned and sequenced nearly 25 years ago (Krusius and Ruoslahti 1986; Day et al. 1987), there was nothing to suggest that this family of proteoglycans and glycoproteins would be implicated in so many biological functions. The SLRPs form an interactive network of extracellular and cell-associated proteins that modulate the activity of key signaling pathways during development and various pathologies. In some cases, SLRPs control bone mass by regulating the number and activity of osteogenic cells and their precursors and affect their ability to utilize growth factors that are critical to skeletal function including TGF-β and BMPs. The SLRP regulation of growth factors is important in many aspects of skeletal cell behavior including proliferation, differentiation, and apoptosis. When these processes are deregulated they cause premature osteopenia leading to an osteoporosis-like phenotype. In other cases, disruption of some SLRP genes causes skin fragility, tendon abnormality, muscular dystrophy, and ocular diseases affecting corneal transparency, visual acuity, or transmission of visual information. The distribution and organization of collagen and other extracellular matrix proteins are compromised in SLRP deficiency, thereby causing abnormal

growth factor distribution and function. In cartilage, TGF-β over-activation induces the production of destructive enzymes that cause osteoarthritis. The use of SLRPs as diagnostic biomarkers for these and other diseases will be an exciting and important future development.

SLRPs are critical components of skeletal stem cell niches, and perhaps other niches. Within tendons, SLRP loss leads to over-activation of BMP signaling in stem cells causing them to form bone instead of tendon. The outcome is a "switch in fate" leading to ectopic ossification and tendon malfunction. It is likely that stem cells in other tissues are regulated by SLRPs; however, the nature of the stem cell and exactly which SLRP is involved will need to be determined.

SLRPs are clearly involved in various aspects of cancer development: from the formation of the tumor microenvironment to cancer growth and metastasis. These multifunctional tasks are achieved by their intrinsic (structure-mediated) ability to interact with and downregulate various tyrosine kinase receptors such as the EGFR, Met, and IGF-IR. In most cases, this interaction results in attenuation of prosurvival signaling pathways and the induction of pro-apoptotic pathways. Some of the SLRPs also directly or indirectly affect angiogenesis.

It is upon this wealth of new information that we need to capitalize and focus our next efforts. Much remains to be learned and discovered about the biology of the SLRPs, and hopefully in the next few years, we will be able to see breakthroughs that will put the latest advances onto a firm footing by better defining the SLRP receptor network and their downstream signaling pathways.

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