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Akira Kudo *Editor*

Periostin

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Periostin

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Preface

In the middle of 2018, the number of periostin-related publications in PubMed was greater than 1000. This number had been increasing every year by the real significance emerged during the past decade. The majority of publications correspond to incurable diseases related to the heart, cancer, bone, and immune systems. Thus, detailed knowledge of periostin would provide opportunities to cure these diseases. Of these diseases, understanding the contribution of periostin on fibrosis is particularly important, since there are not any effective cures. The entire periostin mechanism is an area of ongoing investigation. However, by reviewing our current understanding of periostin within this specialized book, we can hopefully find the clinical solutions to incurable diseases. Finally, as a future perspective, the development of periostin science provides the next three points of view: (1) to provide a new diagnostic marker for unsolved diseases, (2) to provide a new method for curing fibrosis, and (3) to develop a new scientific field by acquiring knowledge of stemness.

Preparation of this book was not possible without the support of the contributing authors who have included the entire area of periostin research with the latest information. The staff at Springer Nature publishing, especially Mr. Selvakumar Rajendran and Dr. Sue Lee, demonstrated great patience with our efforts. This book is the first on periostin and is published in the book series Advances in Experimental Medicine and Biology.

Tokyo, Japan — Akira Kudo

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Part I

Overview of Periostin

Naming, History, Future

1

Akira Kudo

Abstract

The history of periostin and the mechanism of periostin in fibrillogenesis are described. Periostin is a matricellular protein and involved in incurable diseases.

Keywords

Periostin · Periosteum · Periodontal ligament · Fibrillogenesis · Incurable disease

Periostin was named in 1999 as a newly discovered factor for matricellular proteins [2]. Its name is derived from periosteum and periodontal ligament, since this protein had been expected to be sensitive to mechanical stress; the periosteum in bone, periodontal ligaments in teeth, and, as recently found, cardiac valves are very sensitive to mechanical stress during tissue regeneration and development. No clear functions of periostin were discovered until periostin-deficient mice were generated. Thereafter, the first wave of periostin science was found in the field of heart diseases, such as myocardial infarction and cardiac hypertrophy, which revealed some of periostin functions. Periostin is primarily an inducer of fibrillogenesis and acts by stimulating type I collagen production and cross-linking to generate a tight structure [3]. During organ injury followed by its regeneration, overproduction of periostin demonstrates the pathological fibrosis that impairs organ function. Thus, periostin has become a main target in understanding the mechanism of fibrosis, and clinical applications have been initiated to cure fibrosis by blocking the periostin action. The second main action of periostin is as an activator of cell migration. Through periostin binding to integrins, fibroblastic cells proliferate and migrate, which enhances tissue regeneration under physiological conditions and, pathologically, tumor metastasis as observed in cancer research. Finally, periostin function has been investigated in incurable diseases, such as myocardial infarction, hypertrophy, allergy, atopy, tumor metastasis, and stroke [1].

Taken together, the mechanism of periostin function is shown in Fig. 1.1, wherein inflammation or mechanical stress induces the expression of TGF-β and/or IL-4 and IL-13 in macrophages (for inflammation) or in other types of cells (for mechanical stress). These cytokines then induce the expression of periostin as well as other ECM molecules—which are mainly splice variants—such as fibronectin and tenascin-C in fibroblasts, by acting through

Material in this chapter has been adapted from Kudo (2011) [3] and Kudo and Kii (2018) [5] with permission.

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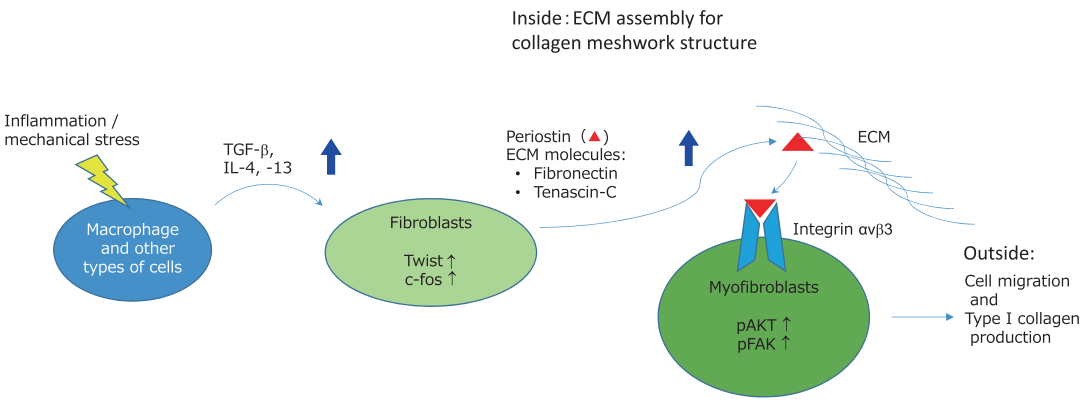

Fig. 1.1 Periostin function at the inside or outside of cells

transcription factors, such as twist and c-fos. The splice variant of periostin is secreted and localized outside the cell in the ECM [4], where it interacts with integrin αvβ3 on myofibroblasts to induce their migration via downstream Akt and FAK phosphorylation-dependent signaling. These myofibroblasts produce type I collagen to repair tissues. During collagen production, periostin, together with tenascin-C found inside the cell, forms a meshwork structure with fibronectin to constitute a scaffold for the cross-linking of type I collagen [5]. This cross-linking is affected by periostin in association with BMP-1 to activate lysyl oxidase (LOX) for enhancement of cross-linking activity inside the cells. During tissue reparation, periostin facilitates the secretion of MMP-9 and expression of Notch1 on the cell surface.

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Part II

Basic Properties of Periostin

2 The Structure of the Periostin Gene, Its Transcriptional Control and Alternative Splicing, and Protein Expression

Akira Kudo

Abstract

Although many studies have described the role of periostin in various diseases, the functions of periostin derived from alternative splicing and proteinase cleavage at its C-terminus remain unknown. Further experiments investigating the periostin structures that are relevant to diseases are essential for an in-depth understanding of their functions, which would accelerate their clinical applications by establishing new approaches for curing intractable diseases. Furthermore, this understanding would enhance our knowledge of novel functions of periostin related to stemness and response to mechanical stress.

Keywords

Periostin gene · Alternative splicing · TGF-β inducible gene · IL-4/IL13 · Periostin protein · Fas1 domain · EMI domain · Proteinase digestion · Heparin-binding site · Heart regeneration

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2.1 The Gene Structure

2.1.1 Mouse or Human Periostin Gene

The osteoblast-specific factor 2 gene, which is the original name for periostin, was cloned using a subtraction library from the mouse pre-osteoblastic cell line, MC3T3-E1, minus the fibroblastic cell line, NIH3T3 [86]. The obtained gene encoded for 811 amino acids (aa), and was found to be the exon 17 deleted-type of periostin. Using this mouse-derived probe, the full-length, human periostin gene was cloned, which encodes for 836 aa (calculated *MW:* 93331), whereas full-length mouse periostin is 838 aa (*MW:* 93159) (Fig. 2.1). The prospective leader sequence encoding a signal peptide is 24 aa for mouse periostin and 22 aa for human periostin, indicating that the mature periostins from both species are both 814 aa with molecular weights around 90 kDa. Comparison of the amino acid sequences between the mouse and human periostin shows 90% homology for the mature form. The mouse periostin gene is located on chromosome 3, whereas the human gene exists on chromosome 13, with both mouse and human genes consisting of 23 exons [29]. In the periostin protein, 4 repeated domains, including two short, highly conserved amino acid sequences (13 and 14 aa, respectively) for one domain, are found to be specific. This region is called the fas1 domain, which

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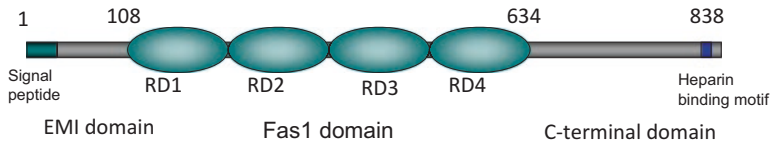

Properties of periostin protein

1. A 90-kDa of TGFβ-induced secreted protein
2. EMI domain: binds to type I collagen, fibronectin and Notch1
3. Fas1 domain (4 RD: repeat domain): binds to tenascin-C and BMP-1
4. C-terminal domain: splice variants and proteolytic cleavage

Fig. 2.1 Periostin protein structure

is conserved in other proteins, including fasciclin 1 in drosophila, βigh3 in humans and mice, Algal-CAM in Volvox, and MPB70 in mycobacterium. These features indicate the presence of the fasciclin 1 family [31]. Drosophila fasciclin 1 functions in growth cone guidance within the nervous system [100].

2.1.2 Alternative Splicing of the Mouse or Human Periostin Gene

The splice variants of periostin are found at the C-terminus in mice between exons 17 and 21, as shown in Fig. 2.2. Initially, three isoforms were found with deletion of one of three exons (b, d, and f), which correspond to exons 17, 20, and 21, respectively, in addition to the full-length periostin [31]. TGF-β1 specifically induces splice variant expression of periostin, fibronectin, and tenascin C. Upon treatment with TGF-β1 in primary osteoblasts, a new band with a smaller molecule was apparent by SDS-PAGE, and its amount was found to increase with increasing TGF-β1 concentrations. Moreover, myofibroblasts produce periostin and also express the splice variants of fibronectin and tenascin-C in response to TGF-β, suggesting the presence of a functional complex involving these three molecules. Han *et al*. reported that TGF-β1 regulates the expression of fibronectin isoforms and the splicing factor, SRp40 [27], suggesting the involvement of a common splicing system (organized by the same splicing factor) in the same cell and developmental stage. In a mouse model of myocardial infarction (MI), the specific splice variant formed by the deletion of exons b and e (exons 17 and 21) from the periostin pre-mRNA is preferentially expressed in the early stage of infarction [77]—the level of full-length periostin is increased later during scar formation. The splice variant has high potential for secretion and induces the phosphorylation of FAK, which is downstream of integrin signaling, suggesting that this variant is fundamentally located in the extracellular matrix to induce cell migration and proliferation. In contrast, full-length periostin is hardly secreted and likely functions within the cell for fibrillogenesis during scar formation. Similarly, 3 days after mouse coronary ligation to induce MI, two isoforms of tenascin-C (220 and 280 kDa) were found [65]; however, tenascin-C-deficient mice showed no significant increase in rupture and a decrease in collagen area after MI. Interestingly, tenascin-C expression during MI is transiently upregulated, which peaks at day 5 and disappears at day 28, indicating that the meshwork structure of type I collagen formed with periostin, tenascin-C, and fibronectin [41] is

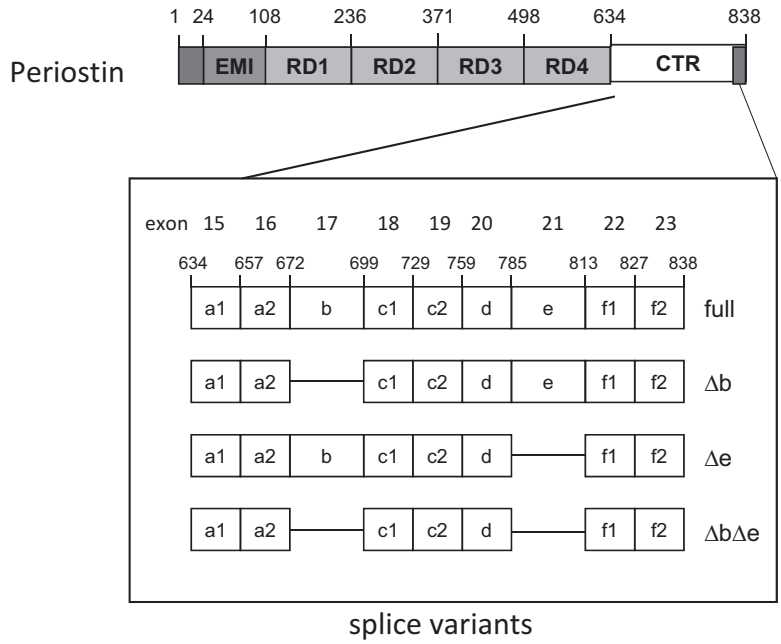

Fig. 2.2 Periostin splice variants

only temporarily present during the early stage of MI. Morra *et al*. found more splice variants of periostin in human tissues and tumors, such as renal cell carcinoma [60] and non-small cell lung cancer [61]. They found five additional splice variants in humans, including two exons (17 and 18 or 17 and 21), three exons (17, 18, and 19 or 17, 18, and 21), and four exons (17, 18, 19, and 21). Taken together, 8 splice variants and full-length periostin have been investigated in human. Furthermore, a tissue and disease-specific periostin variant from mice was described by Litvin *et al*. called the periostin-like factor (PLF), in which exon e (exon 21) is deleted. In retinal neovascularization, two splice variants (deletion of exon 17 or 21) were reported to be specifically expressed [63]. Exon 17 functions in promoting both pre-retinal pathological neovascularization and physiological revascularization in the retina, whereas exon 21 also promotes pre-retinal pathological neovascularization. In another case, one splice variant, termed PDL-POSTN, with deletion of three exons (17, 18, and 21) was shown to be predominantly expressed in the periodontal ligament and found to function in cytodifferentiation and mineralization with strong activation of the integrin αvβ3-focal adhesion kinase (FAK) signaling pathway [96]. In patients with idiopathic pulmonary fibrosis (IPF), exon 21 is the most likely to be spliced out [64].

2.1.3 Other Vertebral Periostin Genes

First, the zebrafish periostin gene was cloned and found to encode for 782 aa and contained the conserved four-fold-repeated fas1 domain. However, this protein also had a novel C-terminal repeat sequence (5 or 6 repeats of 13 aa for one) that was found to be characteristic of fish periostin [49]. Similarly, periostin-a and -b from medaka fish were cloned [37] and it was reported that the gene for type-a was shorter. However, after a 3′RACE experiment, an additional 24 aa at the C-terminus of medaka periostin-a was observed. Thus, full-length medaka periostin-a is 824 aa, whereas medaka periostin-b is 719 aa [48]. Next, the periostin gene from *Xenopus laevis* was reported and found to encode for 794 aa and have the conserved EMI and fas1 domains [90]. Interestingly, the genes proximal to the periostin gene are well-conserved among medaka, mice, and humans, demonstrating the presence of

genes like SAMAD9, ALG5, ExoSC8, Fam48a, and TRPC4 [48]. In medaka, the periostin-a gene is highly similar to mouse and human periostin.

2.2 Transcription Control

2.2.1 Transactivator and Cis-Element

Initially, before generating a knock-out mouse model, periostin had only been characterized by its specific expression patterns in bone-related tissues, such as the periodontal ligament and periosteum. To investigate the function of periostin, one method was to investigate its transcriptional regulation, which was found to be bone-specific and led to the discovery of the bHLH transcription factor, twist [70]. Twist binds to the promoter of periostin and is consistent with the similar expression patterns of twist and periostin in calvarial bones or periodontal ligaments. Furthermore, they are both downregulation due to occlusal hypofunction [1], consistent with the finding that the twist-1 dimer regulates periostin expression due to cranial suture patterning and fusion [9]. Twist behaves as a negative regulator of osteoblast differentiation *in vitro*; therefore, cells overexpressing twist remain in an undifferentiated, osteoprogenitor-like state, and cells expressing twist-antisense progress to more differentiated, mature osteoblasts [53]. *In vivo*, twist suppresses the activity of Runx2 to regulate bone formation as identified by generating twist-1 and -2 deficient mice [4]. In embryonic heart development, BMP-2 induced the expression of twist and periostin in valvulogenesis [34], and twist-1 affects the expression of periostin in endocardial cushion cells [76]. Moreover, in humans, twist-2 binds the promoter of periostin, and its binding affinity is stronger than that of twist-1, which was observed in Setleis Syndrome—a rare autosomal recessive disease characterized as abnormal facial development [17]. Regarding its role in tumorigenicity, periostin is a top target of twist-1 in human glioblastoma, which is a highly lethal and treatment-resistant disease with a median survival time of approximately 12–16 months [59]. Twist-1 in glioma promotes EMT-associated phenotypes of invasion and cancer cell stemness. In another binding protein p73 that is a member of the p53 family in glioblastoma, p73 directly binds to the periostin promoter to activate its transcription [52].

Regarding the transcriptional regulation of cis-elements in the periostin gene during embryogenesis, an evolutionarily-conserved, YY1-binding 37-bp region within a 304-bp periostin core enhancer is found between −2509 and −2205 of the promoter, which is capable of simultaneously regulating expression of the novel, tissue-specific periostin in the cardiac outflow-tract cushion mesenchyme and Schwann cell lineages [54]. Although YY1 is known to be both a transcriptional repressor and/or activator and has been shown to physically interact with more than a dozen proteins, it could also be acting as a novel SMAD-interacting protein that represses SMAD transcriptional activities in a gene-specific manner; therefore, it may be regulating cell differentiation induced by the TGF-β superfamily of pathways [50]. Based on an association method, a tag single nucleotide polymorphism (SNP) revealed that several SNPs of periostin are associated with bone mineral density or vertebral fractures [95]. The most significant polymorphism site, located at −2327 bp upstream of periostin, binds to CDX1 (caudal type homeobox 1), which is a member of the caudal-related homeobox transcription factor family and encodes for a DNA-binding protein that regulates intestine-specific gene expression and enterocyte differentiation. Furthermore, direct interaction between CDX1 and Hoxa-7 affects skeletal formation [79]. Fibrous dysplasia is a benign bone disease characterized by high expression of c-Fos/c-Jun. Overexpression of c-Fos in transgenic mice induces development of sclerotic lesions and periostin expression [38], which is consistent with the fact that there are two potential binding sites for c-Fos/AP-1 in the periostin promoter [70].

2.2.2 Factors Inducing Periostin Expression

Originally, periostin was identified as a TGF-β-inducible gene, indicating that its expression, including the patterns of splice variants, was increased in a TGF-β-dose dependent manner. Furthermore, expression of the lowest molecule derived from the splice variant was significantly increased in the mouse, pre-osteoblast cell line, MC3T3-E1 [31], suggesting that TGF-β itself induces the splice variation. In the human periodontal ligament, treatment with TGF-β1 significantly increases periostin mRNA levels, which are blocked by a focal adhesion kinase (FAK) inhibitor [78]. Concurrently, the Th2 cytokines, IL-4 and IL-13, enhance the expression and secretion of periostin in lung fibroblasts [83], atopic dermatitis (AD), and bronchial asthma [57]. In the human embryonic lung fibroblast cell line, MRC5, three periostin bands were detected at 84, 80, and 78 kDa, which are derived from alternative splicing and generated by the deletion of exons 17, 18, 19, and 21, exons 17, 18, and 21, and exons 17 and 21, respectively. Interestingly in comparison with other matricellular proteins like fibronectin and tenascin-C, IL-4 and IL-13 can dominantly induce expression of periostin splice variants but not those of fibronectin and tenascin-C, whereas TGF-β induces splice variant expression from all 3 genes [83]. IL-4 and IL-13 can enhance periostin expression more effectively compared to TGF-β. In an animal model of asthma, epithelial cell-derived periostin activates TGF-β or sensitizes against TGF-β signaling, resulting in collagen gel elasticity in asthma [93]. This periostin function for activation of TGF-β signaling is coincident with the action of M2 macrophages. Specifically, M2 macrophages are induced by the T helper 2 (Th2) cytokines, IL-4 and IL-13, which is distinct from the interferon-γ (IFN-γ)-mediated classical activation required for M1 macrophages [24]. In glioblastoma (GBM), which is the most common malignant brain tumor, glioma stem cells (GSCs) contribute to GBM tumor growth through periostin that is expressed in GSCs enriched in the perivascular niche and recruit tumor-associated macrophages (TAMs) that are characterized as M2 macrophages [94, 99]. These TAMs are mainly monocyte-derived macrophages from the peripheral blood, and periostin maintains the M2 subtype of TAMs to accelerate tumor growth by promoting cancer cell survival in GBMs [99] and in intrahepatic cholangiocarcinoma stem cells [98]. Similarly, hypoxia enhances the recruitment of TAMs, and polarizes these macrophages toward the M2 subtype by increasing the expression of M-CSFR in macrophages and that of TGF-β in glioma cells [25]. The same action of periostin is observed during injury of the central nervous system. Periostin expression is predominantly induced in the scar-forming pericytes during the spinal cord injury to promote the migration of macrophages for scar formation [97]. Taken together, these results indicate that the Th2 cytokines, IL-4 and IL-13, upregulate periostin expression as well as activation of M2 macrophages. Periostin then recruits these macrophages for tissue repair or tumor growth. Simultaneously, signals from Toll-like receptor 4 (TLR-4), which plays a critical role in initiating inflammatory reactions, activate periostin expression [69].

2.3 Protein

2.3.1 Domains in Association with Other Extracellular Molecules

Periostin has a protein structure composed of an amino-terminal EMI domain, a tandem repeat of four fas1 domains, and a carboxyl-terminal domain that includes a heparin-binding site (Fig. 2.1); therefore, we characterized it as a member of the fasciclin 1 family based on these typical fas1 domains [75]. Fasciclin 1 is a GPI-anchored *Drosophila* protein that functions in axon growth guidance and contains four tandem fas1 domains that are approximately 150 aa residues each, which are not related to any other protein domains from known structures [32]. In humans, fas1 domains are found in βigh3 [43] and stabilins [71] as well as in periostin. Periostin and βigh3 are most similar and share uninterrupted

tandem repeats of four fas1 domains. Interestingly, mutations in fas1 domains of human βigh3 result in corneal dystrophy due to deposition of insoluble protein aggregates in the cornea [62]. The EMI domain, which is a small module rich in cysteine residues that is found in the EMILIN family, is a site for protein-protein interaction [16] and can bind to fibronectin [42], whereas tenascin-C binds to the fas1 domain [41]. During collagen cross-linking in fibrillogenesis, the interaction of periostin with fibronectin, tenascin-C, and BMP-1 (a metalloproteinase for digestion) followed by activation of lysyl oxidase (LOX) for the formation of covalent cross-links in collagen and elastic fibers is essential [56]. In addition to activation of LOX, BMP-1 functions in processing the C-propeptides of procollagen types I-III to yield the major fibrous components of the vertebrate ECM. It also processes the NH_2-terimal globular domains and C-propeptides of types of V and XI procollagen chains to yield monomers that control the diameters of collage type I and II fibrils through their incorporation [20]. Furthermore, BMP-1 processes the precursors of laminin 5 (γ2) and collagen type VII, both of which are involved in securing the epidermis to the underlying dermis. Consistently in wound healing models, periostin is associated with laminin γ2 at the basement membrane, and this association enhances BMP-1-mediated proteolytic cleavage of the laminin γ2 long form to produce its short from [66]. Regarding the importance of BMP-1 in broader fields, its mutation on human chromosome 8p21 or BMP-1-deficient mice show some dentin defects and alveolar bone loss, which have shown to be causal in the development of osteogenesis imperfecta (OI) type XIII [81, 92]. A similarly abnormal alveolar bone loss was observed in periostin deficient mice [82]. Recently, it was found that periodontal ligament cells contribute to alveolar bone formation, and it is encouraging that periostin is a key factor expressed in the stem-like cells of both periodontal ligaments and alveolar bones. During bone metastasis, LOX increases stiffness of the ECM on bones when it is sensed by cancer cells, which in turn focuses the activities of the cancer cells towards invasion and drives them to migrate to distant, metastatic sites [12, 19]. In lung cancer, lysyl hydroxylase 2, which catalyzes the hydroxylation of lysine residues in the telopeptides of fibrillar collagens, leads to collagen cross-links that enhances metastatic propensity by stiffening the tumor stroma through collagen cross-linking [7]. Thus, periostin is possibly involved in bone metastasis through the activation of BMP-1 and LOX.

2.3.2 Proteinase Digestion

At 4 days after induction of myocardial infarction (MI), proteolytic cleavage as indicated by the presence of one major lower band was initially found at the C-terminal site using an antibody recognizing the first fas1 domain at the N-terminal site but not by an antibody recognizing the C-terminal end of periostin. This indicates that at least one site in the C-terminal domain was digested by a proteinase. After 28 days of MI, multiple bands were observed. Similarly, in a model of lung injury by bleomycin administration, several bands were found due to proteolytic cleavage of periostin [46]. The first candidate to determine the action of the shorter periostin is the commercially available, recombinant periostin that is provided by Bio Vender, because this recombinant molecule has 167 aa deleted at the C-terminus. Thus, this construct mainly consists of the EMI domain and the four fas1 domains but does not have almost the entire C-terminal region. In an interesting experiment testing collagen I gene expression induced by periostin in airway fibroblasts, the recombinant periostin could not induce collagen I gene expression even though periostin itself could, suggesting that the C-terminal region enhances collagen I gene expression through activation of TGF-β by periostin [93]. Recombinant periostin markedly induces the elastic modules of gels formed by type I collagen, because addition of recombinant periostin generates a more densely cross-linked gel. In another model of fibrosis in bronchial asthma, the C-terminal region-deleted periostin showed much stronger binding to fibronectin and tenascin-C [83]. Originally, Kii et al. reported

that the cleavage of the C-terminal end of periostin is essential for binding to tenascin-C [41]. These three reports suggest that the truncated form of periostin with the deleted C-terminal region tightly binds to fibronectin and tenascin-C for enhanced cross-linking of type I collagen. Furthermore, the EMI domain of periostin is essential for its multimerization, which facilitates collagen cross-linking through formation of a meshwork structure with fibronectin and tenascin-C [41]. Accordingly, a recombinant periostin easily forms a dimer [83], which is consistent with the crystal structure of human periostin. This indicates that the dimer form functions as a scaffold to bring interacting proteins in close proximity [55]. The analysis of the medaka periostin gene with long or short periostin molecules indicates that the short periostin (periostin-b with 719 aa) shows negative regulation in osteoblast differentiation, whereas the long periostin (periostin-a) behaves in a positive manner for osteoblast differentiation [85]. Taken together, the action of a periostin switch is present, indicating that in the early stage of periostin expression, it behaves as a positive regulator of cell proliferation, movement, and collagen production, and, after cleavage of the C-terminal site, the action of periostin changes to collagen cross-linking (Fig. 2.3).

2.3.3 Heparin Binding Site

At the C-terminal end of periostin, an arginine-rich heparin binding site was observed [80]. Fibronectin contains two heparin-binding sites. The high-affinity heparin II binding domain located at the C-terminal site is thought to interact with cell surface glycosaminoglycans to facilitate cell adhesion and spreading and also plays an important role in matrix assembly. The heparin I domain is also involved in matrix assembly, particularly fibronectin self-assembly [91]. Assembly of dimeric fibronectin into the extracellular matrix involves multiple consecutive binding interactions with integrin receptors, with itself, and with matrix components, such as type I collagen [6].

2.3.4 Elastin Fiber Formation

In fibrillogenesis, elastin fiber formation—wherein the extracellular short fibulins, fibulin-3, -4, and -5, are components of the elastic fiber/microfibril system and are implicated in the formation and homeostasis of elastic tissues—has properties similar to collagen fiber formation: (a) Lox interaction (b) integrin signaling, (c) heparin binding, (d) proteinase digestion, and (e) multimerization [84]. Thus the periostin-like action is possibly utilized in this system. Among fibulins, fibulin-4 can work on proper elastogenesis through its interaction with LOX, which is an

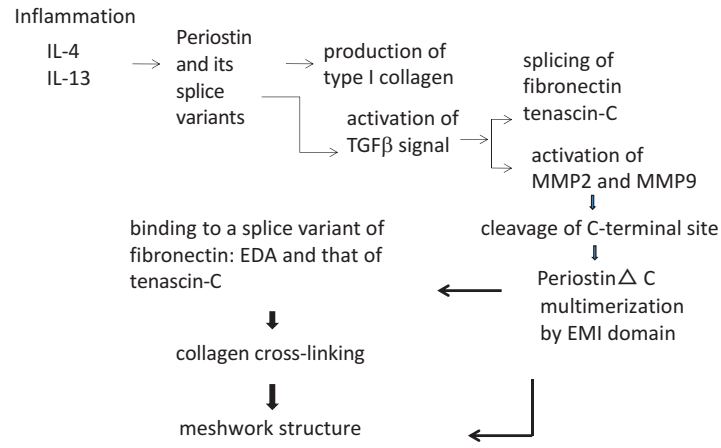

Fig. 2.3 Periostin switch: the mechanistic view of periostin functions

elastin-cross-linking enzyme [26]. These fibulins form multimers, indicating that fibulin-4 behaves like periostin in elastin fiber formation, whereas fibulin-5 binds to human umbilical vein endothelial cells in an RGD-dependent manner via integrins, indicating that fibulin-5 behaves like fibronectin. Moreover, both fibulins may bind cell surface-located heparin sulfate [84]. Taken together, these observations indicate that fibulin-4 and -5 are utilized in the formation of elastin fibers instead of periostin and fibronectin. Thus, knowledge of elastin fiber formation is very useful for understanding the mechanism of collagen fiber formation.

2.3.5 γ-Glutamyl-Carboxylase

Modification of glutamic acid residues to γ-carboxyglutamic acid (Gla) is a post-translational modification catalyzed by the vitamin K-dependent enzyme, γ-glutamylcarboxylase. The most abundant Gla-containing protein secreted by bone marrow-derived mesenchymal stromal cells is periostin, in which the fas1 domain is carboxylated [11]. Since only 12 vitamin K-dependent Gla-containing proteins, such as osteocalcin and matrix Gla protein that play pivotal roles in bone development and repair, have been identified in humans, periostin and βigh3 are the 13th and 14th of these proteins, respectively. Periostin was found to be abundantly deposited in bone nodules in areas where osteoblastic cells are tightly embedded in the mineralized extracellular matrix, suggesting that the Gla residues on periostin might provide hydroxyapatite binding properties that have an important structural role. In contrast, no human periostin extracted fibrotic lung has shown vitamin K-dependent γ-carboxylation, indicating that the question of whether γ-carboxylation of periostin is important [2]. Similarly, no γ-carboxylation of periostin was found in mouse serum *in vivo* [21].

2.3.6 Periostin Paralogue, TGFBI (βigh3)

The interaction between periostin and the periostin paralogue, TGFBI (βigh3), likely results from hetero-multimerization via their EMI domains [44]. This interaction was found to be essential for the proper secretion of a periostin/βigh3 hetero-multimer. Furthermore, it has been demonstrated that βigh3—like periostin—directly binds to collagen types I, II and IV [28] and localizes to the Golgi apparatus [44]. The similarities between both proteins suggest that the functions of these matricellular proteins are to act as modulators.

Periostin and βigh3 generally have different expression patterns. However, in bone cells, periostin and βigh3 are expressed in both osteoblasts and osteoclasts *in vitro* [58], suggesting that these genes possibly act cooperatively in bone formation. In contrast, after myocardial infarction, deletion of βigh3 did not alter cardiac disease—unlike the periostin-deficient mice—indicating a distinct action of βigh3 [74]. In addition, βigh3 lacks an extended region equivalent to the C-terminal domain (CTD), suggesting that the CTD is of functional relevance and could modulate periostin function.

2.4 New Horizon of Periostin Action

2.4.1 Periostin Function in Stemness

Periostin is regarded as a critical molecule that plays a progressive role in severe diseases, such as fibrosis in asthma, scar formation in infarcted myocardium, and tumorigenesis [10]. Interestingly, there are several recent reports of periostin function in stem cells. Periostin has been shown to maintain hematopoietic stem cells (HSCs) in a quiescent state through its interaction with the αv integrin on HSCs in the bone

marrow niche [40]. Another recent report showed that periostin secreted from stromal fibroblastic cells supports not only normal hematopoietic precursor cells but also leukemia-initiating cells [88]. It has also been reported that periostin associates with the matricellular protein, CCN3 (also termed NOV), and that a functional relationship was observed in the periodontal ligament [15]. In this regard, CCN3 acts as a regulator of human HSCs or hematopoietic progenitor cells as demonstrated by the loss of CCN3, which diminishes the functional capacity of the primitive hematopoietic compartment [30]. In mice, CCN3 plays a role in HSC maintenance [35], suggesting a cooperative function of periostin together with CCN3 for HSC maintenance. In addition, the Notch signaling pathway is involved in HSC maintenance, and periostin-Notch1 and periostin-CCN3 interactions indicate possible contributions of the functional complex of these three molecules (periostin-CCN3-Notch1) in HSC stemness. Periostin is secreted from mesenchymal stem cells to support tendon formation, as characterized by the overexpression of periostin [67]. Taken together, it can be speculated that periostin-CCN3 has become functional inside the bone after landing of animals [26], initiating the building of the bone marrow microenvironment that supports hematopoietic stem cells by maintaining the hematopoietic compartment.

Concurrently, bone regeneration relies on the activation of skeletal stem cells (SSCs). Periosteum contains SSCs with high bone regenerative potential, which is maintained with periostin, reconstituting a pool of periosteal cells after injury [14].

2.4.2 Periostin in Mechanical Stress

From expression profiling of periostin in periosteum and periodontal ligament, periostin has been expected to be mechanical stress-sensitive, since the periosteum on the bone and the periodontal ligament in teeth are very sensitive to mechanical stress in order to aid in tissue regeneration and development [31]. However, no direct evidence of stress sensitivity has been reported. Interestingly, cathepsin K controls cortical bone formation by degrading periostin since osteocytes expressing cathepsin K play a central role in the regulation of the biomechanical response in bone tissues, and degradation of periostin by cathepsin K occurs in osteocytes [5]. Hence, cortical bone formation is regulated by the periostin-mediated blocking of random bone formation. Periostin is now known as an essential marker of periodontal ligament; this knowledge has helped to develop research on periodontal ligaments and has clinical applications for periodontal diseases. A recent study demonstrated that periostin maintains stemness of periodontal ligament mesenchymal stem cells and then promotes their osteogenic differentiation through the JNK pathway under inflammatory conditions [89].

A functional link between the signaling cascades in mechanical stress and periostin gene expression was reported. It was found that both periostin and the mammalian target of rapamycin (mTOR) are coordinately up-regulated by mechanical stress during the wound healing to induce cell migration and proliferation [73]. This suggests that the same signal originating from mechanical stress enhances both periostin and mTOR expression and that periostin then activates mTOR signals, probably via integrins. Coincidently, the activated mTOR signaling pathway promotes osteoclast formation for bone remodeling [13], which reasonably suggests that periostin plays a role in the maintenance of bone mass [41]. One of the candidate signals linking mTOR and periostin activation is neuronal nitric oxide synthase (nNOS). In overload-induced skeletal muscle hypertrophy in mice, nNOS is transiently activated in a very short period after overloading, which activates the transient receptor potential cation channel, subfamily V member 1 (TRPV1), resulting in increased intracellular Ca^{2+} concentrations that subsequently trigger mTOR activation [36].

Discoidin domain receptor 1 (DDR1) is a receptor tyrosine kinase that shows similar functions to periostin physiologically and is pathologically specific to chronic kidney disease (CKD). Notch 1 is an interacting partner of DDR1, and type I collagen is a ligand of DDR1,

indicating that activation of DDR1 receptor kinase through type I collagen binding induces Notch 1 signaling to promote cell survival [45]. This activity is highly similar to periostin function [87] and probably involves CCN3 [84]. Further analyses of the synergism between DDR1 and periostin would be very important for understanding apoptosis induced by mechanical stress. In CKD, both periostin and DDR1 are involved in the regulation of inflammation and fibrosis. Thus, both proteins are novel biomarkers and therapeutic targets in CKD [72].

2.4.3 Heart Regeneration

During heart regeneration, different actions of periostin expressed in medaka and zebrafish were revealed [37]. Zebrafish hearts can regenerate, while medaka hearts cannot, though medaka fin is regenerated [39]. In medaka and zebrafish, periostin expression was observed and it was found that a different expression pattern occurred during heart regeneration [37]. In zebrafish, scar resolution is necessary for heart regeneration and is associated with collagenolytic activity [18]. Furthermore, periostin expression in the epicardium generates myofibroblasts [22]. Macrophage migration after injury is delayed in medaka, resulting in no regeneration of the injured heart [51]. This suggests that timely macrophage recruitment is critical for heart regeneration and shows the first clear evidence of distinct periostin actions between zebrafish and medaka. Exploring the different responses of medaka and zebrafish to cardiac injury represents a unique opportunity to identify factors required for heart regeneration [23]. In mice, although the adult heart in mice cannot be regenerated, neonatal hearts can be regenerated, indicating the similarity between medaka hearts and mouse adult hearts and that between zebrafish hearts and mouse neonatal hearts. During further investigation of neonatal heart in mice, macrophages were found to be essential for heart regeneration and also driving angiogenesis [3]. O'Meara et al. [68] then demonstrated that the production IL-13 and STAT3 is important for myocyte proliferation through periostin expression during mouse neonatal heart regeneration and that this process is regulated through the PI3K/p-AKT/cyclin D1 pathway. In neonatal mice, periostin is critical for myocardial regeneration [8]. Periostin function has been elucidated in mouse models of myocardial infarction, and periostin was found to repair injured hearts through scar formation after type I collagen cross-linking [47, 56, 77].

Taken together, it can be speculated that during heart regeneration of mouse neonatal hearts and zebrafish hearts, macrophages secrete IL-13 that induces periostin expression followed by myocyte proliferation and activation of angiogenesis, whereas, in mouse adult hearts and medaka hearts, periostin induces fibrosis and myocyte hypertrophy [33, 47].

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Part III

Function as the Scaffold

Periostin Functions as a Scaffold for Assembly of Extracellular Proteins

3

Isao Kii

Abstract

Periostin is a secretory matricellular protein with a multi-domain structure that is composed of an amino-terminal EMI domain, a tandem repeat of four FAS 1 domains, and a carboxyl-terminal domain (CTD). Periostin has been suggested to function as a scaffold for assembly of several extracellular matrix proteins as well as its accessory proteins (Fig. 3.1, Table 3.1), which underlies highly sophisticated extracellular architectures. This scaffold function is likely due to periostin's multi-domain structure, in which the adjacent domains in periostin interact with different kinds of proteins, put these interacting proteins in close proximity, and promote intermolecular interactions between these proteins, leading to their assembly into a large complex. In this chapter, I introduce the proteins that interact with each of the adjacent domains in periostin, and discuss how the multi-domain structure of periostin functions as a scaffold for the assembly of the interacting proteins, and how it underlies construction of highly sophisticated extracellular architectures.

Keywords

EMI · FAS 1 · Heparin · Fibronectin · Tenascin-C · Collagen · BMP-1 · CCN3 · Lysyl oxidase · Golgi · Endoplasmic reticulum · βig-h3

3.1 The EMI Domain of Periostin Is Responsible for Multimerization and Interacts with Extracellular Matrix Proteins

The EMI domain, named after its presence in the EMI family [16], is always a single copy located at the amino-terminus of secretory proteins [7]. The EMI family contains EMILINs, multimerins, Emu, NEU1/NG3, as well as periostin and its paralog βig-h3 [7, 16]. EMILIN-1 is an extracellular matrix protein involved in elastin deposition [85]. The EMI domain of EMILIN-1 was reported to interact with the C1q domain of EMILIN-2 [5, 17], promoting heterogeneous multimerization between EMILIN-1 and EMILIN-2. It has also been demonstrated that the EMI domain of EMILIN-1 binds to pro-TGF-β1, and prevents its

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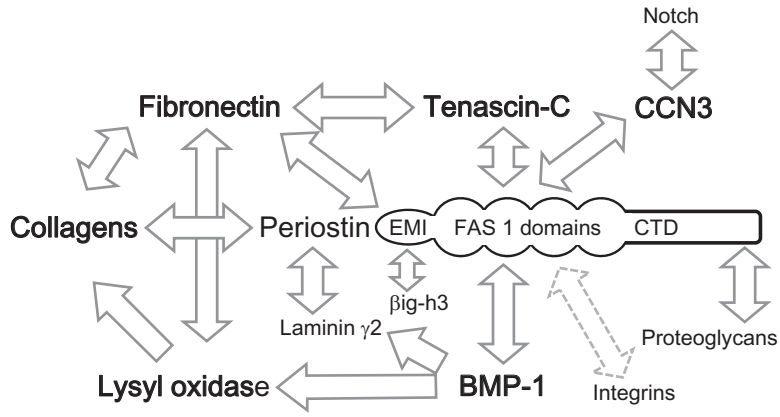

Fig. 3.1 Periostin and its interacting proteins

Table 3.1 Proteins interacting with periostin

	Interacting protein	Domain of periostin	Direct/ indirect/ unknown	References
Extracellular matrix protein	Fibronectin	EMI	Direct	Kii et al. [43] and Norris et al. [58]
	Tenascin-C	FAS 1	Direct	Kii et al. [43]
	Type I collagen	Unknown	Unknown	Kii et al. [42], Norris et al. [58], Suzuki et al. [70], and Takayama et al. [71]
	Laminin γ2	Unknown	Unknown	Nishiyama et al. [57]
Enzyme	BMP-1	FAS 1	Direct	Hwang et al. [32] and Maruhashi et al. [51]
	Lysyl oxidase	EMI	Indirect	Maruhashi et al. [51]
Matricellular protein	βig-h3	EMI	Direct	Kim et al. [45] #377
	CCN3	FAS 1	Unknown	Takayama et al. [73]
Receptor	Integrins	FAS 1	Unknown	Bao et al. [3], Ghatak et al. [22], Gillan et al. [23], Khurana et al. [40], Orecchia et al. [60], Shao et al. [66], Sugiyama et al. [69], and Underwood et al. [80]
	Notch-1	Unknown	Unknown	Tanabe et al. [74]

maturation by furin convertases [64, 84]. EMILIN-3 also binds to TGF-β1 and acts as its agonist [64]. These reports of the EMI domain interactors suggest that the EMI domain is a protein-protein interaction module. But, the interface between the EMI domain and its interactors has not been revealed.

The EMI domain is composed of approximately 80 amino acid residues, and includes six highly conserved cysteine residues [7, 16]. These cysteine residues are likely to be responsible not only for intramolecular disulfide-bridges but also for intermolecular multimer formation. It has been suggested that the first, second, and forth cysteine residues form intramolecular disulfide bond with the fifth, third, and sixth cysteine residues, respectively (Fig. 3.2a) [7]. On the other hand, SDS-PAGE analysis with periostin treated with a reducing reagent DTT showed that periostin forms disulfide-bonded multimers (dimer to hexamer) [43], suggesting that some of these cysteine residues are involved in intramolecular disulfide bond. This intermolecular disulfide bond also prompts hetero-dimerization between proteins in the EMI family. For example, periostin forms hetero-dimerization with its paralog βig-h3 [45]. Thus, the EMI domain functions as homo- and hetero-multimerization core and interface for its interactors [43].

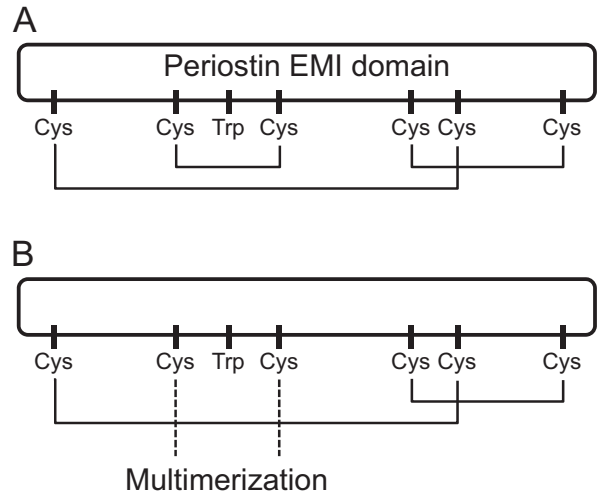

Fig. 3.2 Periostin EMI domain

The whole structure of the EMI domain and the tandem repeat of four FAS1 domains of periostin has been determined by X-ray crystallography [48]. The EMI domain is mainly composed of β-sheets. In this crystal structure of periostin, the arrangement of disulfide-bonds was not consistent with the other reports for the EMI domain of the EMI family [7]. The first and forth cysteine residues formed a disulfide-bond with the fifth and sixth cysteine residues, respectively, which is consistent with the previous report as described above [7]. However, the third cysteine residue in the EMI domain formed a disulfide-bond with the cysteine residue in the second FAS 1 domain (RD2) [48]. In addition, the first and second FAS 1 domains (RD1 and RD2) were disulfide-bonded [48]. Considering the previous report [7], the second and third cysteine residues in the EMI domain of periostin would be involved not only in intramolecular interaction but also in intermolecular binding (Fig. 3.2b).

The EMI domain of periostin has been demonstrated to interact with fibronectin [43]. Fibronectin is the core component of extracellular matrix architecture, in which fibronectin fibrils underlie subsequent assembly of extracellular matrix proteins such as collagens [35]. Purified recombinant periostin protein bound to fibronectin-coated microtiter plates [43, 58], indicating a direct interaction between periostin and fibronectin. But, it has not been known which domain of fibronectin interacts with periostin. The EMI domain that fused to Fc was co-immunoprecipitated with fibronectin in 293T cells transfected with the expression vectors of EMI-Fc and fibronectin [43]. In addition, the proximity ligation assay revealed that a close proximity between periostin and fibronectin was detected inside fibroblastic cells [44], suggesting that the interaction between periostin and fibronectin occurs before its secretion. Furthermore, this interaction in the secretory pathway enhanced secretion of fibronectin into the extracellular milieu [44], suggesting an undetermined role of periostin in fibronectin secretion. Consistently, periostin was localized preferentially in the Golgi apparatus and the endoplasmic reticulum, also indicating its function in the secretory pathway [43–45]. Golgi and endoplasmic reticulum localization of periostin is one of the functional aspects of periostin; however, it has been remained elusive how periostin is retained in these cellular organelles. An anchor sequence in periostin should function to interact with transmembrane proteins in Golgi and endoplasmic reticulum.

The EMI domain possesses a conserved tryptophan residue between the second and third cysteine residue [16, 43]. In periostin, this conserved tryptophan residue locates to position 65 between the second and third cysteine residues (Fig. 3.2). To investigate roles of this tryptophan residue of

periostin, a substituted mutant of this tryptophan residue was generated [43]. The substitution of this tryptophan to alanine impaired the interaction with fibronectin [43], clearly demonstrating that the EMI domain is the interaction domain for fibronectin and that this conserved tryptophan residue is essential for the interaction. In addition, the substituted mutant of Trp65Ala acted as a dominant negative form for the assembly of the interacting proteins [43]. The Trp65Ala mutant is not able to bind to fibronectin, but to other extracellular proteins that interact with tandem repeat of four FAS 1 domains (described below). Thus, the Trp65Ala mutant would abrogate the assembly between fibronectin and the other extracellular proteins. This experiment using the Trp65Ala mutant clearly demonstrates the scaffold function of periostin [43]. In addition, the importance of the tryptophan residue is supported by its codon usage. Tryptophan is encoded only in one codon TGG, suggesting that the tryptophan residue in periostin has been protected from selective pressure in the evolutionary process. The high conservation of this tryptophan residue explicitly demonstrates the functional role of the tryptophan residue in the EMI domain.

Periostin has also been shown to interact with collagens, which is consistent with the localization of periostin on collagen bundles [42, 58, 70]. Periostin was co-immunoprecipitated with collagen type I [58], and a purified recombinant periostin protein bound to collagen type V-coated microtiter plates [71]. However, the binding site for collagen has not yet been identified and further studies of this interaction are required. As described above, periostin interacts with fibronectin inside cells [44]. This also indicates a possibility that periostin binds to collagens in the secretory pathways such as Golgi and endoplasmic reticulum. Collagen fibrillogenesis has been demonstrated to occur in the secretory pathway in tendon, and this collagen fibril secretion mechanism is proposed as fibripositor [9, 10, 30, 36, 38, 76]. It should be quite reasonable that periostin forms a large complex with fibronectin and collagens in the secretory pathway.

Fibronectin has several binding regions for collagen, possibly indicating that periostin directly and indirectly interacts with collagens via the EMI domain. As consequence of the interaction of periostin with collagen and fibronectin that is an essential factor for collagen fibrillogenesis [35], periostin plays a role in promoting collagen fibrillogenesis [58]. Collagen fibrillogenesis is a complicated multi-step process that is still poorly understood [8]. Molecular studies on the interaction of periostin with collagen and fibronectin, both inside and outside cells, would contribute to clarifying the mechanism of collagen fibrillogenesis.

The EMI domain of periostin interacts with the EMI domain of βig-h3 (also known as keratoepithelin and RGD-CAP) that is a protein with structural and sequence homology to periostin [53]. βig-h3 is coded in the *TGFBI* (transforming growth factor-β-induced) gene, mutations of which are associated with corneal dystrophies, progressive eye disorders [37]. *TGFBI* gene knockout mice have been generated, in which tumor development was enhanced in these mice [1]. Thus, *TGFBI* gene encoding βig-h3 acts as a tumor suppressor. The interaction between periostin and βig-h3 likely results from disulfide bond-mediated hetero-multimerization via their EMI domains [45]. This interaction was found to be essential for the proper secretion of a periostin/βig-h3 hetero-multimer [45]. Further, it has been demonstrated that βig-h3 directly binds to collagens type I, II, and IV [25], and localizes to the Golgi apparatus as periostin [45]. These similarities between periostin and βig-h3 suggest redundancy in the molecular functions of these matricellular proteins.

3.2 The Tandem Repeat of Four FAS 1 Domains of Periostin Binds to Tenascin, BMP-1, and CCN3

The FAS 1 domain was originally identified in an insect neural cell adhesion molecule, *Drosophila* fasciclin 1, consisting of the tandem repeat of four FAS 1 domains [13, 14]. The crystal structure of the third and fourth FAS 1 domains of *Drosophila* fasciclin 1 revealed a unique domain

fold, consisting of a seven-stranded β wedge and a number of α helices [14]. Nuclear magnetic resonance spectroscopy was also used to solve the structure of the FAS 1 domain of *Mycobacterium bovis* secretory protein MPB70, which has a structural homology to the FAS 1 domain of fasciclin 1 [11]. The whole structure of the EMI domain or the tandem repeat of four FAS 1 domains of periostin has been determined by X-ray crystallography [48]. This whole structure is consistent with the previously determined FAS 1 domains.

The tandem repeat of four FAS 1 domains of periostin has been demonstrated to bind to tenascin-C [43]. Tenascin is an extracellular matrix protein, and its amino-terminus forms a disulfide-bonded hexamer, resulting in a six-armed oligomer, termed a hexabrachion [52]. Pathophysiological roles of tenascin-C have been investigated. Tenascin-C is expressed during wound healing or in pathological conditions, including chronic inflammation and cancer [52]. These expression patterns of tenascin-C is very similar to those of periostin, indicating functional association between tenascin-C and periostin. Co-immunoprecipitation analysis confirmed the interaction of the FAS 1 domains of periostin and tenascin-C [43]. The purified FAS 1 domains of periostin bound to tenascin-coated microtiter plates [43], clearly demonstrating direct binding between the FAS 1 domains of periostin and tenascin-C. Interestingly, the interaction between the FAS 1 domains and tenascin required cleavage of the CTD of periostin [43]. The CTD of periostin is likely to be cleaved in the secretory pathway, especially in the Golgi apparatus [43]. Although the inhibitory mechanism of the CTD on the FAS 1 domain-tenascin interaction is not currently well understood, inter- and intra-molecular interactions in periostin have been reported [71, 72]. It was shown that recombinant periostin CTD bound to the recombinant tandem repeat of four FAS 1 domains in a solid-phase binding assay [72], indicating a possibility that this intra- or inter-molecular interaction in periostin inhibits interaction of the FAS 1 domains and tenascin-C. Structural analysis of periostin CTD would clarify this auto-inhibitory mechanism.

The four FAS 1 domains of periostin have been reported to interact with bone morphogenetic protein-1 (BMP-1), which is the procollagen C-proteinase that cleaves the carboxyl-terminal propeptides of procollagens I, II, and III [81]. Unlike other BMPs, BMP-1 does not belong to the TGFβ superfamily. BMP-1 is one of the metalloproteinases [81]. Co-immunoprecipitation analysis revealed that the tandem repeat of the four FAS 1 domains of periostin interacted with the metalloproteinase domain of BMP-1 [51]. In addition, a solid-phase binding assay using purified proteins confirmed direct binding between periostin and BMP-1 [32, 51]. Furthermore, three-dimensional docking simulation was performed between the FAS 1 domains of periostin and BMP-1, implicating their binding sites [32]. This direct binding possibly plays a role in enzymatic activity of BMP-1.

This direct interaction between the FAS 1 domains and BMP-1 promoted proteolytic activation of lysyl oxidase (LOX), which was indirectly associated with the EMI domain of periostin through fibronectin [21, 51]. LOX is an enzyme that catalyzes the formation of highly reactive aldehydes from peptidyl lysine residues in collagen molecules [77]. These aldehydes spontaneously react with other aldehydes or with intact lysine residues intermolecularly, resulting in the cross-linking of collagen molecules, which is essential for the stabilization of collagen fibrils. LOX has been focused in cancer progression [19, 20], and recognized as the therapeutic target [4, 18, 34]. The functional role of LOX in collagen fibrillogenesis underlies tumor microenvironment rich in tumor-associated fibroblasts and extracellular matrix proteins. Periostin is also expressed in tumor microenvironment [15, 24, 47], indicating functional association between periostin and LOX in cancer progression. Consequently, targeted deletion of the *periostin* gene in mice caused the reduction of cross-links in collagens [43, 58, 67]. Thus, the periostin-BMP-1-LOX axis underlies the mechanochemical property of the collagen matrix not only in

physiological tissue stiffness but also in tumor microenvironment.

The tandem repeat of four FAS 1 domains in periostin has also been reported to interact with CCN3 [73]. CCN3 is a matricellular protein that belongs to the CCN family. The CCN family is composed of CCN1 (cysteine-rich 61, Cyr61), CCN2 (connective tissue growth factor, CTGF), CCN3 (nephroblastoma overexpressed gene, NOV), CCN4 (Wnt-induced secreted protein-1, WISP1), CCN5 (WISP2/rCOP-1) and CCN6 (WISP3) [61]. CCN3 was initially identified in a chick nephroblastoma [46]. *CCN3* gene knockout mice were established and investigated, resulting in abnormal skeletal and cardiac development, including joint abnormalities, cardiomyopathy, and premature tissue degeneration culminating in muscle atrophy and cataracts in adult mice [46]. CCN3 consists of four domains: the insulin-like growth factor-binding protein-like domain (IGFBP), the von Willebrand type C-like domain (VWC), the thrombospondin type 1-like domain (TSP1), and the carboxyl-terminal domain (CT). A co-immunoprecipitation experiment revealed that the four FAS 1 domains of periostin interacted with TSP1 and CT of CCN3 [73]. Periostin acted as an anchor of CCN3 for its localization in the extracellular matrix in the mouse periodontal ligament [73]. Similarly to tenascin, BMP-1, and CCN3, periostin is likely to act as an anchor for the FAS 1 domain-interacting proteins to the extracellular matrix through the EMI domain.

3.3 The CTD Possesses an Arg-Rich Region Responsible for Binding to Proteoglycans

The CTD of periostin has been demonstrated to interact with heparin [68]. Heparin is a highly acidic polysaccharide with a sulfate group, which is conjugated on transmembrane proteins and secretory proteins called heparan sulfate proteoglycans. The CTD contains basic amino acid residues in its terminal end (Arg-Arg-Arg-Leu-Arg in human) [68], which is a motif common in heparin-binding proteins as B_1-B_2-X-B_3, where B represents a basic residue. This basic amino acid sequence is highly conserved in periostin among species [26], indicating its importance. Thus, periostin is likely to interact with cell-surface heparan sulfate proteoglycans and to regulate cellular processes such as cell migration and growth factor signaling [62]. These interactions have not been extensively investigated, but are likely involved in connective tissue development and disease progression.

The CTD has a variation in the amino acid sequence as a result of alternative splicing [26, 28]. The alternatively spliced region in the CTD has not been a target of biological analyses, and thus its molecular function is not well understood. The periostin paralogue βig-h3 lacks an extended region equivalent to the CTD, suggesting that the CTD is of functional relevance and could modulate periostin function. The CTD was shown to inhibit the interaction of the FAS 1 domains with tenascin-C, and cleavage of the CTD was required for the interaction [43]. This cleavage mechanism would underlie the spatio-temporal regulation of the interaction between periostin and tenascin in the secretory pathway. Identification of the enzymes that catalyze this CTD cleavage would clarify the periostin-specific regulation mechanism for the assembly of extracellular matrix proteins.

3.4 Conclusion

In addition to the proteins described in this review, periostin has been reported to interact with the integrin superfamily. Although periostin is thought to interact with integrins, including αVβ3, αVβ5, and α6β4, which promotes cell proliferation, cell migration, epithelial to mesenchymal transformation, and modulation of the biomechanical properties of connective tissues [3, 22, 23, 40, 60, 64, 69, 80], evidence of the direct interaction between periostin and integrins has not been demonstrated. Periostin has also been found to interact with notch and laminin γ2 [57, 74], and to function as a regulator of these proteins; however, the interacting domain of periostin has not been identified. To understand the

relationship between the interacting proteins and periostin, it is necessary to map the interaction sites on the multi-domain structure of periostin.

The pathological roles of periostin have been focused in a number of published papers. In most cases, inhibition of periostin function has shown a beneficial effect on the prevention and treatment of disease, such as cardiac remodeling, fibrosis, and cancer progression [2, 6, 27, 29, 33, 39, 49, 50, 54–56, 59, 65, 69, 75, 79, 82, 83, 86]. Development of a small organic molecule drug targeting periostin could be useful for disease prevention and treatment. However, structural analyses have not identified a pocket for drug binding in periostin. In addition, drug discovery targeting the protein-protein interaction surface is a promising approach, however this is still difficult to perform. The interacting proteins of periostin described in this review may be potential drug discovery targets. For example, BMP-1 is an enzyme that has been targeted by small molecule inhibitors [78]. Inhibitors of LOX have been tested as a drug for cancer metastasis [12, 31, 63]. Enzymes such as BMP-1 and LOX are involved in the large protein complex based on periostin, and are attractive targets of drug discovery for periostin-related diseases.

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Part IV

Health and Disease in Organs

4 Periostin Reexpression in Heart Disease Contributes to Cardiac Interstitial Remodeling by Supporting the Cardiac Myofibroblast Phenotype

Ian M. C. Dixon, Natalie M. Landry, and Sunil G. Rattan

Abstract

Cardiac muscle (the myocardium) is a unique arrangement of atria and ventricles that are spatially and electrically separated by a fibrous border. The spirally-arranged myocytes in both left and right ventricles are tethered by the component molecules of the cardiac extracellular matrix (ECM), including fibrillar collagen types I and III. Loss of normal arrangement of the ECM with either too little (as is observed in acute myocardial infarction) or too much (cardiac fibrosis in chronic post-myocardial infarction) is the primary contributor to cardiac dysfunction and heart failure. Matricellular proteins exist as non-structural signaling moieties in the ECM, and in the context of cardiac hypertrophy and heart failure, secreted 90 kDa periostin protein has attracted intense scrutiny during the past decade. Secreted periostin is now recognized for its important role in ECM development and maturation, as well as cellular adhesion. The novel mechanisms of periostin function include its role as a mediator of cell-to-matrix signaling, cell survival, and epithelial-mesenchymal transition (EMT). A number of recent studies have examined the hypothesis that periostin is a major contributor to ECM remodeling in the heart, and a number of very recent studies underscore its important role. This review examines recent developments in the mechanisms of periostin function in the normal heart and vasculature, and discusses recent advances which underpin its putative role in the development of cardiovascular disease. Periostin expression is very low at baseline in healthy tissues, but is re-expressed in damaged heart and in vessel walls after injury, in activated cardiac myofibroblasts and vascular smooth muscle cells, respectively. For this reason, periostin may be exploited for investigation of mechanisms of cardiac fibrosis, and we speculate that data generated from studies utilizing this approach may shed light on the timing for application of periostin-specific therapies to quell cardiac fibrosis and associated cardiac dysfunction.

Keywords

Heart · Extracellular matrix · Cardiac interstitium · Periostin · Heart failure · Myocardial infarction

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4.1 Introduction

The heart cycles through systolic and diastolic phases ~2.6 billion times during the average human lifetime [1]. The ability of the diastolic left (LV) and right (RV) ventricles to relax and accept an appropriate volume of blood to preload the myocardium to optimal levels depends on both cardiac myocyte relaxation and the pliability and optimized structural arrangement of the components of the extracellular matrix (ECM or matrix) [2].

Heart disease kills more patients in North America than any other disease, including those dying from cancer [3]. Heart failure (HF) is the primary reason for hospitalization and mortality (800,000 deaths per year in the United States) in adults [4, 5]. HF is a debilitating disorder characterized by reduced myocardial tissue compliance and interstitial fibrosis [2]. Cardiac fibrosis is associated with the primary cause of heart failure having preserved ejection fraction (HFpEF) [6, 7] as confirmed by markers of fibrosis [8] as well as magnetic resonance mapping to assess fibrosis [9]. Furthermore, activated fibroblasts, or myofibroblasts, mediate wound healing and chronic cardiac fibrosis, and thus contribute to ventricular stiffening and dysfunctional ventricular relaxation [10, 11] (Fig. 4.1).

Cardiac fibrosis is not limited to association with lusitropic cardiac dysfunction (HFpEF), but also with heart failure with reduced ejection fraction (HFrEF). With respect to the latter, abnormal accumulation and net expansion of the ECM in the cardiac interstitium is commonly associated with post-myocardial infarction (MI) [2]. Indeed, the now decade-old discovery that fibrosis is a primary cause of heart failure has spurred investigation to discover the mechanisms supporting fibroblast activation and subsequent cardiac fibrosis [12, 13]. Among the myriad of putative mechanisms, the increasing awareness of the putative role of secreted periostin (ie. a non-structural ECM protein) and stimulus for fibroblast activation is gaining acceptance.

Periostin is a 90 kDa peptide which is secreted from resident cardiac fibroblasts and myofibroblasts located in valves, the fibrous barrier between the atria and ventricles, as well as in the ventricular myocardium itself. The primary structure of this peptide is that of four repeated fasciclin domains [14, 15]. Periostin synthesis and secretion in the heart is commonly associated with non-cardiomyocytes, and this peptide is classified as a matricellular protein, as defined in the landmark paper by Bornstein [16]. Thus, periostin is now characterized as a non-structural member of the cardiac ECM, and its expression is

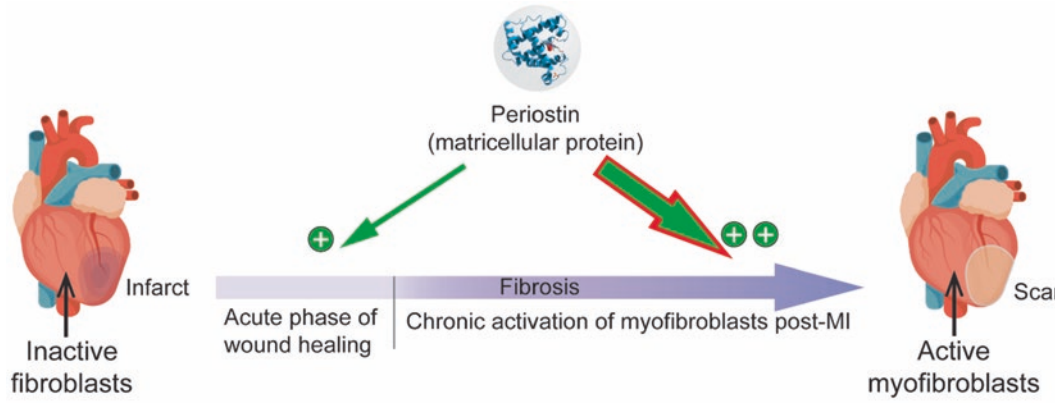

Fig. 4.1 Periostin is re-expressed by cardiac myofibroblasts during the onset of cardiac fibrosis. Periostin is normally expressed at very low levels in baseline conditions in heart. However, following myocardial infarction, fibroblasts are activated to hypersynthetic myofibroblasts, and these cells are responsible for elevated periostin synthesis and release. We suggest that periostin itself is also a stimulus for activation of fibroblasts to myofibroblasts in damaged hearts

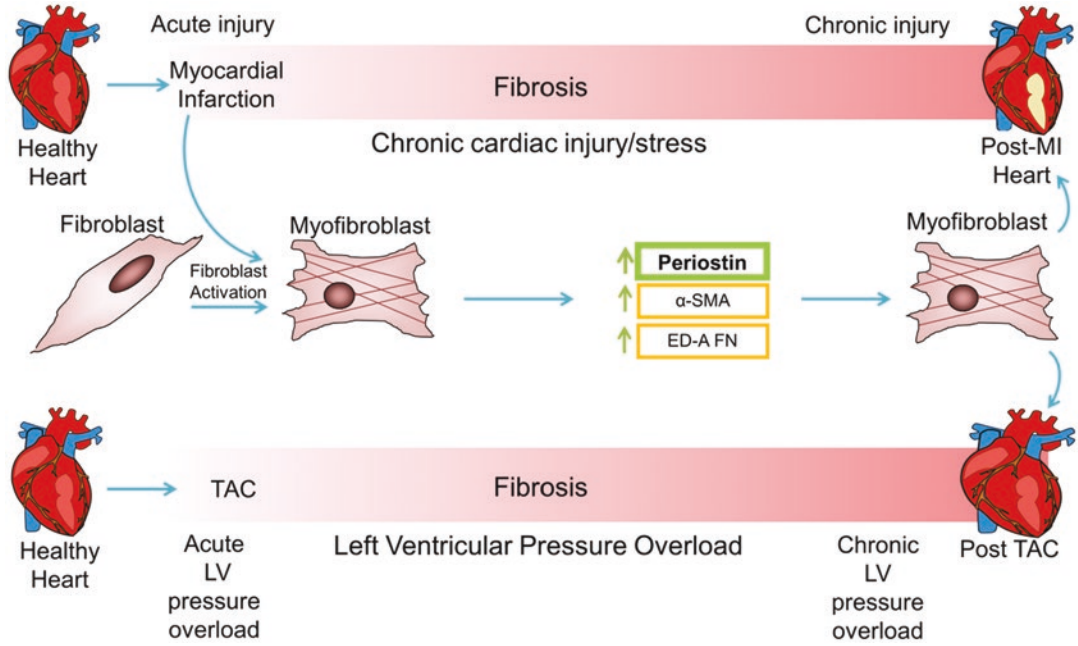

Fig. 4.2 Periostin expression in two common experimental models of cardiac hypertrophy and cardiac fibrosis. In acute cardiac injury, resident fibroblasts are activated to myofibroblasts. During the chronic phase of wound healing after myocardial infarction (MI), or in chronic pressure overload as is seen in the transverse aortic constriction (TAC) model of ventricular pressure overload, periostin synthesis and release to the ECM is chronically elevated. Myofibroblasts express elevated levels of markers such as α-SMA and ED-A splice variant of fibronectin, and are responsible for the redistribution of the cardiac ECM associated with chronic cardiac fibrosis

elevated following myocardial infarction [17, 18] (Fig. 4.2).

In recent years, investigation of the role(s) of periostin has intensified and has revealed it to be a reliable marker for activated cardiac myofibroblasts [19], and by extrapolation, cardiac fibrosis. More than a decade ago, periostin was discovered to be critical for the maturation and normal development of cardiac tissues [13, 20]. More recently, various research groups have linked periostin to fibrogenesis [21]. Whether periostin is important as an activator of cardiac fibroblasts in cardiac hypertrophy and overt cardiac failure is an open debate. This review will provide details of recent investigation of periostin with the purpose of adding consensus as to its specific role in the development of heart failure. Further, we address the recent and novel work to describe the role of periostin in the regeneration of cardiomyocytes.

4.2 Periostin as a Regulator of Cardiac ECM

Matricellular proteins are peptides normally residing in the interstitial microenvironment and are effective in regulating the activity and behavior of resident cells, including resident cardiac fibroblasts [20, 22, 23]. Periostin is known to be important for normal natal development of cardiac valves [20]. It is a unique matricellular protein in the ECM of the heart after the completion of heart development. In the adult heart, periostin expression is largely absent until after injury [20, 24] where it participates in the dynamic reorganization of the extracellular matrix. Following chronic or acute insult, the ECM responds more rapidly to a multitude of biomechanical and hormonal stimuli, over and above that of normal matrix turnover [25]. More than a decade ago,

matricellular periostin was proposed as a relatively specific marker for labelling resident cardiac fibroblasts. This method allowed for tracking non-myocytes during cardiac development so as to explore the role of the fibroblast in cardiac development [20]. In particular, while periostin knockout (*Postn*$^{-/-}$) mouse lines result in significant perinatal lethality (~14%) and higher risk of rupture of the ischemic ventricle following ischemic insult, those animals surviving ischemia to adulthood are resistant to chronic cardiac fibrosis and improved heart function if they survive [24].

4.2.1 Previously Unrecognized Interstitium in the Vascular Adventitia – A New Look at the Role for Periostin in Vascular Disease?

The novel revision of the anatomical concepts of the submucosa, vascular adventitia, dermis, and fascia by Benias and colleagues [26] suggest that these structures are fluid-filled interstitial spaces rather than densely packed barriers of collagen. While periostin is known to be involved in vascular repair following injury [27], there was little evidence to determine how vascular myofibroblasts (or other cells) might contribute to this process. The new findings of Benias et al. are intriguing as they point to the existence of a shock-absorbing interstitium (useful in organs that undergo episodic spatial deformation), and also supports the suggestion of intra- and/or inter-interstitial communication within these structures [26]. The suggestion of contiguous perivascular spaces in large arteries and veins, and particularly in the adventitial layer of these structures may impact on our understanding of normal cardiovascular function as well as progression of fibrosis in these structures. The data by Benias *et al*. indicates that the extracellular fluid in the vascular adventitia is in direct contact with the matrix of the novel vascular interstitium in the perivascular space of large arteries and veins [26]. While periostin appears to be an important matrix regulator in the myocardium, we suggest that periostin may also play a role in signaling within the collective vascular interstitium, by regulation of fibrillar collagen synthesis and degradation, as well as other ECM components within the fluid-filled interstitium.

4.2.2 Cardiac Regeneration and the Putative Role of Periostin

Cardiac regeneration is sought after as a putative solution to cardiac injury following MI. Recently, and not without a modicum of surprise to the investigators, it was demonstrated that ECM-producing cells in zebrafish heart are important participants in cardiac regeneration [28]. Cardiac regeneration in mammals also appears to utilize optimal distribution of cardiac ECM. With respect to this issue, neonatal mice may regenerate myocardium *de novo* at 1 day post-partum following resection of the left ventricular (LV) apex [29]. Notari *et al*. found that the ability of the neonatal mice to regenerate myocardium beyond 1 day from birth declined rapidly, so that after 48 hours, resection of the myocardium led to fibrosis rather than cardiac regeneration [29]. In a comparison of transcriptomes of 1- and 2-day-old hearts from these experimental animals, it was discovered that the protein components of the cytoskeleton and ECM were differentially regulated, in contrast to proteins normally found in myocytes. The authors concluded that alterations to the ECM microenvironment were a critical determinant of the facilitation of cardiac regeneration [29]. They also found that the efficacy of this phenomenon declines with time post-partum. This paper raised the possibility that a matricellular protein with the ability to influence the ECM microenvironment post-partum, may contribute to the regenerative capacity of neonatal myocardium. In addition to the conclusions of this paper, another recent study provides a connection of periostin's role in cardiac regeneration, using a model of chronic MI in transgenic periostin knockout mice [30]. Whereas the induction of MI in neonatal wildtype mice led to the complete regeneration of infarcted myocardium at 21 days post-MI with minimal fibrosis, the ablation of periostin in neonatal mice after induction of myocardial infarction led to abnor-

mal deposition and rearrangement of cardiac ECM, 3 weeks following MI [30]. While one may speculate as to the precise nature of the impact of periostin and the cardiac ECM in cardiac regeneration, consideration of both studies supports the broad hypothesis that the normal maturation of the cardiac ECM may be influenced by periostin, and in turn, influence cardiac regeneration. Although differences in experimental design and models in these studies exist, it is tempting to speculate that periostin may be implicated in neonatal cardiac regeneration via its influence on the cardiac ECM microenvironment. Further, a similar pattern of dependency upon optimized ECM distribution and cardiac regeneration among disparate species [28, 29] speaks to a generalized mechanism. It is notable that, in the context of cardiac regeneration, anti-fibrotic therapies may be less effective when compared to those aimed at targeting fibroblast inactivation [28].

4.2.3 Periostin as a Marker of Activated Myofibroblasts

The recent literature that is focused on cells responsible for cardiac fibrosis is marked with controversy due to the lack of a specific cardiac myofibroblast marker. The periostin allele identifies activated myofibroblasts in the heart, and is regarded as a necessary component of the post-MI cardiac wound healing response [19, 20]. Xiang and colleagues have recently employed a strategy using periostin to target activated myofibroblasts in the heart to drive the knockout of β-catenin in a mouse model, and showed that this intervention effectively reduces interstitial fibrosis in the heart while preserving cardiac function, with no effect on activated myofibroblast numbers *in vivo* [31]. Periostin is expressed at very low levels in healthy mesenchymal cells in heart, including valvular interstitial cells (VIC's) [32, 33], vascular smooth muscle cells (VSMCs) [34] and in cardiac fibroblasts [21]. However, in damaged heart, periostin is a prominent component of the activated myofibroblast secretome, and as such it may be useful for manipulation of myofibroblasts, as the ECM undergoes active rearrangement [19]. Furthermore, while activated myofibroblasts are not normally found in normal healthy hearts, but are very prominent in damaged hearts following MI, the concomitant re-expression of periostin in these cells is of considerable interest. While periostin is a novel marker in heart disease, it has been speculated that its rapid secretion and localization to the ECM adjacent to source myofibroblasts may limit its utility as a biomarker [35]. Nonetheless there can be little doubt that this matricellular protein is among the most specific products of activated myofibroblasts [19]. Likewise, periostin's re-expression in heart disease lends itself as a sentinel gene for the creation of inducible knockout transgene models where one may study the impact of myofibroblast-restricted expression of target proteins in the context of cardiac hypertrophy or myocardial infarction.

4.3 Heart Disease and Periostin – Future Research and Treatment Modalities for Heart Failure

The use of biologic therapies in the treatment of cardiac fibrosis and heart failure is promising, but is still in its infancy [36]. The discovery of periostin as a player in cardiac fibro-proliferative diseases provides rationale to specifically target cardiac fibrosis *per se*, as there is no current approved therapy to meet this need [37–41].

4.3.1 Myocardial Infarction – The Current Problem and Possible Research Directions

Prior to the development of overt heart failure following MI, acute wound healing to replace lost myocytes is required at the infarct site to avoid ventricular rupture and sudden cardiac death, and therefore ongoing chronic cardiac fibrosis is distinguished from acute infarct scar maturation [2, 42]. Chronic cardiac fibrosis is associated with impaired cardiac relaxation (lusitropic dysfunction) as well as cardiac contraction (inotropic dysfunction), and thus the timed, stra-

tegic treatment of post-MI cardiac fibrosis in the clinic must account for both acute ("good") and chronic ("bad") cardiac fibrosis. Current pharmacological therapies to treat heart failure with attendant cardiac fibrosis are inadequate. While the degradation of patients' cardiac performance may be delayed with standard therapies of β-adrenoceptor antagonism, effecting on ACE inhibition and angiotensin receptor blockade, which is the majority of this cohort, spiral to dysfunction and manifest heart failure [2]. The occupation of the infarct scar and surrounding myocardium with activated myofibroblasts is not directly addressed by common therapeutic approaches, and thus the expansion of the extracellular matrix remodeling continues unabated. The development of a periostin knockdown-based strategy to inhibit periostin release in the heart after the infarct scar is healed, which is warranted to "switch off" this signal for autocrine activation of fibroblasts, ensuing chronic wound healing, subsequent cardiac fibrosis, and thus to delay the onset of heart failure associated with cardiac fibrosis. In this respect, as periostin predominantly exists in the ECM as a secreted protein, specialized *in vitro* methodologies incorporating cardiac myofibroblasts residing in three-dimensional matrices that include fibronectin and integrins should be considered in the experimental design. The inclusion of integrins and cell-associated fibronectin will allow the binding of periostin both components in such a way that better emulates the characteristic bioavailability of periostin *in vitro* [43], when compared to traditional cell culture conditions.

4.3.2 Future Directions

The use of adeno-associated virus (AAV) to direct gene transfer in various tissues is now well-known and widely applied [44]. Recently, this technology was used to drive Cre recombinase expression in a myofibroblast cell lineage in mouse hearts after myocardial infarction [45]. This experiment is significant because it does not involve the creation of a transgenic animal, and furthermore may facilitate the development of new means to delivery genetic therapy to post-MI patients.

4.4 Synopsis

The developing field of periostin biology has recently yielded new discoveries which have advanced our understanding of how this matricellular protein contributes to heart disease. Activation of myofibroblasts is a key step in the pathogenesis of heart failure, and it appears that periostin may contribute to the persistence of these cells in heart failure, and specifically, to the persistence of myofibroblasts in the healed infarct scar. New modalities of treatment of cardiac fibrosis may be foreseen, as methods are developed to take advantage of the highly inducible gene, in the setting of damaged and failing hearts.

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Periostin in Bone Biology

5

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Abstract

Periostin is specifically expressed in periosteum that functions in bone modeling and remodeling and bone repair, and is sensitive to mechanical stress. Thus periostin has been expected for controlling these crucial systems in bone. The results from periostin deficient mice demonstrate that periostin acts on bone remodeling though detailed mechanisms are unknown. Recent findings have revealed that periostin is essential for bone repair. In this chapter, I introduce expression and function of periostin in bone.

Keywords

Bone biology · Bone repair · Mechanical stress · Periosteum · Fibrillogenesis · Collagen cross-linking · Lysyl oxidase · BMP-1 · Fibronectin assembly · ECM architecture

5.1 Expression of Periostin in Bone

In mouse bone at 5-week-old, strong expression was revealed in the periosteum, which is restricted to the ALP positive cells, but not endosteum nor bone matrix [14]. Moreover, no expression was observed in cartilaginous tissue. In mouse fetal femurs, strong expression of periostin is found in the fibrous layer of the periosteum and perichondrium, then at postnatal 1–2 weeks, this expression is restricted to the periosteum [13]. As mice grow, periostin expression gradually comes to be restricted to the osteoblastic layer. Consistently with mice, in human normal bone**,** there is strong expression of periostin in the cambium layer but not the fibrous layer of the periosteum nor on the surface of articular cartilage [16], whereas there is no expression for periostin in normal lamellar cortical and cancellous bone, osteocytes, and bone lining cells [5]. The periosteum is comprised of two layers; the outer fibrous layer is consisted of fibroblasts, collagen, and elastin along with a nerve and microvascular network, and the inner cambium layer is consisted of mesenchymal skeletal progenitor cells, osteoblasts, smaller fibroblasts, and sympathetic nerves, acting for bone formation and repair [1]. In the mouse calvarial bone, mRNAs of periostin together with Twist are localized at the osteogenic fronts of calvarial bones, and Twist can bind to the promoter of periostin gene [23].

Material in this chapter has been adapted from Kudo [19] with permission.

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Pathologically, in neoplastic and non-neoplastic bone forming lesions, periostin was found to be strongly expressed in osteoid/woven bone [5] and in the fibrous component of fibrous dysplasia [16]. In osteoarthritis patients, a high periostin expression was observed in chondrocytes and their periphery matrix near the erosive area in cartilage [7].

5.2 Abnormal Bone in Periostin Deficient Mice

From the expression pattern of periostin, we expected to find that periostin functions in mechanical stress-induced regeneration of periosteal bone. Periostin is specifically expressed in the periosteum, which is a complex structure composed of an outer fibrous layer lending structural integrity and an inner cambium layer possessing osteogenic potential [10]. In periostin deficient mice, cortical bone in the femur shows a decrease in area and thickness, suggesting that periostin functions in intramembranous bone formation [17]. Moreover, ectopically mineralized deposits are observed in periostin deficient mice following a thickening of the periosteum, which abnormal phenotype resembles periostitis. A similar phenotype was observed in tenascin-C-deficient mice, suggesting that periostin is associated with tenascin-C to elaborate an ECM meshwork architecture that includes fibronectin and type I collagen. This architecture generates a microenvironment for mechanical stress-dependent bone formation. Consistently, we observed reduced collagen cross-links for fibrillogenesis in the periosteum from periostin deficient femurs [17]. Defective cross-linkage is a common feature of periostin function reported in periostin deficient mouse skin [22] and heart tissues after a myocardial infarction [24]. In a further experiment, periostin deficiency significantly reduced the bone strength of fatigued tibia, resulting generation of cracks. This result indicates that periostin appears to influence bone properties such as damage accumulation and repair, including the local remodeling process [3]. Similarly in human, the study to measure circulating periostin during skeletal modeling, and assess the relationship with cortical measures in the young age group demonstrates that periostin is higher in young adults with ongoing cortical modeling, but lower in individuals who are nearer to peak bone mass where cortical consideration is complete and modeling has slowed [26].

5.3 Periostin in Collagen Cross-Linking

The molecular mechanism of periostin action in collagen cross-linking has been investigated, and the results showed that periostin enhances the proteolytic activation of lysyl oxidase (LOX), which is an enzyme responsible for cross-link formation; and it is caused by interacting with BMP-1 to promote the collagen cross-linking [20]. In this activation of LOX, we propose the involvement of a special ECM structure based on fibronectin to which precursor LOX and periostin can bind; then periostin stabilizes the precursor LOX to allow it to be effectively proteolyzed to generate the mature LOX. According to this scenario, periostin functions in high stiffness-collagen formation with effective collagen cross-linking. ECM proteins are synthesized in the endoplasmic reticulum (ER) to assemble into the extracellular structure. Periostin plays a role in fibronectin secretion from ER through the EMI domain of periostin that binds to fibronectin [18].

What is the molecular mechanism for generating this collagen structure? In tendon, bone and skin, type I collagen is the major component of these fibrils. In fibrillogenesis, collagen fibril formation in cell culture is dependent on the prior assembly of fibronectin into fibrils. Therefore, collagen fibrillogenesis that occurs on the cell surface is downstream of fibronectin assembly; and it is mediated by interactions with cell-surface integrins [6]. Tenascin-C forms a typical disulfide-linked hexamer, called the hexabrachion, in which six flexible arms emanate from a central globular particle and are associated with the dimer form of fibronectin to establish a meshwork structure (Fig. 5.1). This structure

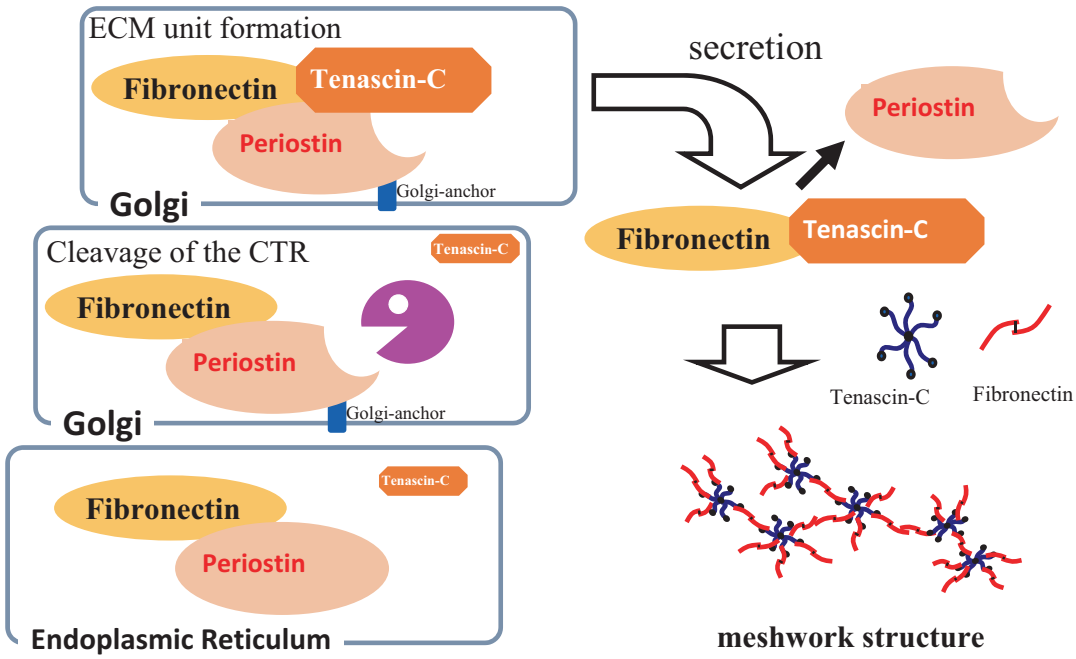

Fig. 5.1 Architecture of mesh work structure

underlies the extracellular meshwork architecture composed of type I collagen in ECM fibrils. Periostin and tenascin-C expressions are coordinately induced by mechanical stress, with transcription of these genes being regulated by cytoskeletal actin [8], indicating that periostin-mediated incorporation of tenascin-C into the ECM architecture maintains structural homeostasis when adapting to a changing mechanical environment.

5.4 Periostin in Mechanical Stress

Mechanical stress stimulates bone formation and suppresses bone resorption, resulting the increase of bone mass. From expression profiling of periostin in periosteum and periodontal ligament, periostin has been expected to be mechanical stress-sensitive, since the periosteum on the bone and the periodontal ligament in teeth are very sensitive to mechanical stress in order to aid in tissue regeneration and development [14]. Although no direct evidence of stress sensitivity has been reported, cortical bone microarchitecture and bending strength were altered in periostin deficient mice [2]. Interestingly, cathepsin K controls cortical bone formation by degrading periostin since osteocytes expressing cathepsin K play a central role in the regulation of the biomechanical response in bone tissues, and degradation of periostin by cathepsin K occurs in osteocytes [4]. Hence, cortical bone formation is regulated by the periostin-mediated blocking of random bone formation. In conclusion, with the response to mechanical stress, periostin expression is enhanced and activates cellular functions to improve the irregular collagen fibrillogenesis and ECM organization to maintain tissue homeostasis.

In traditional tissue-engineered cartilage, a biodegradable polymer scaffold can be used for shape retention; periostin contributes to this shape retention by enhancing the mechanical strength of the surrounding fibrous tissues consisted of periostin-mediated collagen structure [15]. Moreover, periostin enhances chondrogenesis in the 3D culture embedded within the collagen gel, indicating that conformational changes

in collagen induced by periostin can alter the cell-substratum adhesion and affect the signal transduction of chondrocytes [15], which opens a new possibility of periostin function in cartilage.

5.5 Periotin in Future

Periostin is now known as an essential marker of periodontal ligament; this knowledge has helped to develop researches on periodontal ligaments and has clinical applications for periodontal diseases. A recent study demonstrated that periostin maintains stemness of periodontal ligament mesenchymal stem cells and then promotes their osteogenic differentiation through the JNK pathway under inflammatory conditions [25].

Concurrently, bone regeneration relies on the activation of skeletal stem cells (SSCs). Periosteum contains SSCs with high bone regenerative potential, which is maintained with periostin, reconstituting a pool of periosteal cells after injury [9].

As for another aspect of LOX function, a new finding of LOX regulation for bone metastasis was reported. Interestingly, breast tumor cells secrete LOX that activates osteoclasts to enhance bone resorption for the formation of bone lesions [11]. Consistently, in a mouse model of human breast cancer bone metastases, an overexpression of periostin was shown in the stroma surrounding the bone metastases [21]. Periostin is possibly involved in this bone metastasis with activation of LOX on the bone surface. In this occasion, since periostin increases stiffness and tensile strength through collagen cross-linking, this increased stiffness of the ECM is sensed by the cancer cells which change to focus their activities towards invasion [12].

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Periostin in Bone Regeneration

6

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Abstract

Bone regeneration is an efficient regenerative process depending on the recruitment and activation of skeletal stem cells that allow cartilage and bone formation leading to fracture consolidation. Periosteum, the tissue located at the outer surface of bone is now recognized as an essential player in the bone repair process and contains skeletal stem cells with high regenerative potential. The matrix composition of the periosteum defines its roles in bone growth, in cortical bone modeling and remodeling in response to mechanical strain, and in bone repair. Periostin is a key extracellular matrix component of the periosteum involved in periosteum functions. In this chapter, we summarize the current knowledge on the bone regeneration process, the role of the periosteum and skeletal stem cells, and Periostin functions in this context. The matricellular protein Periostin has several roles through all stages of bone repair: in the early days of repair during the initial activation of stem cells within periosteum, in the active phase of cartilage and bone deposition in the facture callus, and in the final phase of bone bridging and reconstitution of the stem cell pool within periosteum.

Keywords

Periosteum · Periostin · Bone repair · Stem cell

6.1 Introduction

Following a fracture, bone regeneration is initiated. Bone tissue has a great capacity to regenerate, creating new bone which is indistinguishable from the uninjured bone, without leaving a scar. Although the steps of bone regeneration largely recapitulate those observed during bone development, bone regeneration is also regulated by other factors such as the inflammatory response and the mechanical environment that can influence the repair process [1–3]. The origins of skeletal stem cells and the factors regulating their functions in bone repair have been investigated using mouse models. While systemic recruitment of cells is minimal, bone marrow stromal cells/skeletal stem cells (BMSCs) have a local osteogenic potential and mostly have trophic and immunomodulatory roles within the bone marrow compartment. Periosteum is another local source of skeletal stem cells for bone repair [4–6].

The osteogenic potential of the periosteum has been highlighted since 1742 when Duhamel placed a periosteum on a silver ring around a bone and observed new bone formation coming from the periosteum [7]. One century later, Dupuytren suggested that periosteum and bone

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marrow played a role in cartilage formation in the fracture callus [8]. It is now well established that the periosteum plays many roles during bone development, growth, repair and aging, and its functions may be affected in some pathologies. The ECM composition of the periosteum is likely to influence its functions. The matricellular protein Periostin is expressed within periosteum and periodontal ligament and was first identified as Osteoblast-specific factor 2 (OSF-2) in a mouse osteoblastic calvarial cell line (MC3T3-E1) and in primary osteoblasts [9–11]. In bone, Periostin acts as a structural component of the matrix regulating collagen cross-linking and as a signaling molecule via interaction with integrin receptors [12]. Functional analyses of periosteal cells and Periostin using *Periostin* KO mice have now revealed several roles of Periostin in bone regeneration.

6.2 Bone Regeneration after Fracture

Bone regeneration generally occurs through a combination of endochondral and intramembranous ossification. The regeneration process can be summarized into four critical stages: the inflammation phase (hematoma formation and skeletal stem cell recruitment), the soft callus phase (neo-angiogenesis and cartilage/bone formation), the hard callus phase (cartilage resorption and/or active bone deposition) and the remodeling phase [13, 14]. Following a fracture, cells coming from the local bone marrow and the vasculature form the hematoma and the inflammatory process is initiated [15]. Pro-inflammatory mediators are secreted and initiate the repair cascade [16]. Neutrophils are first mobilized at the fracture site and secrete interleukins and chemokines like IL-6 (interleukin-6) and CCL2 (chemokine ligand 2) [15, 17]. Interleukins-1β (IL-1β) [18], IL-6 [19, 20], IL-11 [15], IL-17 [21], IL-18 [15], IL-23 [18] as well as Tumor Necrosis Factor-α (TNF-α) [22] promote angiogenesis and attract inflammatory cells like macrophages that remove necrotic tissues [16]. The inflammatory phase of healing is generally completed after 7 days following a fracture in mice [23]. In cases of bacterial infection, the inflammation phase persists and the healing process fails [24]. The inflammatory cells, macrophages and platelets secrete growth factors like Platelet-Derived Growth Factor (PDGF), Vascular Endothelial Growth Factor (VEGF) and Bone Morphogenetic Proteins (BMPs) to initiate the recruitment of skeletal stem/progenitor cells at the fracture site that are activated around day 3 [13, 16, 25]. During the soft callus phase, skeletal stem cells differentiate into chondrocytes or directly into osteoblasts depending on the mechanical tensions. In the center of the callus, where the mechanical tensions are high, bone regeneration occurs via endochondral ossification while at the periphery, bone regeneration mainly occurs through intramembranous ossification. During endochondral ossification, skeletal stem cells first differentiate into chondrocytes, proliferate and secrete a cartilaginous matrix rich in Type II Collagen and Aggrecan. These cells are then replaced by osteoblasts that produce a bone matrix rich in Type I Collagen. During the hard callus phase, the cartilage matrix is mineralized. Chondrocytes undergo hypertrophy and secrete VEGF to allow vascular invasion of hypertrophic cartilage. Due to osteoclast activity, cartilage is resorbed and replaced by woven bone. While it was generally recognized that hypertrophic chondrocytes die by apoptosis allowing vascular invasion and endochondral ossification, recent studies have shown that some hypertrophic chondrocytes can transdifferentiate into osteoblasts as observed at the level of the growth plate [26–30]. The cascade of events during this transition between mineralized cartilage and new bone is strictly regulated and any disruption of this transition leads to delayed or impaired healing [31, 32]. During the remodeling phase of repair, primary bone in the callus is degraded by osteoclasts and replaced by lamellar bone to restore the anatomical structure of bone that supports mechanical loads [23]. In mice, the replacement of cartilage by bone is complete by day 28 and the bone remodeling process carries on until after 2 months.

6.3 Role of Periosteum in Bone Repair

The periosteum from Greek *peri:* surrounding and *osteon*: bone, is a thin membrane of connective tissue on the external surface of most bones. The periosteum is attached to the bone cortex through Sharpey's fibers and serves as an attachment site for the muscles, tendons and ligaments [33]. The periosteum is composed of two layers, an outer fibrous layer rich in fibroblasts, Type I Collagen and Elastin fibers and an inner layer named the « cambium » layer in direct contact with the bone cortex [5, 34, 35](Fig. 6.1). The cambium layer is rich in osteoblasts, fibroblasts, osteoprogenitor cells and putative skeletal stem cells in a sparse collagenous matrix. The periosteum is highly vascularized and innervated [36]. The periosteal arteries supply blood to the adjacent bone and muscles [37]. The presence of sensory nerves endings that penetrate from the periosteum into bone canals makes the bone sensitive to pain [38].

Due to its external localization on bone, the periosteum is extremely sensitive to mechanical stimuli. When mechanical tensions or increased loading are applied, new bone formation deriving from the periosteum is observed, as well as periosteal hypertrophy, DNA synthesis, and cell proliferation [39–42]. During childhood and through adulthood, periosteal appositions allow bone to grow in width and cortical bone modeling/remodeling in concert with osteoclasts maintain bone structure in response to mechanical strain. In the context of fracture, it has been long known that removal/stripping of the periosteum impairs bone regeneration [43]. In models of diaphyseal fracture in rats, disruption of the periosteum impairs early chondrogenesis [43, 44]. Transplantation of Rosa26-LacZ segmental grafts into critical bone

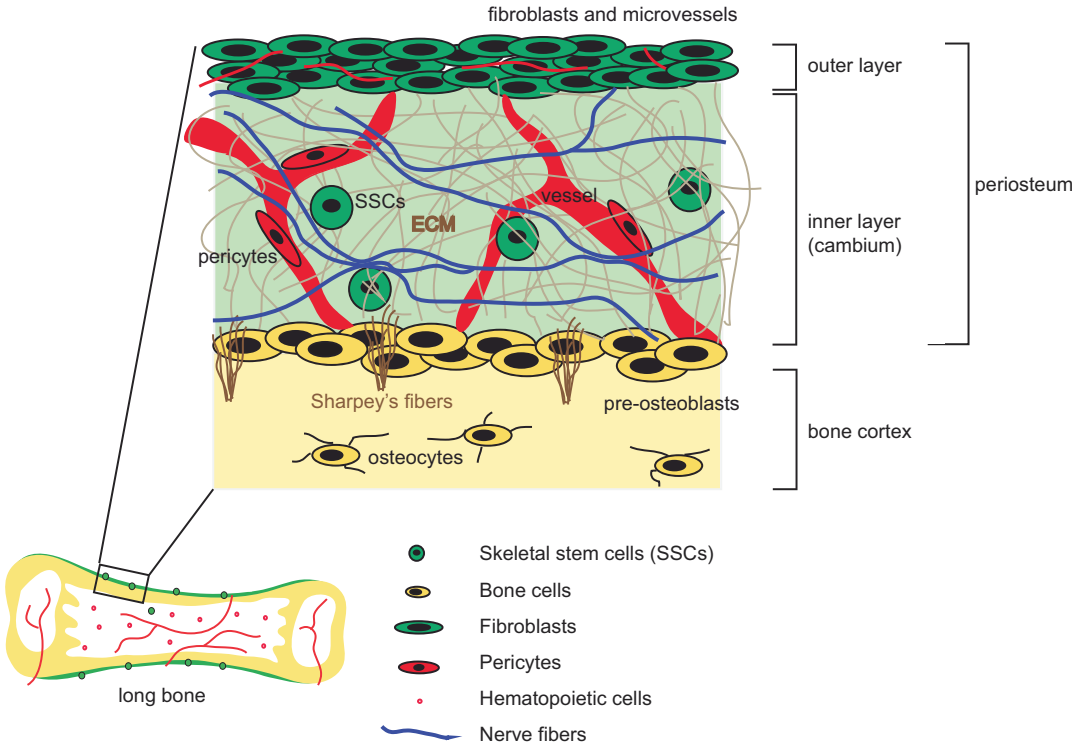

Fig. 6.1 Composition of the periosteum. The periosteum is composed of two layers, an outer fibrous layer containing fibroblasts and microvessels, and an inner (cambium) layer highly vascularized and innervated, containing skeletal stem cells and in contact with the bone surface covered by pre-osteoblasts. (Adapted from Refs. [5, 35])

defects or periosteum grafts into fractures showed that periosteum was a major contributor to cartilage and bone in the callus compared to bone marrow and endosteum [4, 45–47].

The periosteal response to the fracture can be observed histologically as early as 24 h following injury with a cellular proliferation leading to thickening of the periosteum near the fracture site. The size and the cellular content of the callus depend on the periosteal response to the mechanical environment. If the fracture is left unstabilized, the periosteal response to mechanical strain is very efficient, leading to bone regeneration via endochondral ossification. In the presence of mechanical stability or if the fracture is reduced, the periosteal response is less efficient leading to direct ossification [2, 32, 48]. These studies highlight the high regenerative potential of the periosteum. However, the absence of specific marker(s) to follow periosteum-derived cells (PCs) during bone regeneration makes it difficult to distinguish its endogenous potential compared to bone marrow. Therefore, many studies have aimed to isolate cells from the periosteum to study their in vitro and in vivo properties.

6.4 Periosteal Cells Express Periostin in Response to Bone Injury

Bone marrow stromal cells/skeletal stem cells (BMSCs) are the most described skeletal stem cells in the literature. In vitro, BMSCs can differentiate into osteoblasts, adipocytes and chondrocytes, and form colonies at low density. In vivo, the regenerative potential of BMSCs has been tested using cell transplantation in subcutaneous sites, where they form bone ossicles and support hematopoiesis [49]. However, these ectopic sites do not recapitulate the environment of a fracture (i.e. inflammation and mechanical stimuli). Self-renewal was shown using clonal expansion in vitro followed by ectopic subcutaneous transplantation. Many studies report transplantation of BMSCs in bone defects or at injury sites, but few include lineage tracing to follow their fate. BMSCs rapidly disappear after transplantation and have poor osteogenic potential. BMSCs rather stimulate repair via their ability to secrete growth factors and inflammatory factors [50, 51]. Several lineage studies using Cre reporter mice have aimed to define markers for BMSCs and follow these cells during bone repair [52–54]. However, there is no clear evidence regarding the specificity of these markers that may also label cells in periosteum and other tissues. Indeed, periosteal cells (PCs) express markers described initially for BMSCs such as *PDGFRα, Gremlin 1, Cxcl12* and *Nestin*. PCs and BMSCs exhibit similar differentiation characteristics in vitro, but PCs have increased clonogenicity and cell growth. After transplantation in fractures, PCs have a higher integration potential in cartilage and bone in the callus compared to BMSCs and persist within bone indicating the higher regenerative potential of PCs compared to BMSCs [55].

Microarray analyses of PCs and BMSCs have revealed an over expression of *Periostin* (*Postn*) and *Periostin*-linked genes in PCs in response to injury (Fig. 6.2). Some of these genes encode matricellular proteins (Postn and Thrombospondin 2) and other extracellular matrix (ECM) proteins (Col3a1, Lumican, Asporin, Decorin, Fibrillin 1 and Dermatopontin) (Fig. 6.2). Studies have also reported *Postn* up regulation in the fractured tibias along with other genes encoding ECM proteins such as SPARC, Biglycan and Decorin [55–58]. The expression of matricellular proteins is tightly regulated throughout life. Their constitutive expression in adult tissues is limited, but is elevated during development and in response to injury or stress [59]. Postn expression can be detected in the developing embryo by E9.5 in the first bronchial arch epithelium that is later the site for odontogenesis [60, 61]. Postn and Periostin-like-factor (PLF) are expressed at E12.5 in and around mesenchymal cells that condensate to form cartilage templates in the vertebrae, by E13.5 in cartilaginous vertebrae and ribs and by E16.5 in proliferating and hypertrophic chondrocytes in the limbs [62]. In the adult, Periostin is specifically expressed is collagen-rich tissues submitted to mechanical stress like the periodontal ligament and periosteum [11].

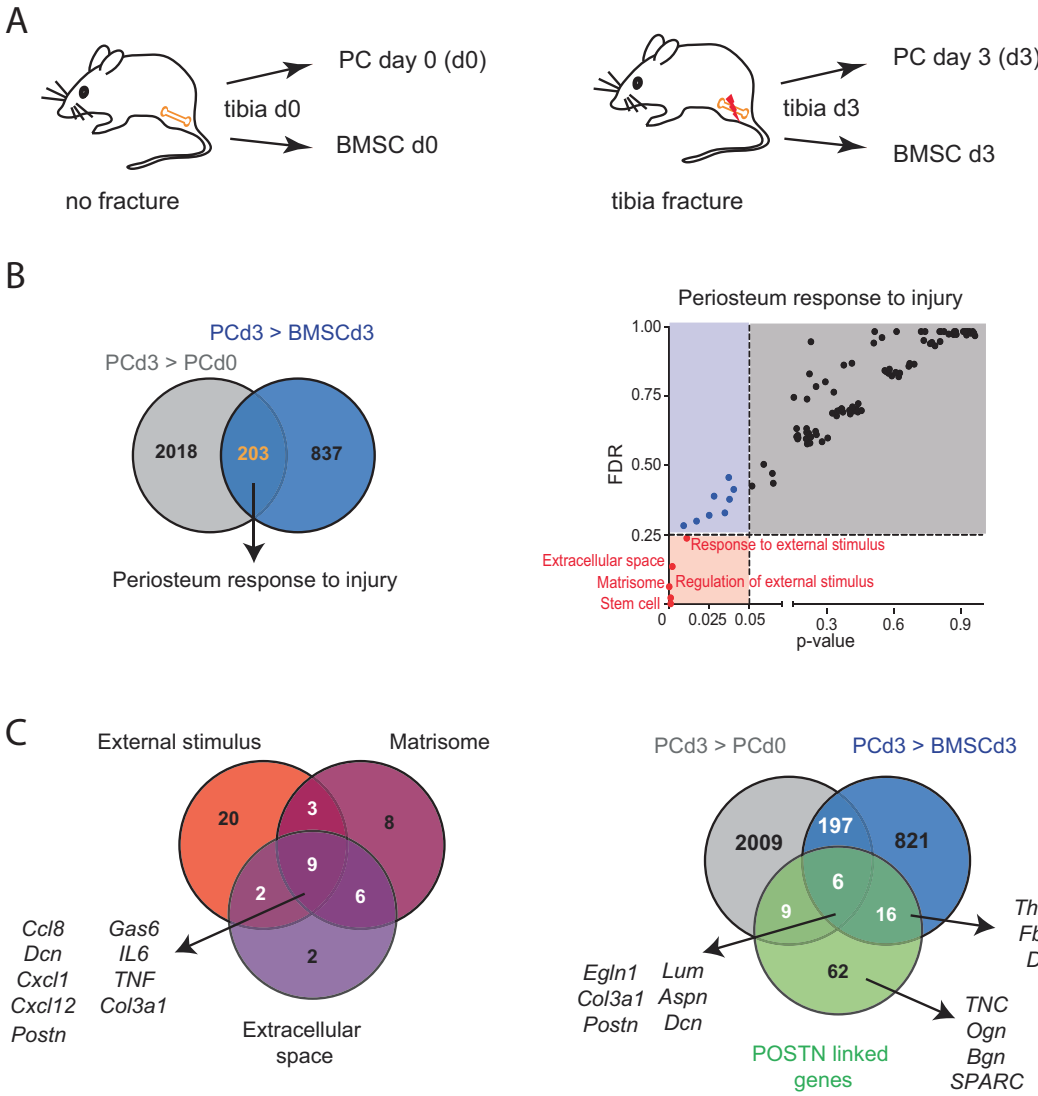

Fig. 6.2 Periostin is a marker defining the periosteum response to bone injury. (**a**) Schematic representation of primary cultures of periosteal cells (PCs) and bone marrow stromal cells (BMSCs) from un-injured (day 0, d0) and injured (day 3, d3) mouse tibia. (**b**) Comparative molecular response of PCs and BMSCs to bone injury (d0 versus d3) via microarray analysis: (left) Venn diagram showing 203 genes defining the periosteum response to injury (PRI); (right) Five significant functions are identified via GSEA analyses in the PRI "response to external stimulus", "extracellular space", regulation of external stimulus", "matrisome" and "stem cell". (**c**) (left) Periostin (Postn) and 8 other genes are found in common among the five significant functions. (right) Six genes including *Postn*, *Egln1* (*Endoglin1*), *Col3a1* (*Collagen3a1*), *Lum* (*Lumican*), *Aspn* (*Asporin*) and *Dcn* (*Decorin*) belonging to the list of *Postn* linked genes are also found in the PRI. (**d**) Periostin expression (left) in the intact periosteum and (right) in the activated periosteum at day 3 post-fracture. Scale bar: 0.5 mm. SO: SafraninO staining, c: cortex, m: muscle, bm: bone marrow, po: periosteum, cl: cambium layer, fl: fibrous layer, orange arrow indicates the fracture site (Adapted from Ref. [55])

6.5 Periostin Is Required for Bone Repair

Matricellular proteins have diverse functions such as promoting cell adhesion that may induce cell migration, they interact with growth factors that facilitate cell-matrix interactions, and bridge inorganic matter and proteins of the ECM [63]. Matricellular proteins are crucial regulators of cell phenotype, and consequently tissue function. While mice knockout models of structural ECM proteins generally induce severe phenotypes that can be lethal during development, gene deletion of matricellular proteins induces mild phenotypes that are exacerbated upon injury [64]. Like other matricellular proteins, *Postn* gene deletion in mice does not lead to embryonic lethality [65]. *Postn* KO mice show no apparent phenotype at birth, but exhibit growth retardation and weight loss within 1 month. The *Postn* KO bone phenotype is marked by the reduction of the size of the skull, ribs and cartilaginous growth plates and less trabecular bone in the limbs suggesting a role of Postn in bone development and homeostasis [65]. The absence of Postn also leads to loss of alveolar bone in the tooth probably due to increased osteoclasts activity in the periodontium. Thus the phenotype of *Postn* KO mice might at least, in part, be due to feeding disabilities caused by lesions in the in periodontal ligament, and under soft diet, growth retardation was attenuated [65].

Mice lacking *Postn* have impaired bone healing that is characterized by decreased callus and bone volumes throughout the stages of repair, as well as decreased cartilage formation and delayed cartilage removal (Fig. 6.3) [55]. During the remodelling phase of repair, *Postn* KO mice show absence of consolidation with reduced bone bridging in the callus (Fig. 6.3). This is correlated with defective periosteum shown by decreased contribution to bone repair of GFP labelled *Postn* KO periosteum grafts transplanted at the fracture site of wild type hosts compared to wild type grafts (Fig. 6.4) [55]. The absence of Postn that is normally highly expressed in the cambial layer of the activated periosteum compared to un-injured periosteum thus impairs periosteal activation and callus formation (Figs. 6.2 and 6.4). Downstream pathways regulated by Postn and mediating skeletal stem cell (SSC) activation still remain to be analysed. Cells maintain their normal functions in tissues by adhering to ECM proteins through integrin receptors. Postn may regulate SSC function in periosteum via its ability to bind to integrin receptors at the cell surface that has been previously described to enhance cell proliferation, survival and migration [66–69].

During the soft and hard callus phase of bone repair, Postn is also expressed in hypertrophic cartilage at the junction between cartilage and bone within the callus and in osteoblasts within new bone trabeculae [55]. Postn inactivation may thus impact bone formation directly during bone repair as Postn has been shown to regulate osteoblast proliferation, adhesion and differentiation [11, 60]. Although we did not detect expression of Postn in TRAP-positive osteoclasts in the fracture callus (data not shown), Postn deposition in ECM may also indirectly influence osteoclast function [66]. A conditional knockout approach may therefore be useful to determine the role of Postn in various cell populations during bone repair. At later stages of repair, fibrosis is observed in the callus of *Postn* KO mice (Fig. 6.3) [55]. Postn has been shown to promote fibrosis in allergic and respiratory diseases [70, 71]. The cellular origin of this fibrosis and the specific role of Postn in fibrosis deposition and remodelling in the fracture callus remain to be established.

6.6 Periostin Regulates the Skeletal Stem Cell Niche Within Periosteum

In addition to regulating periosteal cell activation in the inflammatory phase of repair, and periosteal cell differentiation during the soft callus phase of repair, Postn also regulates the niche of periosteal cells during the remodelling phase of repair. This was first suggested by the expression of Postn in the inner layer of the newly formed periosteum [55]. A niche for stem cells regulates their quiescence, self-renewal and differentiation potential in normal tissues and in response to injury. To

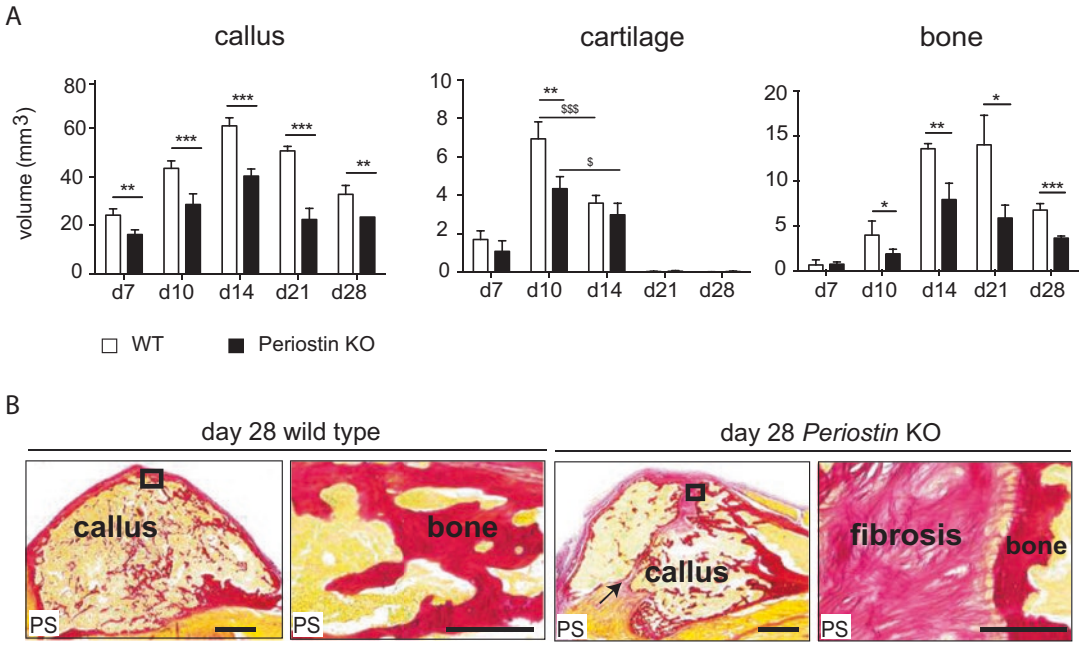

Fig. 6.3 Periostin is required for bone repair. (**a**) Histomorphometric analyses showing callus, cartilage and bone volumes at days 7 through 28 post-fracture in wild type and *Periostin* KO mice. (**b**) Representative sections of fracture calluses stained with Picrosirius (PS) red showing callus bridging in wild type callus at day 28 but decreased bone bridging in *Periostin* KO mice and fibrosis deposition. Scale bar: 1 mm. Statistical differences between the groups were determined using Mann-Whitney test (*p ≤ 0.05, **p < 0.001, ***p < 0.0005) (n = 3 to 5). All data represent mean ± SD. (Adapted from Ref. [55])

demonstrate the presence of stem cells with self-renewing potential within periosteum, GFP labelled periosteal grafts were transplanted at the fracture site and graft contribution to repair was evaluated over 3 consecutive cycles of injuries. The results show that PCs within periosteum could be activated to participate in callus formation, reintegrate their niche in the newly formed periosteum at the end of the repair process and be reactivated after several injuries to contribute again to bone repair (Fig. 6.4, left panels) [55]. To test whether the absence of Postn had an impact on the capacity of PCs to reintegrate their niche and reconstitute a pool of SSCs, GFP labelled periosteum grafts from Postn KO mice were transplanted in wild type hosts. Although the fracture callus was mostly wild type host-derived, bone regeneration was impaired around the *Postn* KO graft with no cartilage formation and local induction of fibrosis. Moreover, *Postn* KO PCs could not reintegrate their niche in the new periosteum at later days and did not contribute to cartilage and callus formation after a second injury (Fig. 6.4, right panels). PCs isolated from *Postn* KO mice show less clonogenicity as well as decreased osteogenic and adipogenic potential in vitro. Together these data indicate that Postn is required in the periosteal niche to reconstitute the pool of PCs at the end of the repair process. Interestingly, transplantation of Postn KO graft in *Postn* KO hosts caused an aggravation of the repair phenotype after a second injury, further supporting the role of Postn in re-establishing the stem cell pool in periosteum [55].

The nature and role of ECM proteins and cells that constitute the SSC niche within periosteum are unknown. The periosteum is composed of many cell types (fibroblasts, pre-osteoblasts, pericytes, skeletal stem cells and osteoprogenitor cells) entrapped in the ECM. Changes in other ECM molecules are found in *Postn* KO PCs, suggesting complementary roles of these ECM factors in regulating SSC activation and periosteum function during bone repair [55]. Further

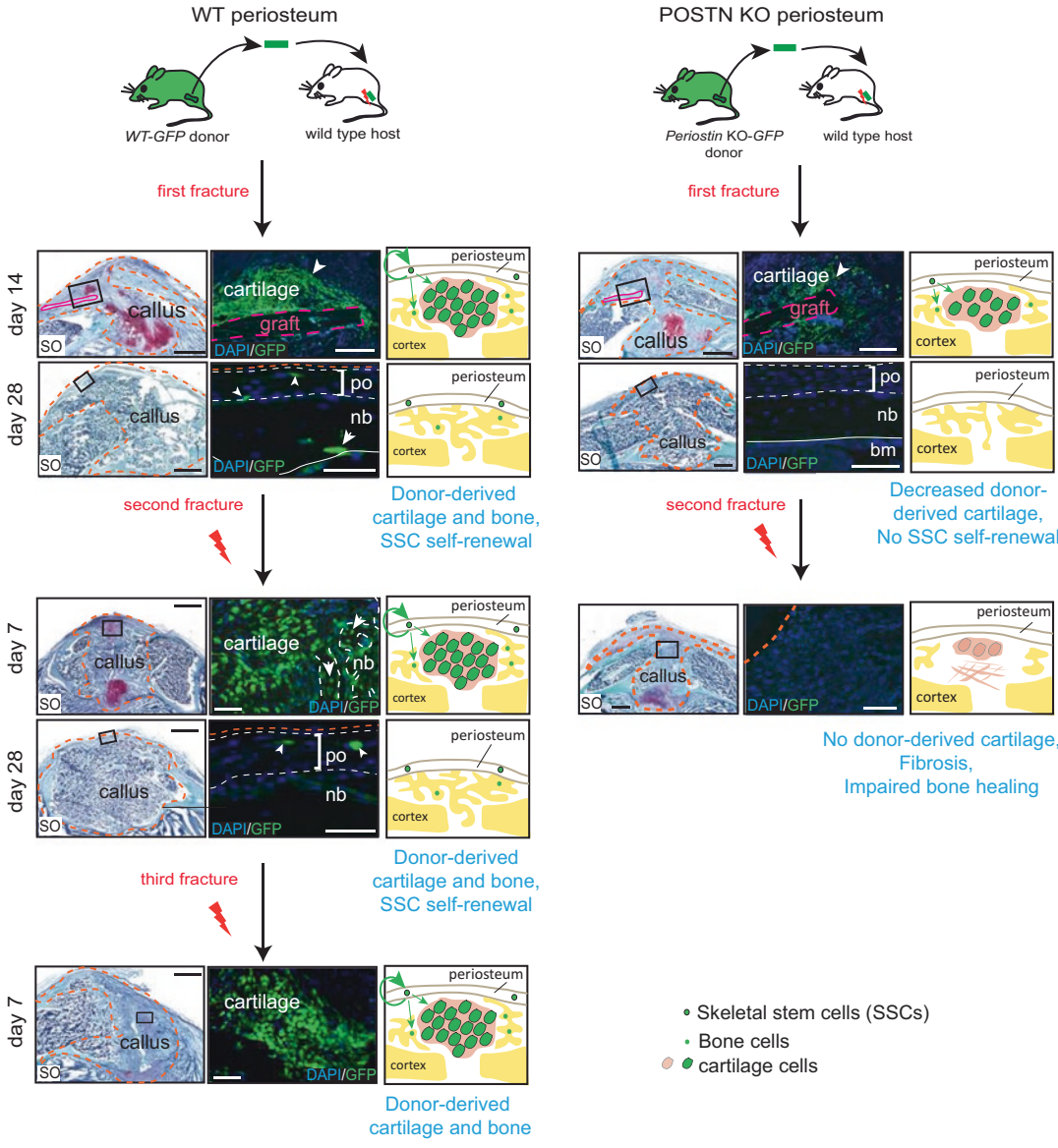

Fig. 6.4 Periostin regulates the skeletal stem cell niche within periosteum. (Top) Experimental design for the transplantation of periosteum grafts from wild type *GFP* donor or *Postn* KO-GFP donor mice at the fracture site of wild type hosts. (Left panels) Safranin-O (SO) and DAPI/GFP staining on longitudinal callus sections at day 14 showing contribution of wild type *GFP* grafts to cartilage and bone. At day 28 post-fracture, rare periosteum-derived GFP+ cells integrate in the new bone (nb) to form osteocytes (white arrow) and in the new periosteum (po, white arrowheads). After a second fracture, abundant periosteum-derived GFP+ cells are found in the callus and form cartilage (white arrowheads) and bone (white arrows) at day 7 and few GFP+ cells reintegrate the new periosteum at day 28 (white arrow heads). After a third fracture, periosteum-derived GFP+ cells can again form cartilage efficiently in the callus by day 7 indicating SSC self-renewal in periosteum. (Right panels) Few GFP+ *Postn* KO grafts-derived cells are detected in the cartilage at day 14 but no GFP+ *Postn* KO cells can be detected in the new periosteum at day 28. After a second injury, no GFP+ *Postn* KO chondrocytes contribute to the callus by day 7 indicating absence of SSC self-renewal. These *Postn* KO-GFP grafts induce fibrosis at the fracture site of wild type hosts. SO Scale bars = 1 mm. po: periosteum, nb: new bone, bm: bone marrow (Adapted from Ref. [55])

investigation on Postn and identification of other ECM molecules that regulate PCs activation in response to injury and long-term maintenance in the periosteum will be crucial to better understand periosteum and PC functions in bone homeostasis and repair (Fig. 6.5).

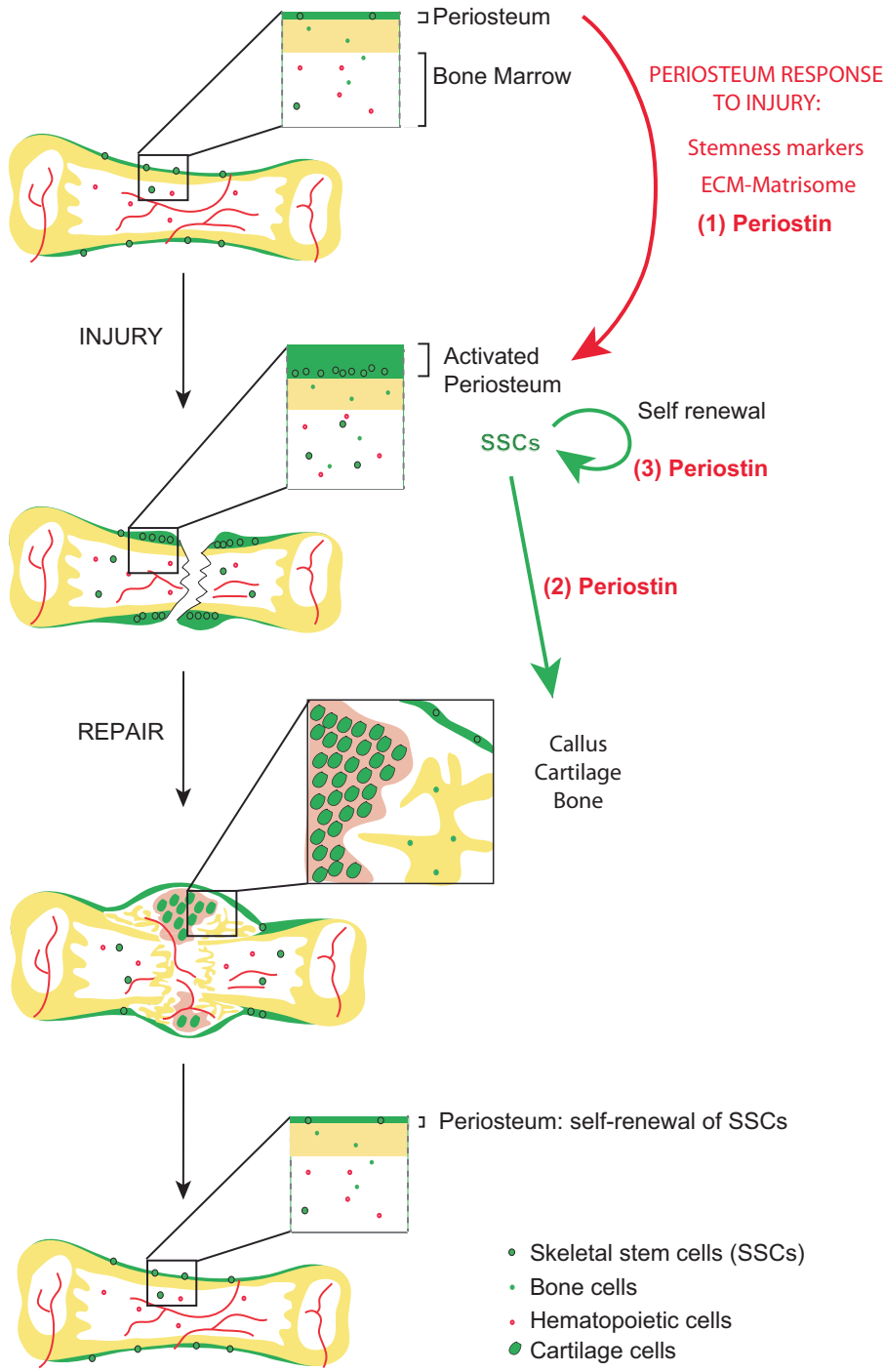

Fig. 6.5 Schematic representation of Periostin functions during bone repair [1]. Periostin is up-regulated in the activated periosteum in response to injury, [2] is required for callus, cartilage and bone formation, and [3] allows self-renewal of SSCs within the periosteum. (Adapted from Ref. [55])

6.7 Clinical Applications Using Periosteal Cells and Periostin in Bone Repair

The increased regenerative capacities of PCs compared to BMSCs make the periosteum a good target to stimulate repair or an attractive source of cells for therapy. For clinical applications in orthopaedics, several concerns are raised regarding the use of SSCs such as their site and method of isolation, ex vivo expansion, mechanism of activation as well as their osteogenic potential and long term integration after transplantation. For cell-based approaches aiming to treat complex fractures or critical size defects, a two-step surgery to isolate PCs would be necessary. The resection methods as well as culture conditions could influence stem cell number and regenerative potential. Moreover, types of bones from which periosteum could be isolated and inter-individual differences (age, sex) could also impact PCs potential [72, 73]. Cell-based approaches using PCs need to be explored or these cells could be reactivated in their native environment (periosteal niche) using pharmacological drugs that would act on signalling pathways to activate these cells or to modulate the ECM niche.

The enhanced regenerative potential of PCs marked by an augmented molecular response to injury and Periostin up regulation is essential for adequate bone repair, PCs self-renewal and the reconstitution of the periosteal niche [55]. Periostin could be used therapeutically to stimulate cell migration and contribution to repair in cell-based approaches. In a model of critical size defect in rabbit, Zhang and collaborators (2017) transplanted osteogenic-induced modified BMSCs and showed increased bone formation, bone mineral density and bone volume concomitant with increased Periostin/β-catenin protein expression. In vitro, Periostin increased ALP and alizarin red S production by BMSCs with increased levels of associated proteins (p-LRP-6, p-GSK3, β-catenin and Runx2) [74]. This suggests that Periostin may be delivered to directly activate Wnt/β-catenin signaling to stimulate bone formation in the callus [75, 76]. In another study, recombinant Periostin stimulated human adipose tissue-derived stromal cells (hASC) proliferation and migration, and augmented hASC-mediated repair in a critical size calvarial defect model [68]. The multiple roles of Periostin in regulating matrix composition and cellular functions during bone repair may be mediated by various isoforms, therefore the roles of these different isoforms need to be defined to target Periostin or select appropriate Periostin fragments for tissue engineering approaches in bone repair.

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7 Functions of Periostin in Dental Tissues and Its Role in Periodontal Tissue Regeneration

Juan Du and Minqi Li

Abstract

The goal of periodontal regeneration therapy is to reliably restore teeth's supporting periodontal tissue, while aiding the formation of new connective tissue attached to the periodontal ligament (PDL) fibers and new alveolar bone. Periostin is a matricellular protein, primarily expressed in the periosteum and PDL of adult mice. Its biological functions have been extensively studied in the fields of cardiovascular physiology and oncology. Despite being initially identified in bone and dental tissue, the function of Periostin in PDL and the pathophysiology associated with alveolar bone are scarcely studied. Recently, several studies have suggested that Periostin may be an important regulator of periodontal tissue formation. By promoting collagen fibrillogenesis and the migration of fibroblasts and osteoblasts, Periostin might play a key role in the regeneration of PDL and alveolar bone after periodontal surgery. In this chapter, the implications of Periostin in periodontal tissue biology and its potential use in periodontal tissue regeneration are reviewed.

Keywords

Periostin · Periodontium · Periodontal ligament · Alveolar bone · Periodontal regeneration

7.1 Periostin and the Periodontium

Periostin is a 90-kDa glutamate-containing matricellular protein. It was identified in the mouse osteoblastic cell line MC3T3-E1 and initially named osteoblast-specific factor 2 (OSF-2) [1]. Subsequently, OSF-2 was renamed as Periostin, based on its localization in the periosteum and periodontal ligament [2]. Periostin is preferentially expressed in collagen-rich fibrous connective tissue which undergoes constant mechanical strain, such as the periosteum, PDL, tendons, heart valves, and skin. As a multifunctional protein, Periostin is thought to be involved in tissue remodeling by promoting cell adhesion, cellular differentiation, cell survival, and fibrogenesis.

The periodontium is a specialized tissue that surrounds and supports the teeth, ensuring their

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stability in the maxillary and mandibular bones. The periodontium consists of four principal components: gingiva, PDL, cementum, and alveolar bone. By definition, periodontal tissue engineering/regeneration must achieve the regeneration of all tooth-supporting structures, supporting the reproduction of alveolar bone, cementum, and PDL, and ensuring adequate sealing by gingival tissue [3]. As Periostin is abundantly expressed in the periodontal ligament, it may become a potential stock in promoting periodontal tissue regeneration.

7.2 Expression of Periostin in Dental Tissues

The expression of Periostin in PDLs was initially demonstrated by immunohistochemistry of 5-week-old mice mandible in 1999 [2]. The spatiotemporal localization of Periostin was then revealed within developing and maturing dental tissues. Kruzynska-Frejtag et al. [4] found that both its mRNA and protein were asymmetrically expressed at the lingual/palatal and buccal side during early epithelial-to-mesenchymal interactions. Meanwhile, Suzuki et al. [5] found that, in tooth germs at the cap stage, Periostin immunoreactivity was recognizable at the interface between the inner enamel epithelium and preodontoblasts, as well as in mesenchymal tissues around the cervical loop and dental follicles. They also found that Periostin is present in dental papilla cells and within trans-differentiating odontoblasts during the bell and hard tissue formation stages of tooth development. Additionally, the expression level of Periostin in periodontal tissue was found to vary directly with the maturation stage of the periodontal ligament [6]. Therefore, it seems that the distribution of Periostin is shifting in space over time, during dental tissue development. However, further research is needed to determine the underlying mechanisms of this diversification.

In the same study, An et al. [6] observed that Periostin expression was restricted to the periodontium in adult rodents. Following postnatal day 7, immunoreactions of Periostin become uniformly localized to fibrous bundles in the PDLs in accordance with the organization of the periodontal fibers, indicating its role in the morphogenesis of PDLs. In the incisors of both 7- and 21-day-old mice, Periostin immunoreactions were discernible in the lingual PDL and labial fibrous tissue adjacent to the papillary layer [5]. During physiological tooth movement, Periostin mRNA expression was found to be uniformly distributed in the PDL surrounding the mesial and distal roots of molars in 7-week-old Sprague-Dawley rats [7]. Most importantly, Periostin was also found to be expressed on the alveolar bone surface by both methods of immunoreactivity and in-situ hybridization [5–7]. Furthermore, regarding its distribution in cellular substructures, immunoelectron microscopy showed immunolocalization of Periostin in the membrane of cytoplasmic extensions of periodontal fibroblasts, but not in their cytoplasm or the ground substance of mature PDL. Periostin is present at sites of close association between cells, including periodontal fibroblasts and cementoblasts, as well as in the adjacent collagen fibrils [5]. The Periostin protein is markedly present in the extracellular matrix (ECM), and possibly secreted by periodontal fibroblasts. In contrast, there is no detectable Periostin in the enamel, dentin, cementum, dental pulp, or alveolar bone [8]. This finding suggests that Periostin plays a key role in tooth development, and may be linked to the deposition and organization of other ECM adhesion molecules. Moreover, Periostin might modulate and maintain the integrity of adult teeth by mediating cell-to-matrix interactions, particularly at sites of hard-soft tissue interfaces (Fig. 7.1).

7.3 Functions of Periostin in Dental Tissues

The functions of Periostin have been classified into two major categories based on its molecular properties and protein interactions [9]. One is fibrillogenesis, which occurs inside the cell; the other is cell migration, which includes its extracellular activity [9, 10]. First, Periostin promotes

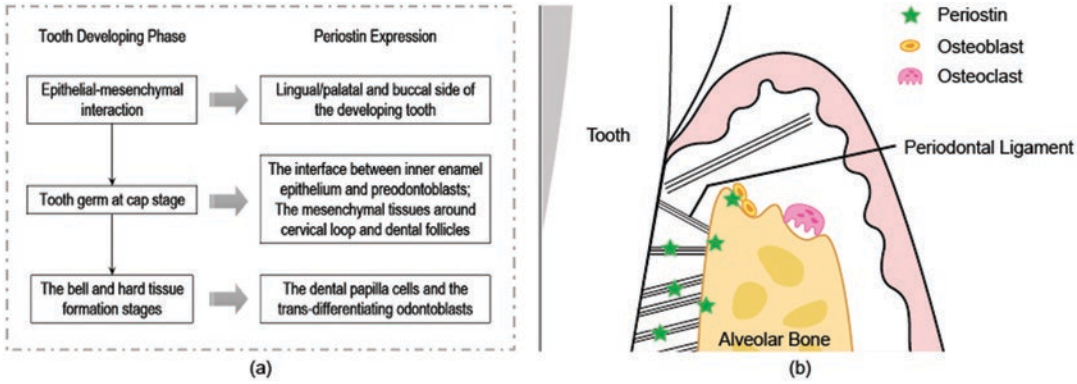

Fig. 7.1 The expressions of Periostin in dental tissues. (**a**) The expression of Periostin during dental development. (**b**) The expression of Periostin in periodontal tissues

the proteolytic activation of lysyl oxidase for collagen crosslinking. This process is mediated by the ability of Periostin to directly interact with type I collagen [11], fibronectin [12], and Notch1 [10] through its EMI domain, as well as with tenascin-C [12] and BMP-1 [13] through the Fas-1 domain. Periostin also serves as a ligand of integrins αvβ3 and αvβ5, facilitating cell motility by activating the actin/myosin contractile machinery [14]. Thus, it is reasonable to assume that Periostin could serve as an adhesive between these cells and collagen fibers, bearing mechanical stress in mature tissues and determining the strength and rigidity of these tissue.

7.3.1 Periostin Affects Dental Tissue Development as a Fetal Protein

Periostin has been regarded as a fetal protein involved in the morphogenesis and subsequent development of various tissues, including bone and periodontal tissues [4, 15, 16]. Periostin expression has demonstrated intense immunoreactivity in the cervical loop and dental follicles of molar tooth germs at cap and bell stages, which then disappears in the advance stages of tooth development [5]. In addition, Periostin immunoreactions have been found in the dental follicles of developing teeth, as well as in the developing sites of incisors, suggesting that this protein could be widely distributed in various fetal tissues in which the ECM undergoes active remodeling. Moreover, Periostin could inhibit precocious cell differentiation to maintain the proliferative potential of these cellular elements at the fetal stage. This change in the expression pattern of Periostin may be explained by the theory that Periostin is produced as a kind of fetal protein [5].

7.3.2 Functions of Periostin in the PDL

The PDL, which lies between the tooth and alveolar bone, is important for many functions such as proprioception, tooth support, and tissue remodeling in response to physiological or pathological conditions. The PDL consists of cells (*e.g.* fibroblasts, epithelial cells, mesenchymal cells, and bone and cementum cells) and extracellular compartments of dense fibrous connective tissue that confer resistance against mechanical stress. The extracellular compartments are formed of type I, III, and V collagen fibers, fibronectin, and tenascin-C embedded in the intercellular substance. It is important for bone and PDL to maintain the local histological architectures and the integrity of the ECM, and several ECM-associated proteins could play pivotal parts in regulating cell proliferation, adhesion, migration, differentiation, survival, *etc*. [17].

Periostin has been reported to be part of the ECM and contribute to the regulation of bone homeostasis, which is crucial in maintaining the integrity of PDL as well as that of the neighboring alveolar bone. Periostin null mice develop an early onset periodontal disease-like phenotype [18]. Widened PDLs and damaged alveolar bone coupled with enhanced osteoclast activity were discovered in Periostin null mouse incisors, leading to abnormal remodeling. Moreover, ameloblast functions are abnormal in Periostin null mice, leading to improper amorphous matrix secretion postnatally. These mice also present with compressed and disordered enamel and dentin of the incisors, and abnormal jaw bone, which ultimately result in enhanced tooth wear [18]. Furthermore, the periosteum of Periostin null mice exhibits alterations in collagen fibrillogenesis, fibril diameter and collagen crosslinking as observed in the skin, tendons, and heart [11, 19]. Similar findings from Kii et al. [20] using Periostin null mice showed abnormal incisor eruption. Their results also suggested that Periostin has crucial functions during the remodeling of the collagen matrix in the shear zone. Another experiment using Periostin mutant mice performed by Norris et al. [11] demonstrated the reduced diameters of collagen fibrils compared with those of wild-type mice. These results indicate aberrant collagen fibril maturation and assembly, as well as disorganized collagen crosslinking. Several crucial ECM proteins (type-I collagen, fibronectin, and tenascin C) in the PDL of Periostin null mice also showed altered distribution [21]. In the absence of Periostin, the collagen bundle organization was random, with abnormal localization of fibronectin and tenascin C. In addition, the expressions in the incisor dentin of several non-collagenous proteins, such as dentin sialophosphoprotein, dentin matrix acidic phosphoprotein-1, bone sialoprotein, and osteopontin, were also found to be altered in Periostin mutant mice compared to the wild-type mice [21]. Furthermore, soluble Periostin treatment increases the expression of osteogenic markers in bone marrow mesenchymal stem cells, including Runx2, collagen 1, osteocalcin, osterix, alkaline phosphatase and calcium nodules, and this effect was weakened when the soluble Periostin was neutralized [22]. Taken together, these findings suggest that Periostin might play a crucial role in the cross-linkage and distribution of collagenous and non-collagenous ECM proteins, implying that Periostin is critical in preserving the integrity of PDLs and plays a significant role in the postnatal development.

7.3.3 Periostin Regulates Alveolar Bone Cells

Bone tissue, including alveolar bone, is continuously remodeled through the concerted action of bone cells. This process consists of bone formation by osteoblasts and bone resorption by osteoclasts, while the osteocytes serve as a mechanosensor and an orchestrator during bone remodeling [23]. Periostin was found to be expressed on the alveolar bone surface *in vivo*, suggesting its role in the regulation of osteoblast functions. In Periostin-deficient mice, the crestal alveolar bone was decreased, and the PDL appeared to be enlarged. In addition, despite not belonging to the periodontal tissue, the basal bone of the mutant mice was also affected, causing a decrease in bone volume on tissue volume (BV/TV) and enhanced fibrous areas [24]. Consistent with these observations, other studies of osteoblasts in long bone show that Periostin deficiency results in defective attachment of osteoblasts to the bone matrix, which affects their differentiation into mature osteoblasts as shown by a severe reduction in the expression of type I collagen, osteocalcin, osteopontin, and alkaline phosphatase, and by a decrease in mineralization processes *in vitro* [25, 26]. Conversely, Cobo and colleagues demonstrated that the overexpression of Periostin inhibited the migratory capacity of MC3T3-E1 osteoblastic cells, but increased their adhesion capacity [27]. The results of Periostin overexpression affecting the RNA expression profiles of MC3T3-E1 cells confirmed that many genes associated with processes such as cell migration, adhesion, and bone metabolism have impaired expression, except for the genes involved in bone differentiation [27]. In addition,

Periostin overexpression in rats by injection of an adenovirus could increase the bone formation rate and bone mass [28]. Further studies are necessary to determine whether Periostin could directly regulate the osteoblastic cell functions.

Different from osteoblasts, studies on osteoclasts are scarce. There is currently no direct evidence of Periostin expression in osteoclasts of alveolar bone. However, Periostin null mice incisors showed a significant enhancement of osteoclast activity in the periodontium, with abnormal bone remodeling and a defect of alveolar bone [18], suggesting that Periostin affects osteoclast function. Recently, an *in vitro* study suggested that osteoclasts from mouse long bones could express a low level of Periostin during osteoclastogenesis, and the expression level is assumed to increase with the stage of differentiation [29]. In addition, Periostin-deficient mice have a higher number and activity of osteoclasts, with lower bone formation indices in alveolar bone (jaw) [24] and femurs in response to unloading [30].

Periostin is considered to be a marker of immature osteocytes because its mRNA expression is not observed in osteocytes [2, 7]. Consistently, Periostin mRNA has been shown to be expressed in preosteocyte-like cell line MLO-A5 cells, but not in osteocyte cell line MLO-Y4 cells [31]. However, increased basal sclerostin expression, abrogation of sclerostin down-regulation with loading, and reduced load-related bone formation were observed in Periostin knockout mice [32]. Sclerostin is an osteocyte-specific factor. By antagonizing the canonical Wnt pathway in osteocytes, sclerostin reduces bone formation [33]. Periostin knockout mice have shown abnormal skeletons and decreased alveolar bone volumes, which are the results of an increased expression of sclerostin. Moreover, the disordered alveolar bone phenotype of Periostin knockout mice could be normalized by crossbreeding with sclerostin knockout mice [34]. Similarly, Bonnet demonstrated that Periostin mutant mice show higher apoptosis levels of osteoblasts and osteocytes. Stimulated intermittent parathyroid hormone (PTH) can upregulate Periostin expression at the periosteal surface and in osteocytes, but reduces the expression of sclerostin [25]. In addition, the number of osteocytes in Periostin knockout mice decreased, while the number of empty osteocyte lacunae increased with the administration of high-dose zoledronate [24]. Although the above-mentioned evidence indicates that Periostin may play an anti-apoptotic role in osteocytes, it remains to be determined whether Periostin affects other functions of osteocytes.

7.3.4 Role of Periostin in Periodontium Responses to Mechanical Loading

Mechanical forces are prevalent in various biological processes, stretching the cytoskeleton within cells, assembling the original fibers that connect the cells, and regulating ligand-receptor binding on the cell surface. Cells from the bone, ECM architecture and PDL can activate mechanosensory signaling systems and adjust the cytoskeleton in order to respond to mechanical force stimulation. Accordingly, there are many proteins involved in this process. Considering its functions in osteoblasts, osteocytes, and the PDL matrix structure, Periostin is expected to be involved in periodontium remodeling in response to mechanical stress. It has been proved that Periostin withstands mechanical forces loaded onto the PDL, such as occlusal forces and/or tooth eruption. Consequently, Periostin null mice have severe periodontal defects after tooth eruption [18]. Alleviating the mechanical strain on the PDL by removing masticatory forces with a soft diet could partially rescue both the enamel and periodontal-disease-like phenotypes [35]. Similarly, a study of 45 Wistar rats showed that the PDL fiber system undergoes degradation and Periostin levels decrease in the absence of mechanical stress [36].

Orthodontic tooth movement is achieved by reiterated alveolar bone resorption on the pressure side and new bone formation on the tension side. Periostin is essential during orthodontic tooth movement processes, and deletion of this gene significantly alters collagen and bone remodeling in the periodontium. Divergent expression of

Periostin mRNA was observed, compared with control specimens, during experimental tooth movement from 3 to 96 h. It has been reported that the expression of Periostin protein on pressure sides is stronger than that on tension sides [7]. In studies involving Periostin silencing, the mutant mice showed a wider residually-compressed PDL compared to wild-type littermates, while several bone-remodeling-related factors were also affected [37–39]. Immunolocalization of cathepsin K, matrix metalloproteinase (MMP) 1, and MMP2 decreased greatly in the compressed PDL of Periostin null mice after orthodontic tooth movement at 1 and 3 days [37]. High mobility group box 1 (HMGB1), a late inflammatory cytokine, could be regulated by PDL cells during tooth movement. HMGB1 expression in Periostin knockout mice was found to have a high basal level, but on the compression side, a weak response level was shown compared with the wild-type mice, suggesting a correlation between HMGB1 and Periostin following the action of a mechanical force [38]. Furthermore, during tooth movement, sclerostin in alveolar bone displayed divergent expression, with an increase on the compression side and a decrease on the tension side. However, this phenomenon was not observed in Periostin knockout mice, which suggests an interaction between Periostin and sclerostin during tooth movement [39]. The mRNA expressions of Periostin and twist, which is an upstream molecule regulating Periostin in the PDL, could be inhibited by removing mechanical forces [8]. In addition, the delayed bone remodeling on the compression side during tooth movement might be attributed to the reduced osteoclast activity in Periostin null mice [34]. However, a subsequent study of the same group showed that the expressions of both RANKL (a potent stimulator of osteoclasts) and osteoprotegerin (a strong inhibitor of osteoclasts) decreased in Periostin null mice, which complicates the mechanism underlying the reduced osteoclast number in Periostin null mice [39]. Taken together, these results suggest that Periostin is essential for the periodontium in response to orthodontic tooth movement. However, further studies are required to fully elucidate the role of Periostin during this process (Fig. 7.2).

7.4 Periostin and Periodontal Tissue Regeneration

Inflammatory responses to bacteria can initiate the destructive process of periodontitis, leading to both a loss of connective tissue and alveolar bone around teeth and an apical shift of the junc-

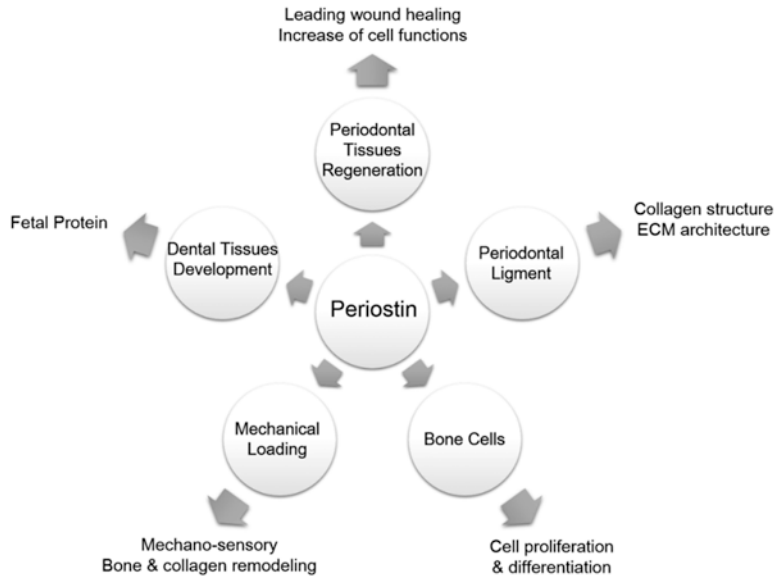

Fig. 7.2 The multiple functions of Periostin in dental tissues

tional epithelium. Untreated periodontitis results in loss of function, tissue destruction, loosening, and subsequent loss of teeth [40]. The preferential expression of Periostin in collagen-rich tissues submitted to mechanical strains, such as the PDL, and its increase during fracture healing, suggest that it might play a critical role in periodontium maintenance and regeneration. Periostin has successfully been used as a periodontal regeneration marker [41–43]. Padial-Molina et al. [44] designed a case-control study to determine the expression profile of Periostin that facilities wound stability and maturation. Periostin increased after periodontal surgery in gingival crevicular fluid (GCF)/wound fluid, which is higher in periodontitis patients. The decline of chronic inflammatory stimuli and bacterial challenge caused by the periodontal surgical procedure could potentially explain the Periostin increment. Moreover, the expression levels of Periostin in GCF/wound fluid moderated to baseline levels as the wound matured, possibly resulting from an increase in Periostin deposits in the ECM as the collagen structure matures [44].

As indicated above, periodontal tissue engineering/regeneration must achieve the regeneration of all tooth-supporting structures [3]. In this particular healing process, a temporal sequence and specific spatial distribution of multiple cells, scaffolds, matrix interactions, and signaling molecules must be followed [45]. The application of biological agents can regulate and promote the natural processes in the healing area, facilitating tissue regeneration [46]. Periostin is a matricellular protein, thus an extracellular protein. Consequently, it is assumed that Periostin plays a part in the cell–matrix interactions and cell functions, but is not directly involved in the formation of structural elements [47]. In light of these characteristics, Periostin is supposed to regulate cell migration, recruitment, adhesion, proliferation and attachment to healing areas of various tissues. By promoting the migration of fibroblasts and osteoblasts, Periostin might play an essential role in the remodeling of the PDL and its surrounding bone.

Periostin could regulate cell functions to favor tissue regeneration through several signaling pathways. For example, Periostin enhances the migration and proliferation of human PDL fibroblasts subjected to tumor necrosis factor-α and *Porphyromonas gingivalis* lipopolysaccharides through the PI3K/Akt/mTOR pathway [48]. Moreover, Periostin expression in human PDL fibroblasts promotes the migration of human mesenchymal stem cells through the αvβ3 integrin/FAK/PI3K/Akt pathway *in vitro* [49]. Additionally, Periostin regulates angiogenesis through the enhancement of vascular endothelial growth factor and MMP-2, which could occur through the activation of the αvβ3 integrin/extracellular-related kinase signaling pathway in human PDL cells [50].

At both mRNA and protein levels, Periostin expression is rapidly enhanced during fracture healing [51, 52], suggesting its role in bone regeneration at various phases. Despite its initial identification in the MC3T3-E1 osteoblastic cell line, the functions of Periostin have not been sufficiently studied in bone-remodeling cells (osteoblasts and osteoclasts). In MC3T3-E1 cells and primary rat osteoblasts, Periostin promotes proliferation and differentiation by increasing Runx2, alkaline phosphatase, and osteocalcin levels [26, 53]. These data indicate that Periostin could be expressed by immature osteoblasts and participate in the differentiation process, which favors bone regeneration. Recently, a study using a murine calvarial defect model showed that Periostin administration was able to promote the survival and bone-healing capacity of transplanted human adipose tissue-derived mesenchymal stem cells [54]. In addition, Periostin has been found to be expressed in ameloblasts, subodontoblasts, and odontoblasts. An enormous increase of dentin mass in Periostin null incisors and defects of enamel in these null molars support its direct role in the modulation of postnatal tooth formation [55].

7.5 Conclusion

Considering its spatial localization in both the PDL and alveolar bone of the periodontium, and its pivotal role in the regulation of the functions of these tissues, Periostin may become a promising agent to promote the regeneration of periodontal tissues in the future.

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Periostin and Human Teeth

8

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Abstract

Periostin is a secreted matricellular protein that primarily interacts with type I collagen and fibronectin extracellular matrix proteins, and is widely distributed in tissues rich in collagen-rich connective tissues, including the periodontal ligament. Its expression in these tissues is especially regulated by mechanical load. While the expression and regulation of periostin in the teeth of murine models and cell lines is well known, its presence in human teeth is poorly documented. Here we update and summarize the available data on the distribution of periostin in the human periodontal ligament, gingiva and dental pulp.

Keywords

Periostin · Periodontal ligament · Gingiva · Dental pulp · Subodontoblastic layer · Orthodontic movements · Human

8.1 Introduction

The periostin (POSTN) is a glutamate-containing secreted matricellular protein with an estimated molecular weight of 90 kDa highly homologous to fasciclin-1, an insect axonal guidance protein [11]. At least five human isoforms POSTN have been identified [10], and recently a new isoform has been characterized in the periodontal ligament [38]. POSTN directly interacts with extracellular matrix proteins and matricellular proteins, as well as membrane receptors, growth factors and cytokines [4, 13, 16, 21, 29]. POSTN is widely distributed in collagen-rich connective tissues where it is involved in tissue remodeling by promotion of cell adhesion, differentiation, and survival, as well as fibrogenesis [15]. Its expression in these tissues is especially regulated by TGFβ and mechanical load, and this also occurs in periodontal and dental tissues [9].

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This chapter updates the knowledge about the localization and roles of POSTN in the human periodontium (gingiva and periodontal ligament) and teeth.

8.2 Periostin in Human Periodontal Ligament and Gingiva

The occurrence and distribution of POSTN in the adult human periodontal ligament and gingiva was reported by Cobo et al. [6] and Menéndez-Díaz et al. [20] using immunohistochemistry.

The periodontal ligament displays a strong POSTN-immunoreactivity without differences between the different segments of the periodontal ligament or teeth (Fig. 8.1a–h). The pattern of the POSTN distribution is fibrillary and roughly matched with the arrangement of the fibroblasts (Fig. 8.1a, e; b, f). Double immunostaining for periostin and vimentin (used to label fibroblasts in the periodontal ligament) shows no co-localization in the periodontal ligament, such as vimentin being detected in the cytoplasm of the fibroblasts and POSTN in the pericellular space (Fig. 8.1c, d; g, h). These data in humans are in good agreement with previous reports in rodents and cell lines [11, 28, 33, 36]. At the ultrastructural level, POSTN is presented in the cytoplasmic extensions of periodontal fibroblasts membrane, but neither in the cytoplasm, thus suggesting that it is secreted from periodontal fibroblasts [31].

The role of POSTN in the human periodontal ligament is unknown but presumably matches some of functions reported in rodents. In the murine periodontal ligament mechanical load, a result from mastication activates latent TGF-β1, which leads to up-regulation of POSTN levels [28]. Furthermore, because POSTN interacts with extracellular matrix proteins, it may regulate collagen fibrillogenesis and the migration, proliferation and adhesion of fibroblasts [23]. On the other hand, POSTN promotes the migration and osteogenic differentiation of periodontal ligament mesenchymal stem cells in both normal and inflammatory conditions [32]. Interestingly, POSTN deficient mice show periodontal alterations [21], and the progression and intensity of periodontal diseases is paralleled with reduction of POSTN in gingival crevicular fluid [3]. Therefore, POSTN may be involved in the maintenance of the structure and participate in reparative processes in adult human periodontal ligament.

Regarding the gingiva, Cobo et al. [6] used western blot analysis and immunohistochemistry to investigate the presence and localization of POSTN in this part of the periodontium. They found a protein of 94 kDa, consistent with that expected for the human POSTN, which is the relatively higher levels corresponded to the non-bone attached periodontal ligament segment. The immunohistochemical detection of POSTN shows that it was detected just under the epithelium (presumably associated with some basement membrane components, since it co-localized with type I collagen) and was never localized within the cytoplasm of the basal epithelial cells; POSTN was also found around the gingival collagen fibers of the non-bone attached portion of the periodontal ligament (Figs. 8.1o, p). In agreement with the matricellular protein filiation of POSTN in the human gingiva it is localized extracellularly. Nevertheless, the presence of POSTN immunoreactivity in the keratinocytes of the healthy human skin [12, 22] or in the basal layer cells of the human corneal epithelium [24] has been reported.

POSTN plays key roles in the interactions of the connective tissue with the epithelial cells [8]. In the skin, POSTN controls collagen fibrillogenesis and cross-linking, and regulates the formation of extracellular matrix meshwork. Whether or not POSTN evolves identical roles in the gingiva remains still unknown.

8.3 Periostin in Human Dental Pulp

Menéndez-Díaz et al. [20] have recently demonstrated the occurrence of POSTN in human teeth using immunohistochemistry. POSTN immunoreactivity detected forming a meshwork below the odontoblasts layer in the so-called cell-free

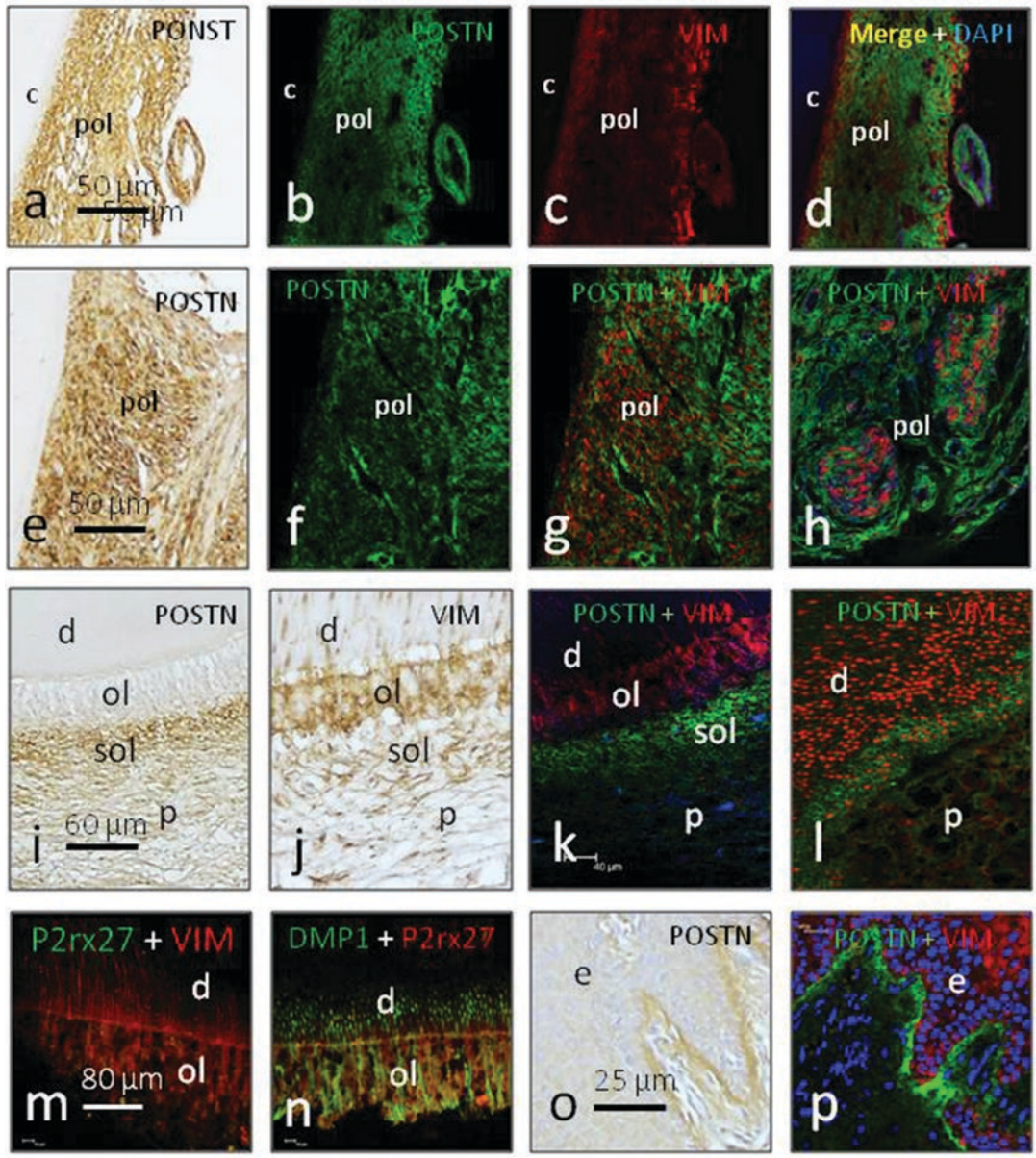

Fig. 8.1 Immunohistochemical and inmunofluorescence detection of periostin (POSTN) in the human periodontal ligament (**a–h**), dental pulp (**i–l**) and gingiva (**o–p**). Images in **m–n** show the localization of DIM-1 and P2x27 in the human dental pulp. *VIM* vimentin, *c* cement, *d* dentin, *e* epithelium, *ol* odontoblasts layer, *pol* periodontal ligament cement, *sol* subodontoblast layer

zone (see Avery and Chiego [2]; Fig. 8.1i–l). Conversely, POSTN immunoreactivity is observed neither in the odontoblasts nor in the dentine (Fig. 8.1k). This localization in humans matches that described in the murine teeth [19, 40], and is supported by the expression of POSTN in human pulp cell lines [35].

Recently it was observed that POST regulates the expression of DMP-1 and P2rx7 [7]. The study of the distribution of those proteins in the human teeth demonstrated that POSTN do not co-localize

with P2rx7 in human pulp, although P2rx7 can be detected occasionally in the odontoblasts layer and the initial segments of the odontoblast processes [20]. Conversely, POSTN co-localizes with DMP-1 in the periodontal ligament (Fig. 8.1m, n).

In animal models, POSTN is expressed during tooth development in the dental papilla and dental pulp cells, while it is restricted to the periodontal ligament and gingiva in the postnatal life [11, 14, 27, 31, 36]. Moreover, experimental data suggest that POSTN may play a role in dental pulp repair [14, 19].

The function of POSTN in the cell-free zone of the human dental pulp is completely unknown, however, since dental pulp is rich in type I collagen, POSTN presumably can be involved in fibril genesis and arrangement. Moreover, data obtained from POSTN deficient mice suggest that POSTN could participate in maintenance of mineralizing dental tissues during embryogenesis and in the reparative processes of the dentin in adult tissue [19, 27]. In this way, POSTN is present at minimal levels in dental pulp tissue, but POSTN overexpression induces down-regulation of odontoblasts markers *in vitro*, whereas silencing induced up-regulation of odontoblasts markers, thus suggesting that POSTN may be a negative regulator of odontoblast differentiation and/or mineralization (Zhou et al. 2015). Recent studies *in vitro* demonstrate that it regulates the dentin mineralization, bone biology, inflammation and dentine sensitivity [7, 32].

8.4 Mechanical Regulation of Periostin Expression in the Periodontal Ligament

TGFβ regulates the expression of POSTN in the periodontal ligament [11, 30, 34, 35], as well as mechanical forces [5, 28] or tooth movement [25, 36]. Strong mechanical stretch causes apoptosis and increased levels of POSTN protect osteoblastic cells against mechanical stretch-induced cell death [39]. Conversely, the absence of mechanical stimulation results in degradation of the periodontal fiber system concomitantly with a reduction in POSTN [1, 5]. When POSTN has been deleted, the mutant mice showed a wider residually compressed periodontal ligament [17, 18].

Orthodontic tooth movement occurs as a result of external tensile forces acting on a tooth, and in this process, POSTN may be essential for remodeling of periodontal ligament and bone [37]. During orthodontic treatments, cells from the bone and the periodontal ligament activate mechanosensory signaling systems to respond to mechanical force stimulation (see Rangiani et al. 2016). Thus, it is expected that POSTN is involved in periodontium remodeling in response to mechanical stress. In addition, in the POSTN null mice, bone remodeling is delayed in the compression side during tooth movement, which can be related to a reduction of osteoclast activity in absence of POSTN [26].

Taken together, it is apparent that POSTN present in the human periodontium is essential for the normal biology of the periodontal ligament and gingiva, but the roles of POSTN in the human dental pulp remain to be elucidated.

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9 Ability of Periostin as a New Biomarker of Idiopathic Pulmonary Fibrosis

Masaki Okamoto, Kenji Izuhara, Shoichiro Ohta, Junya Ono, and Tomoaki Hoshino

Abstract

The primarily pathogenesis of IPF, an incurable respiratory disease is believed to over-repair to lung injury. The development of new drugs for IPF has increased the necessity of identifying biomarkers for predicting clinical behavior and the selection of the appropriate treatment strategy for individual patient.

We and another group found that periostin, a matricellular protein expressed specifically in areas of ongoing fibrotic lesions, such as fibroblastic foci in lung tissues from human IPF or murine bleomycin-induced lung injury models. Murine bleomycin-induced lung injury was improved by the constant suppression of periostin expression and treatment with neutralizing anti-periostin antibodies at the fibroproliferative phase. Moreover, total periostin can predict both short-term declines of pulmonary function and overall survival in IPF patients. Our group also established a new enzyme-linked immunosorbent assay (ELISA) kit that is more specific for IPF compared with the conventional kit. This new periostin ELISA kit specifically detects monomeric form, whereas the conventional kit detects both monomeric and oligomeric forms. The monomeric periostin levels can be used to predict pulmonary function decline and to distinguish IPF patients from healthy controls.

In conclusion, periostin may play an important role in fibrogenesis and could be a potential biomarker for predicting disease progression and therapeutic effect in IPF patients.

Keywords

Monomeric periostin · Matricellular protein · Idiopathic pulmonary fibrosis · Fibroblast · Myofibroblast · Biomarker · Interleukin-13 · Interleukin-4 · TGF-β

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9.1 Idiopathic Pulmonary Fibrosis Is a Chronic Respiratory Disease with Unknown Etiology and a Grave Prognosis

Interstitial lung disease (ILD) is one of respiratory disorders with significant morbidity and mortality [1]. Patients with ILD present mainly with chronic progressive exertional dyspnea, a restrictive pulmonary dysfunction, and a radiological diffuse lung infiltration and/or fibrotic change. ILDs are classified into (1) idiopathic interstitial pneumonias (IIPs) with unknown etiology and (2) secondary ILD with known and heterogeneous etiology [2]. The most common underlying disease of secondary ILD is a connective tissue disease, such as rheumatoid arthritis, scleroderma, or polymyositis/dermatomyositis [3].

Idiopathic pulmonary fibrosis (IPF), a pathologically usual interstitial pneumonia (UIP), is the most common and incurable form of IIPs [4, 5]. Estimates of the IPF prevalence per 100,000 people were reported as ranging from 2 to 43 cases worldwide [4]. Patients with IPF have a grave prognosis with a median survival of 2–5 years [2, 4, 5] and the major causes of death being acute exacerbation (AE), gradual respiratory failure, and cardiovascular disease [6]. AE is a complication of IPF that shows rapid and lethal respiratory failure at a frequency of 8.5–14.2% per year during the clinical course of this disease [4, 7–9].

9.2 Understanding of Pathogenesis and Development of Molecular Biomarkers for IPF

Although the pathogenesis of IPF has not been fully elucidated, the primarily mechanism is believed to attribute to aberrant wound healing responses to repetitive lung injury that targets alveolar epithelial cells (AECs). The death and apoptosis of AECs trigger abundant fibroblast recruitment, proliferation, and activation, as well as extracellular matrix (ECM) protein secretion. Moreover, fibroblasts frequently differentiate into myofibroblasts that express α-smooth muscle actin (α-SMA) in IPF. Myofibroblasts have the highly activated contractile ability, and they secrete ECM proteins with characteristics that are intermediate between fibroblasts and smooth muscle cells [10–12]. Small aggregates of proliferating myofibroblasts and fibroblasts, termed fibroblastic foci (FF), play an important role in ongoing fibrogenesis and have been reported to be relevant for predicting the prognosis of IPF patients [13, 14].

The over-repairing environment in IPF is activated by innate and adaptive immune responses involving the activation of type 2 T helper (Th2) cytokines, M2-like macrophages, and/or growth factors. TGF-β1 is an important growth factor in the fibrotic process of IPF; it promotes myofibroblast differentiation as well as an anti-apoptotic phenotype in fibroblasts and myofibroblasts [10–12, 15]. An inappropriate shift in the Th1/Th2 cytokine balance, favoring the Th2 profile, can contribute to lung fibrosis in IPF [10]. A previous report revealed that the expressions of interleukin (IL)-4 and IL-13 receptors in the lungs were increased in IPF patients compared with patients having other IIP subtypes [16]. Lee et al. reported a model for lung fibrosis in which the overexpression of IL-13 in CC10-IL-13 transgenic mice caused lung fibrosis by selectively stimulating and activating TGF-β [17].

Recently, an evolving understanding of IPF pathogenesis has contributed to the development of two therapeutic drugs, pirfenidone and nintedanib, for which New Drug Applications had been approved by the U.S. Food and Drug Administration [18, 19]. Moreover, ongoing or recently completed clinical trials of new drug candidates targeting lung over-repair have been reported. Human monoclonal antibodies targeting connective tissue growth factor (CTGF), a matricellular protein (FG-3019), a selective autotaxin inhibitor that reduces plasma concentrations of lysophosphatidic acid (GLPG1690), and pentraxin 2 that inhibits M2-like macrophage differentiation (PRM-151) were all found to slow

the disease progression of IPF in phase 2 randomized, double-blind, placebo-controlled trials [20–22]. On the other hands, these paradigm shifts in the available therapeutic strategies for IPF treatment have raised the new problem of selecting an appropriate treatment type and intervention time for individual patient.

The natural course of IPF has been described as a progressive decline in pulmonary function until eventual death [4]. The short-term progression measured via the decline of pulmonary function from baseline values has been reported as a promising prognostic predictor. A decline of 10% for forced vital capacity (FVC) and that of 15% for diffusing capacity of the lung for carbon monoxide (D_{LCO}) over 6 months were associated with the survival of IPF patients in some previous reports [23, 24]. It should be noted that there is heterogeneity in the clinical behavior of individual IPF patients, including gradual or accelerated progression or AE development [2, 4, 25]. There is no established predictor that can accurately determine the clinical behavior or survival of IPF patients. Some epithelial or macrophage-related proteins, such as CC-chemokine ligand-18 (CCL-18), matrix metalloproteinase-7 (MMP-7), Krebs von den Lungen-6 (KL-6), surfactant protein D (SP-D), and SP-A, were reported as biomarkers for declining pulmonary function or those for survival [2, 26–31]. Molecular biomarkers may allow the application of "precision medicine" by aiding in the selection of a suitable treatment for individual IPF patients, such as an anti-fibrotic drug, lung transplantation, or palliative therapy [2, 25, 26, 32].

9.3 Periostin, a Matricellular Protein, Is Involved in the Pathogenesis of IPF

Periostin is an ECM protein belonging to the fasciclin family that acts as a matricellular protein modulating cell–matrix interactions via the $\alpha_v\beta_1$, $\alpha_v\beta_3$, or $\alpha_v\beta_5$ integrin receptor [33]. Periostin is secreted from fibroblasts, epithelial cells, and endothelial cells via stimulation by IL-4, IL-13, TGF-β, angiotensin II, CTGF, bone morphogenetic protein 2, mechanical stretch, and cancer-derived factors [33]. Periostin contributes to tissue development and wound healing by stabilizing collagen cross-linking and fibrotic disease progression [34]. Takayama et al. suggested that periostin secreted from lung fibroblasts is involved in subepithelial fibrosis via binding to other ECM proteins in a murine ovalbumin-induced allergic asthma model [35]. Periostin also contributes to the development of skin fibrosis in scleroderma [36]. Experiments in vitro showed that periostin can cooperate with TGF-β to promote the expression of messenger ribonucleic acid (mRNA) for α-SMA and procollagen type-I alpha 1 on dermal fibroblasts in a bleomycin (BLM)-induced murine scleroderma model. These mechanisms depend on the phosphatidylinositol 3-kinase/Akt (PI3K/Akt) pathway [37].

We and other groups attempted to clarify whether periostin is involved in the fibrotic mechanism in IPF [38–44]. We hypothesized that periostin was strongly expressed in fibroblasts, especially in FF areas, but not in regenerative alveolar epithelium, inflammatory cells, or areas showing established fibrosis with dense collagen depositions in IPF lungs (Fig. 9.1) [38]. In histochemical analyses of other subtypes of IIP, periostin was strongly expressed in fibrotic non-specific interstitial pneumonia (NSIP), whereas the periostin expression was weak in cellular NSIP and cryptogenic organizing pneumonia, as well as in normal lungs [38]. Uchida et al. also reported the high expression of periostin in lung fibrotic tissues, particularly in the α-SMA-positive myofibroblasts, from BLM-administered mice that are a representative murine model of IPF [39]. Naik et al. demonstrated that periostin localizes to FF areas as well as to subendothelial and subepithelial regions in lung tissue from IPF patients [40]. Some experiments in vitro revealed that lung fibroblasts and circulating fibrocytes are sources of periostin in the circulation of IPF patients [40, 41]. Together, these results suggest that the expression of periostin is localized specifically to areas of ongoing fibrotic lesions in IPF lungs.

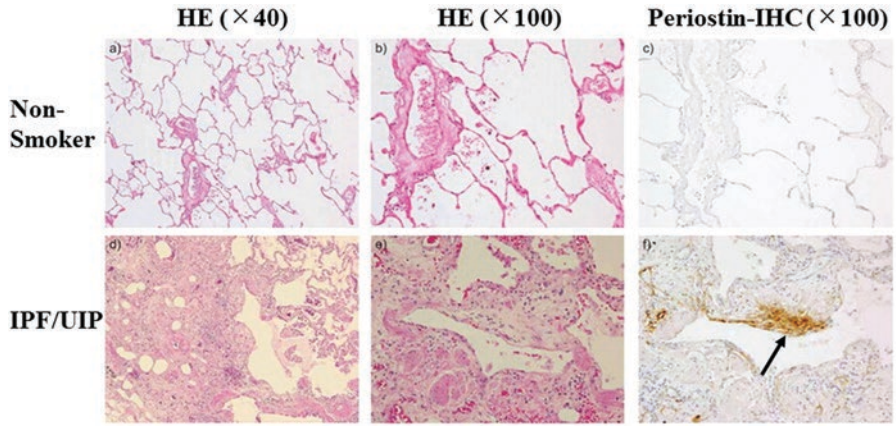

Fig. 9.1 Immunohistochemical (IHC) findings. (This figure was quoted from Ref. [38] and modified)
Expression of periostin in lungs of a 69-years-old female nonsmoker (**a**–**c**), a 64-years-old male with usual interstitial pneumonia (UIP) (**d**–**f**). The tissues were stained with haematoxylin and eosin (HE) 40×, HE 100× or periostin 100×, as shown

Uchida et al. used periostin-deficient mice in which periostin expression was constantly suppressed for clarifying the pathogenesis of IPF. Periostin-deficient mice administered with BLM exhibited less lung fibrosis and mortality compared with wild type mice that were treated similarly [39]. Naik et al. reported that treatment of wild type mice with neutralizing antibody against periostin (OC-20) on 10 and 15 days after BLM administration protected them from lung fibrosis and improved survival [40]. Therefore, periostin suppression at only the fibroproliferative phase of the disease was able to improve murine BLM-induced lung injury similarly to constant suppression.

Previous studies suggested that periostin induces type 1 collagen production from lung fibroblasts and/or circulating fibrocytes and promotes collagen deposition, mesenchymal cell proliferation, and wound closure in the lungs [40, 41]. Periostin and TGF-β upregulate the production of one another by fibroblasts and fibrocytes [41]. Ashley et al. demonstrated that periostin promotes CTGF production from fibrocyte and myofibroblast differentiation, leading to pulmonary fibrosis [41]. Periostin is thought to augment ECM protein deposition in IPF lungs via activating soluble factors, such as growth factors and other matricellular proteins, and mesenchymal cells. Nance et al. sequenced mRNA from the lung tissues of eight IPF patients and seven healthy controls, and their results suggested that the spliced-out exon 21 of periostin gene (POSTN) occurred more highly in IPF samples than in control samples [42].

Some drugs, particularly chemotherapeutic agents, often cause an acute and lethal subtype of ILD with the typical histopathological features of diffuse alveolar damage (DAD). We revealed that periostin was expressed not only in IPF lungs but also in human lung tissue with BLM- or gefinitib-induced DAD. Periostin staining was evident in the thickened alveolar walls adjacent to α-SMA-positive cells in the lung tissues with either drug-induced ILD [39]. During day 1–7 of BLM administration, mouse lungs showed acute lung injury-like features; histologically, the accumulation of inflammatory cells and upregulation of chemokines and pro-inflammatory cytokines were observed [45]. Uchida et al. found that the increase of chemokines, pro-inflammatory cytokines, and neutrophil and macrophage recruitment in the lung tissues or bronchoalveolar lavage fluids collected at day 7 of a murine BLM-induced lung injury model were impaired in periostin-deficient mice [39]. Therefore, periostin promotes the production of chemokines and pro-inflammatory cytokines, followed by the recruitment of neutrophils and macrophages, subsequently leading to lung injury. Periostin

may be important in rapidly progressive ILD with acute onset or AE of IPF showing a histological DAD pattern. We will investigate these issues in future analyses.

9.4 Periostin Is a Potential Prognostic Biomarker of IPF

We first reported that periostin is a potential prognostic biomarker for predicting the short-term progression of IPF patients in a single-center study. In that work, baseline serum levels of periostin were positively correlated with a decline of vital capacity (VC) and D_{LCO} over 6 months (VC: N = 26, r = −0.498, $p < 0.01$; D_{LCO}: N = 21, r = −0.467, $p < 0.05$) [38]. Moreover, we attempted to clarify whether periostin is useful for predicting long-term survival by analyzing 29 IPF subjects who were observed for at least 2 years and for up to 5 years [43]. Log-rank tests revealed that a higher serum periostin level was a predictor of a shortened overall survival (OS) and time-to-events (TTE) defined as a complicating AE or a relative decline in VC of ≥10% from the baseline (OS: relative risk (RR) = 3.6, 95% confidence interval (CI) = 1.3–9.9, $p < 0.01$; TTE: RR = 6.0, 95%CI = 1.8–21.1, $p < 0.01$) [43]. In that study, the baseline periostin levels significantly correlated with an increase in the extent of honeycombing visible on high resolution computed tomography images during 6 months [43]. Naik et al. demonstrated that the baseline periostin level was able to predict progression-free survival within 48 weeks for IPF patients, as determined by the time until any of the following: death, complicating AE, lung transplant, or relative decline in FVC of ≥10% or in D_{LCO} of ≥15% (N = 54, hazard ratio = 1.47, 95%CI = 1.03–2.10, $p < 0.05$) [40]. Thus, the serum level of periostin can predict short-time disease course and survival.

Unfortunately, periostin is not a specific biomarker for IPF because it is upregulated in various diseases other than IPF [36]. This fact may affect its accuracy as prognostic biomarker for IPF in patients who complicated other high-periostin diseases. Izuhara et al. and the Shino-Test established a new enzyme-linked immunosorbent assay (ELISA) kit that is more specific for IPF compared with the conventional kit [44]. The new periostin ELISA kit specifically detects the monomeric form (SS20A × SS19D, the capture and detection antibody), whereas the conventional kit detects both the monomeric and oligomeric forms (total periostin, SS18A × SS17B). We found that the index of total periostin/monomeric periostin was significantly lower in IPF patients (2.1, N = 40) than that in either patients with atopic dermatitis (14.2, N = 224), systemic sclerosis (11.7, N = 37), or bronchial asthma (7.3, N = 143), all of which are also high-periostin diseases [44]. These results suggest that a high ratio of monomeric periostin to total periostin is characteristic of IPF patients. Serum periostin mostly exists in the oligomeric form assembled by intramolecular disulfide bonds, with only small amounts existing in the monomeric form. The fact that monomeric periostin is predominantly upregulated compared with the level of total periostin may be explained by the aberrant redox status in IPF. We will investigate this issue in future work.

We conducted a multi-center analysis to examine the ability of monomeric periostin to serve as a prognostic biomarker of IPF. The changes in VC and D_{LCO} were inversely associated with both the monomeric periostin level (VC: r = −0.492, $p < 0.01$; D_{LCO}: r = −0.587, $p < 0.001$) and the total periostin level (VC: r = −0.428, $p < 0.01$; r = −0.460, $p < 0.01$) (Fig. 9.2) [44]. We also suggested that periostin could be a useful diagnostic biomarker of IPF. The receiver operating characteristic curve analyses for distinguishing IPF patients (n = 60) from healthy control donors (n = 137) revealed that monomeric periostin had the highest area under the curve (AUC, 0.958) among the investigated biomarkers (total periostin: 0.843, KL-6: 0.948, SP-D: 0.953, lactate dehydrogenase [LDH]: 0.898). When we set the cut-off values for monomeric periostin at 11.2 ng/mL and total periostin at 77 ng/mL, the sensitivities and specificities were respectively evaluated as 90.0% and 91.2% for monomeric periostin and at 73.3% and 79.6% for total periostin (Fig. 9.3). Therefore,

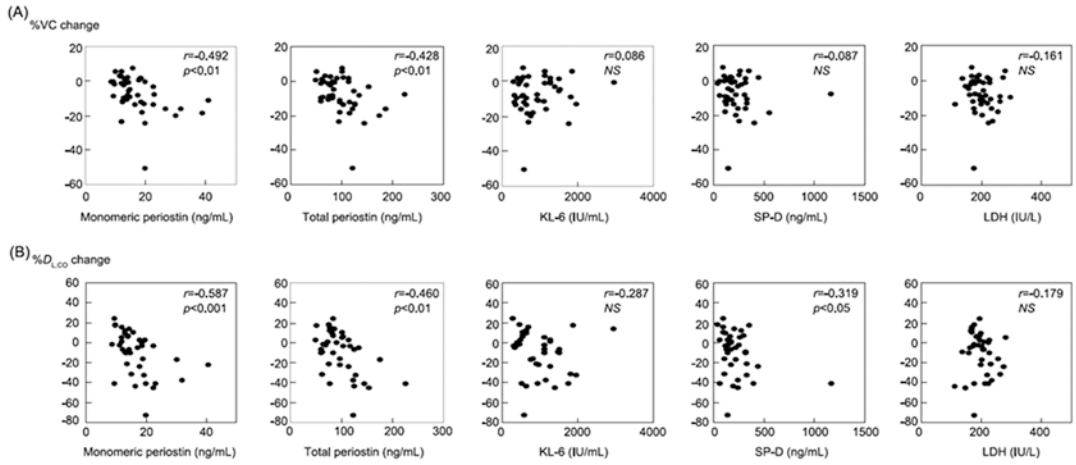

Fig. 9.2 Ability of each biomarker to predict the short-term progression of idiopathic pulmonary fibrosis. (This figure was quoted from Ref. [44])
Correlations between monomeric periostin, total periostin, Krebs von den Lungen-6 (KL-6), surfactant protein D (SP-D) or lactate dehydrogenase (LDH) and short-term change of vital capacity (VC, A) or diffusing capacity of the lung for carbon monoxide (D_{LCO}, B) in idiopathic pulmonary fibrosis patients (N = 44 for VC and 39 for D_{LCO})

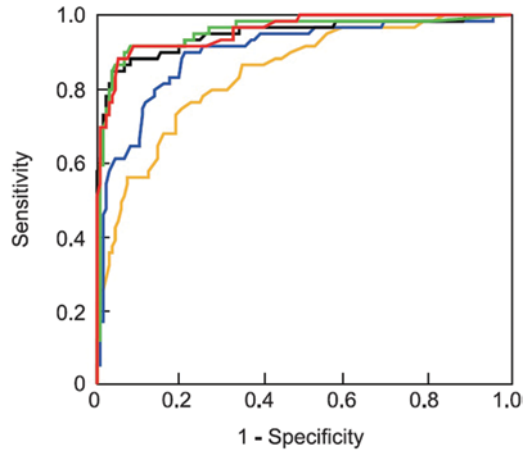

Fig. 9.3 Abilities of each biomarker to diagnose as idiopathic pulmonary fibrosis. (This figure was quoted from Ref. [44])
A receiver operating characteristic curve analysis of each biomarker between idiopathic pulmonary fibrosis (IPF) patients and healthy donors. Monomeric periostin (red), total periostin (orange), Krebs von den Lungen-6 (KL-6), (black), surfactant protein D (SP-D) (green), and lactate dehydrogenase (LDH) (blue) between IPF patients (n = 60) and healthy donors (n = 137)

monomeric periostin is a potential diagnostic and prognostic biomarker for IPF patients that is less influenced by complicatied high-periostin diseases.

Neighbors et al. performed post-hoc analyses of two replication IPF cohorts from the clinical trials for pirfenidone (CAPACITY and ASCEND). That was a comprehensive study evaluating the properties of biomarkers useful for predicting FVC changes as well as the therapeutic effect as assessed by a difference in the FVC decline between the pirfenidone and placebo groups over 12 months [46]. Total periostin measured using the Elecsys® periostin assay (Roche Diagnostics, Penzberg, Germany) was included among the analyzed biomarkers. The baseline serum levels of periostin and many other biomarkers were able to predict the specified parameters in the CAPACITY cohort but not in the ASCEND cohort [46]. These findings raise the problem that the utility of a prognostic biomarker may be affected by the characteristics of the analyzed IPF population. Their study indicated that evaluations with a combination of CCL18, C-X-C motif chemokine ligand 14, and total periostin were better at predicting the prognosis and therapeutic effect compared with any single biomarker [46]. The evaluation of a combination of biomarkers associated with different pathologies of IPF may be useful for prognostic prediction. Periostin is also expected to be applicable as a

biomarker for predicting the therapeutic effect and stratification of subjects in clinical trials on IPF patients.

9.5 Periostin as a Therapeutic Target

We and another group reported the improvement of murine BLM-induced lung injury via the constant suppression of periostin expression [39] or treatment with neutralizing anti-periostin antibodies at the fibroproliferative phase [40]. A previous report additionally suggested that osteopontin reduced murine BLM-induced lung injury via the α_V integrin [47]. Periostin or the α_V integrin may be a potential target in new treatments for IPF.

9.6 Conclusion

Periostin may play an important role in fibrogenesis and be applicable as a potential biomarker for predicting both the disease progression and therapeutic effect in IPF patients. The further study of periostin is expected to accelerate the development of new diagnostic and treatment strategies for IPF.

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Involvement of Periostin in Skin Function and the Pathogenesis of Skin Diseases

10

Yutaka Kuwatsuka and Hiroyuki Murota

Abstract

Skin is a large organ that is susceptible to damage by external forces, chronic inflammation, and autoimmune reactions. In general, tissue damage causes alterations in both the configuration and type of cells in lesional skin. This phenomenon, called tissue remodeling, is a universal biological response elicited by programmed cell death, inflammation, immune disorders, and tumorigenic, tumor proliferative, and cytoreductive activity. During this process, changes in the components that comprise the extracellular matrix are required to provide an environment that facilitates tissue remodeling. Among these extracellular matrix components, periostin (a glycoprotein secreted predominantly by dermal fibroblasts) has attracted much attention. In normal skin, periostin localizes mainly in the papillary dermis and basement membrane of the epidermis. However, it is expressed at higher levels in the dermis of lesional skin of those with atopic dermatitis, scars, systemic/limited scleroderma, melanoma, and cutaneous T cell lymphoma; expression is also increased by damage caused by allergic/autoimmune responses. Furthermore, periostin induces processes that result in development of dermal fibrosis; it also activates or protracts the immune response. The aim of this review is to summarize recent knowledge about the role of periostin in the pathogenesis of dermatoses.

Keywords

Periostin · Hypertrophic scar · Keloid · Scleroderma · Atopic dermatitis · Melanoma · Mycosis fungoides

10.1 Introduction

Skin is a large organ that covers the body surface and is exposed to the extraneous environment constantly [33]. Therefore, while forming a strong barrier that prevents invasion of foreign components, the skin also functions as a site for immune responses against various external and/or internal stimuli. The skin is divided into two compartments: the epidermis and the dermis. The dermis is roughly divided into an interstitial fibrous component and a cellular component made of the interstitial component. Mainly, the interstitial component comprises collagen fibers, although it also contains other elements such as

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extracellular matrix (ECM) components, elastic fibers, and substrates. In addition to physically supporting the cellular components, the ECM provides a foothold for attachment of cellular substrates [3].

Periostin, a component of the ECM, is an N-glycoprotein that was identified initially as osteoblast specific factor-2 [44]. Subsequent studies revealed that its expression is confined to the periosteum and periodontal ligament; therefore, it was renamed periostin [17]. From the embryonic through neonatal stages of development, periostin is expressed mainly in the dermis, the basement membrane of the epidermis, and in hair follicles; however, its expression is reduced in adults, where it is limited mainly to the basement membrane, papillary dermis, and hair follicles [47]. Periostin binds to other ECM components such as type 1 collagen, fibronectin, and tenascin-C, and is involved both in maintaining the structure of the dermis and in tissue fibrosis. Interestingly, periostin harbors the characteristics of a matricellular protein. Such matricellular proteins (MPs) are found in the non-structural components of the ECM and are important transmitters of information between the extracellular and intercellular components. Mainly, MPs are expressed during and after birth and are required for proper growth and development. Moreover, their expression is regulated in several post-natal conditions. Some MPs are expressed primarily during development and in response to injury and wound healing [24]. For example, integrins, a type of MP, are translocated to cell surfaces where they play a role in immune cell rolling and adhesion [24, 47]. Previous reports show that periostin harbors several functional domains, including a cysteine-rich EMI domain and four tandem-fascilin-like domains. With respect to the function of MPs, the EMI domain is important for association between proteins while the tandem-fascilin-like domain is important for binding to integrins αvβ3 and αvβ5 [24]. Moreover, mRNA splicing of the C-terminal domain of MPs yield several variants [24]. The type and number of these splice variants varies in each organ; however, the characteristic variant present in skin is unclear [24]. The role of periostin as a unique MP was first analyzed in studies of cardiovascular disease [6]. Subsequently, its role in diseases such as kidney fibrosis, asthma, and skin was examined. The role of periostin in skin has been recognized throughout of skin tissue repair. Mast cells play an important role in this process; histamine secreted by mast cells induces production of periostin by fibroblasts [31, 48, 55]. Interestingly, recent reports show that periostin also plays a role in atopic dermatitis, scleroderma, and skin carcinoma.

Here, we review the function of periostin in the skin and discuss recent studies of its involvement in the pathology of skin diseases.

10.2 Role of Periostin in Wound Healing and Scar Tissue Formation

Skin damage, which is often caused by trauma, inflammation, or tumor progression, initiates a series of physiological processes, including production of humoral factors, mediators, cytokines, and growth factors. In addition, cells involved in tissue repair (e.g., mastocytes and T cells) are recruited to damaged skin; these cells then induce production of various MPs [52]. The wound healing process is divided into several phases: inflammatory, proliferative, and remodeling/regenerative [47, 52]. The inflammatory phase begins after cessation of bleeding due to formation of blood clots. During the inflammatory phase, cells necessary for tissue repair are recruited into the site of damage where they proliferate and differentiate to generate "granulation tissue". This process is characterized by angiogenesis, tissue balls, and invasion of inflammatory cells [47, 52]. In particular, mast cells and Th2 cytokines contribute to wound healing during tissue remodeling from the inflammatory to the proliferative stages [51] (Fig. 10.1). Histamine secreted by mast cells induces production of periostin by activating extracellular signal-regulated kinase 1/2 via the H1 receptor, which is expressed by dermal fibroblasts [55]. Comprehensive DNA microarray analyses revealed that Th2 type cytokines (IL-4/

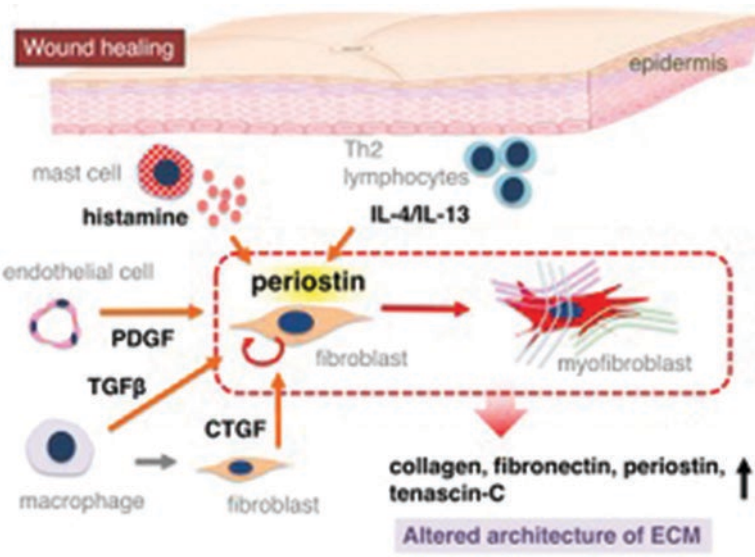

Fig. 10.1 Schematic illustrating the relationships between periostin and tissue remodeling. Fibroblasts and endothelial cells secrete periostin in response to a traumatic wound. Histamines (derived mainly from degranulated mast cells), Th2 cytokines, and fibrogenic cytokines (e.g., TGFβ, CTGF, and PDGF) promote production of periostin by fibroblasts. Periostin induces differentiation of dermal fibroblasts into myofibroblasts, which then remodel the extracellular matrix. In addition, periostin, together with other matricellular proteins, activates immune cells

IL-13) induce production of periostin by lung fibroblasts, resulting in binding of periostin to other matrix molecules such as tenascin-C, fibronectin, and collagen V [43]. Similarly, IL-4/IL-13 might contribute to production of periostin by dermal fibroblasts; however, because the expression of periostin is lower during the inflammatory phase than during other phases, it is thought that periostin is involved mainly in tissue repair from the proliferation stage onwards.

During the proliferative phase, an emergency matrix comprising fibrin and fibronectin forms a temporary scaffold, which is required for tissue remodeling [14]. At the end of the inflammatory phase, macrophages infiltrating the granulation tissue produce TGF-β, which promotes wound healing [4]. TGF-β activates dermal fibroblasts and induces their migration into granulation tissue where they transform into myofibroblasts; these cells are capable of producing ECM components [15], including type I and III collagen, which are major components of the dermal ECM and contribute to maintaining skin elasticity and strength. Activated fibroblasts and myofibroblasts also produce tenascin, fibronectin, various proteoglycans, and periostin. As remarked previously, periostin binds to other ECM components and plays a role in maintaining tissue structure and in fibrosis. At the same time, it contributes to activation of dermal fibroblasts and to transformation of fibroblasts to myofibroblasts [8].

During the remodeling/regenerative phase, the surface of the granulation tissue of the skin ulcer is covered by a newly generated epidermis; this process is called re-epithelialization. In normal skin, periostin is localized in the dermis (just under the epidermis in a region termed the papillary dermis); however, after wounding it is distributed widely in all dermal layers of the scar/lesion [36]. Previously, it was estimated that periositin affects re-epithelializiation in *Postn* knockout mice, which exhibits delays in wound healing, and re-epithelialization in the remodeling/regenerative phase ([35, 47]). It suggests that periostin induces proliferation and differentiation of epithelial cells, resulting in appropriate re-epithelialization.

Overproduction of ECM components (e.g., type I and III collagen, periostin, and tenascin) occurs in hypertrophic scars and keloids, and the ratio of I/III collagen in these lesions is higher than that in normal scar tissue [39]. Keloid is characterized by abnormal deposition of ECM components such as versican, which is expressed at low levels in normal skin. A previous report shows extensive and intense staining of periostin in abnormal scar and keloid tissue [36]. This indicates that periostin contributes to the pathogenesis of hypertrophic scars and to keloid formation. Zhang et al. argue that expression of periostin in keloid cells is higher in hypoxic environments than under normal conditions [56]. Moreover, inhibiting periostin reduces the number and proliferative capacity of keloid cells, and production of collagen fibers, under hypoxic conditions [56]. Thus, periostin plays an important role during the proliferative and remodeling/regenerative phase of wound healing, and its aberrant expression contributes to abnormal scarring after wounding of the skin.

10.3 Periostin and Scleroderma

Scleroderma is an autoimmune connective tissue disease in which various organs, including the skin, become fibrotic and sclerotic [34]. Sclerosis usually begins in the deep dermis and is defined as accumulation of ECM components; this accumulation arises from a vicious cycle involving excessive synthesis and attenuated degradation of ECM components. This cycle exacerbates disease progression [34]. Deposition of ECM components such as collagen, hyaluronic acid, glycosaminoglycan, and fibronectin, destroys the original structure of the skin and impairs proper function [46]. In addition, activation of myofibroblasts and resistance of fibroblasts to apoptosis are observed. Although in scleroderma, the myofibroblast precursors in lesional skin have not been determined, interstitial fibroblasts (as well as cells such as pericytes, endothelial cells, and bone marrow-derived fibroblast-progenitor cells) can differentiate into myofibroblasts [9]. Adipocytic progenitor cells have also been identified as a source of myofibroblasts [27].

Previous reports reveal that several mediators contribute to activation of fibroblasts in skin lesions. Among these, TGF-β plays a central role in sclerosis [25]. In general, the harmful impacts of over-exposure to TGF-β are suppressed by the negative feedback function provided by the orphan nuclear receptor, NR4A1 [37]. However, in scleroderma, continuous activation of TGF-β is mediated by abnormalities in its transcriptional and post-transcriptional regulation, or reduced feedback via NR4A1. Dysfunction of the feedback loop may result in high susceptibility to fibrosis [25, 37]. Moreover, connective tissue growth factor (CTGF, also known as CCN2, a member of the CCN matrix protein family), which is produced by endothelial cells stimulated with TGFβ, endothelin-1, and angiotensin II, also promotes the skin fibrosis process in co-operation with TGF-β [1, 42, 49]. Platelet-derived growth factor (PDGF), a potent mitogen for mesenchymal cells, also plays a role in scleroderma-related fibrosis [19]. PDGF is produced by endothelial cells, platelets, macrophages, and fibroblasts. Its receptors are expressed at high levels in the skin and lungs of patients with scleroderma.

The role of periostin in the pathology of scleroderma was confirmed in animal models. In mouse models, scleroderma-like skin sclerosis is induced following subcutaneous administration of bleomycin for several weeks. Surprisingly, *Postn* KO mice do not develop scleroderma-like skin sclerosis and show no increase of type 1 collagen expression despite increased expressions of TGFβ and CTGF (similar to those seen in the wild type) [54]. In other words, periostin is necessary for induction of TGF-β- and CTGF-mediated type 1 collagen expression during pathogenesis of scleroderma. Furthermore, TGF-β-mediated induction of myofibroblasts does not occur in *Postn* KO mice [54]. Periostin acts on αv-integrin expressed by fibroblasts and induces expression of type 1 collagen via the PI3K/Akt signaling pathway [54]. This suggests that periostin creates a suitable environment for fibrosis. By contrast, a recent *in vitro* study investigating the effects of crenolanib (an inhibitor of PDGF

receptor signaling) on the fibrotic activity of TGF-β-stimulated cultured dermal fibroblasts derived from scleroderma patients found that crenolanib attenuated expression of CTGF and periostin [26]. These results indicate that periostin orchestrates the direction of the fibrotic response mediated by TGF-β, CTGF, and PDGF.

Periostin is localized throughout the dermis of the scleroderma lesion; indeed, immunostaining for periostin in scleroderma lesions is stronger than that observed in keloid and hypertrophic scars [54]. Similarly, periostin is present throughout the dermis in individuals with morphea, which is characterized by localized skin sclerosis [22]. Interestingly, another report indicates the utility of serum periostin levels as a biomarker for scleroderma disease severity [53]. Thus, periostin is involved in both the pathogenesis and pathology of scleroderma and is a possible molecular target for scleroderma treatment.

10.4 Periostin and Atopic Dermatitis

Atopic dermatitis presents with clinical manifestations that are characteristic of age of onset and disease duration. The childhood atopic dermatitis in the typical clinical picture appears mainly at eczematous lesions of the ear, lower neck, elbow, and knee. Eczema is accompanied by strong itching, and scratching of which will lead to exacerbation of eczema. If disease duration lasts into adolescence, eczematous lesions and pruritus develop into chronic dermatitis, resulting in lichenification of the skin [21, 31], which is a major skin manifestation of chronic atopic dermatitis [31]. Chronic inflammation and addictive scratching result in acanthosis of the epidermis, prolongation of dermal papilla, proliferation of fibroblasts, and an increase in the number of thickened collagen fibers. Chronic intractable lesions are formed, which are characterized by basement membrane thickening and increased production of ECM components. This skin remodeling contributes to homing of inflammatory cells in the skin, leading to prolongation of chronic inflammation by inhibiting drug delivery to skin lesions. Tissue remodeling is not limited to atopic dermatitis; it is also seen during allergic inflammation of other organs (e.g., in asthma and allergic rhinitis). When managing the chronic inflammatory disease it is necessary to pay attention to both remodeling and symptomatic treatment. Mast cells play an important role in tissue remodeling, and their specific actions have been identified [29].

High numbers of mast cells are present in atopic dermatitis lesions [32]. Mast cells undergo degranulation upon antigen challenge, after infection followed by stimulation by scratching of the skin. As a result, various inflammatory mediators are released into the tissues, and then act on constituent skin cells and increase proliferation [30]. Among these mediators, histamine acts on the cells expressing histamine receptors; such cells include epidermal cells, fibroblasts, vascular endothelial cells, antigen-presenting cells (e.g., Langerhans cells, dendritic cells, and macrophages) and neurons. Histamine causes an itching sensation, recruits inflammatory cells, and triggers vasodilation and leakage of plasma into the tissues [30].

Histamine also activates the innate immune system and tissue remodeling by activating H1 receptors [29]. Engagement of these receptors on skin fibroblasts, vascular endothelial cells, Langerhans cells, and eosinophils results in production of inflammatory mediators and causes inflammatory cells to adhere to vascular endothelial cells; histamine also alters the composition of the ECM. This alteration of the ECM drives the pathogenesis of chronic inflammatory diseases by promoting infiltration by leukocytes [29, 30]. Histamine induces collagen synthesis by fibroblasts [32]; therefore, hardening of the skin is frequently observed in lichenoid lesions [21]. Indeed, *in vitro* studies show that histamine causes an increase in type 1 collagen synthesis by fibroblasts 48 h after stimulation, a response that might involve one or more second messengers [31].

Previous reports that used genome-wide association studies and quantitative mRNA expression analysis to explore genes related to atopic disease reveal that several MPs, including

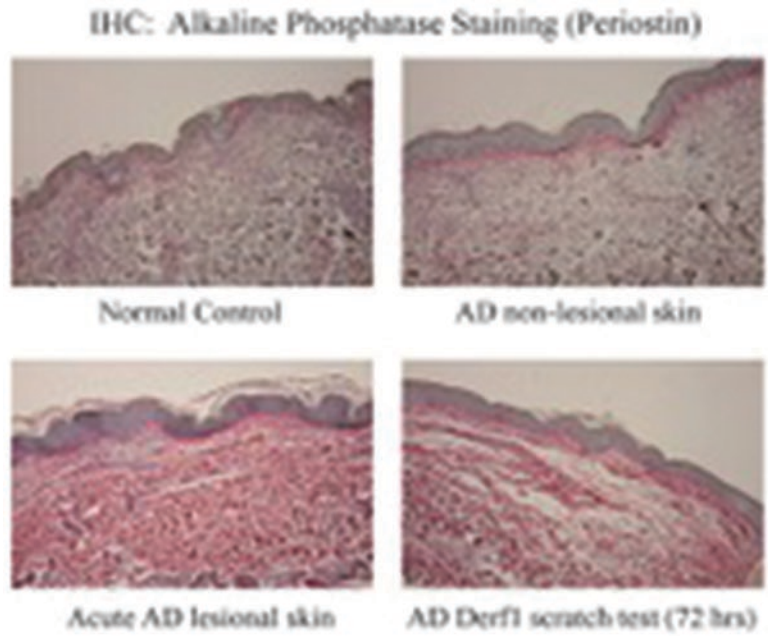

Fig. 10.2 Localization of periostin in lesional skin in atopic dermatitis. A skin specimens from a normal control (non-atopic dermatitis) (upper left), from atopic dermatitis non-lesional skin (upper right), and an atopic dermatitis acute lesion (lower left) 72 h after a scratch test with Derf1 (lower right). Periostin was stained with alkaline phosphatase (red). Magnification, ×100. (This figure was reproduced from Murota et al. (with permission from Elsevier Publishing Group))

periostin, are expressed strongly at lesion sites [16, 50]. Both wild-type and *Postn* knockout (KO) mice develop atopic dermatitis-like skin inflammation after topical application of mite extract. However, unlike wild-type mice, *Postn* KO mice show a relatively milder skin phenotype in terms of acanthosis and infiltration by inflammatory cells [28].

Shiraishi *et al.* report that periostin plays a role in chronicity of allergic skin inflammation by inducing Th2 chemokines, such as TSLP, production by fibroblasts and keratinocytes [38]. Indeed, we confirmed that large amounts of periostin are deposited in lesions in patients with atopic dermatitis [55] (Fig. 10.2). It is thought that periostin plays a role in tissue remodeling as well as in the chronic pathology of atopic dermatitis and lichenification (Fig. 10.3).

As described above, we confirmed that histamine derived from mast cells induces periostin production by fibroblasts via the H1 receptor, and that expression of type 1 collagen occurs via an autocrine pathway [55]. These new findings about the relationship between mast cells and tissue remodeling have increased our understanding of the mechanism underlying lichenification. Thus, therapeutic strategies that target mast cells could regulate tissue remodeling, which might in turn inhibit excessive expression of periostin.

10.5 Periostin and Melanoma

Melanoma is widely recognized as a life-threatening malignant tumor of the skin because it metastases easily to internal organs. Once melanoma gains metastatic potential and spreads to other organs, patient prognosis is poor. Thus, it is imperative that we understand how melanoma acquires metastatic and invasive characteristics. Recently, proteome and genome initiatives have increased our knowledge about several gene products such as BRAF, FAK, and ERK1, which are deregulated in invasive and metastatic melanomas [13].

In addition, the relationship between tumor cells and the surrounding ECM (called the tumoral stroma) plays an important role in invasion of proximal tissue by tumor cells and their subsequent metastasis to other organs. With respect to melanoma, analyzing the cytoskeletal structure and relationships between melanoma cells and the surrounding ECM is helpful [13]. Naka and colleagues explored factors related to

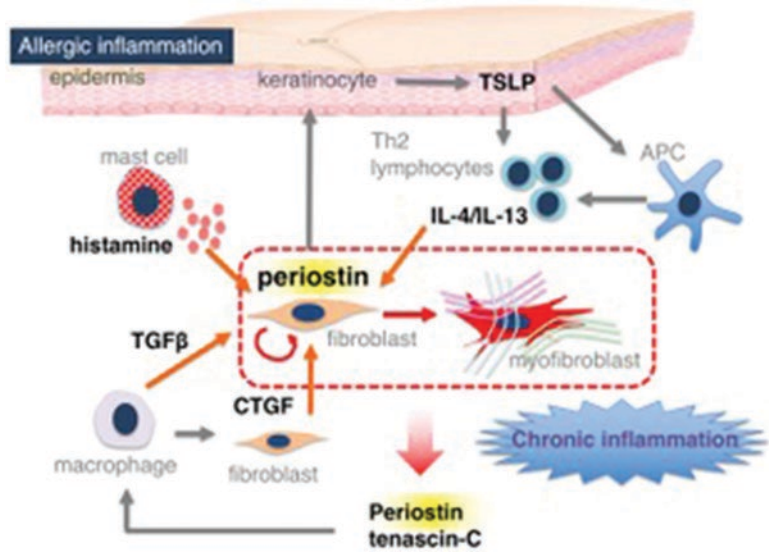

Fig. 10.3 Schematic illustrating the relationship between periostin and atopic dermatitis. In allergic dermatoses, chronic inflammation triggers tissue remodeling and continuous activation of dermal fibroblasts or keratinocytes. Periostin activates keratinocytes, which then secrete chemokines such as TSLP; these cytokines induce continuous activation of immune cells

development of melanoma using a quantitative proteomic analysis method called "isobaric tags for relative and absolute quantitation (iTRAQ)" [23]. They found that periostin was expressed at high levels by invasive melanomas. A relationship between melanoma and periostin was also reported by Tilman and colleagues, who found increased transcription of *Postn* in some melanoma cell lines [45]. Naka and colleagues observed an increased expression of periostin when melanoma cells were co-cultured with normal human dermal fibroblasts [23]. In addition, periostin derived from fibroblasts promotes proliferation of melanoma cells by increasing expression of integrins [23]. This tumor growth-promoting effect of periostin was confirmed in *in vivo* experiments; *Postn*/*Rag2* double knockout mice inoculated with melanoma developed significantly smaller tumor masses than in *Rag2* knockout mice [23]. Fukuda and colleagues reported that periostin acts as a chemotactic factor during metastasis of melanoma cells [11]. Their findings confirmed that periostin secreted by wounded skin promotes migration of melanoma cells into the lesion [11]. However, periostin did not affect proliferation of melanoma cells [11]. Although the impact of periostin on proliferation of melanoma cells remains unclear, these results indicate that an increase in periostin expression contributes to formation of a pro-tumor microenvironment and promotes progression of melanoma to a more serious stage. Periostin expressed in the melanoma microenvironment is thought to be derived from fibroblasts stimulated with CTGF produced by melanoma cells [18]. In summary, periostin derived from the tumor stroma appears to play a role in invasion of melanoma cells by creating a tumor microenvironment, which in turn contributes to proliferation and metastasis of melanoma cells.

The next task is to understand how MPs, including periostin, promote invasion by melanoma cells. A study by Spatz et al. reported a new animal model that closely reproduces the conditions that support melanoma invasion *in vivo* [40]. Such animal models will enable us to better understand how MPs involved in melanoma progression affect the motility of melanoma cells, and their interaction with the ECM, stromal cells, and blood vessels.

10.6 Periostin and Other Dermatoses

Several studies suggest a possible association between periostin and pathogenesis of certain dermatoses. For example, because periostin is a

downstream signaling molecule for Th2 cytokines (e.g., Il-4 and Il-13), Bae et al. measured serum periostin levels in subjects with chronic spontaneous urticaria [5]. The results showed, somewhat surprisingly, that serum periostin levels were significantly lower in cases of severe chronic spontaneous urticaria with high levels of serum IL-13 [5]. Likewise, although the role played by periostin in the pathogenesis of dermatoses remains obscure, abnormal immunohistochemical staining for periostin has been reported in cases of pemphigus vulgaris, bullous pemphigoid, mycosis fungoides, and lichen sclerosus et atrophicus [10, 12, 20].

Mycosis fungoides (MF) comprises the majority of cutaneous lymphoma cases and accounts for up to 40% of all cutaneous lymphoma cases [41]. Usually, patients with MF exhibit a chronic clinical course and suffer persistent symptoms. Most patients with MF remain at the early patch stage; however, some are at risk of gradual progression from the patch stage to the plaque and/or tumor stage [2]. Histopathological findings suggest that the intensity of periostin-positive staining is more prominent at the early stage of disease. Recent reports identify an apparent increase in the number of tissue-infiltrating M2 macrophages in lesional skin. Monocyte-derived macrophages stimulated with periostin show phenotypic characteristics typical of tumor-associated macrophages at the early stage of MF, and express high levels of CXCL5 and CXCL10 [12]. As these chemokines affect formation of cutaneous T cell lymphoma, increased expression of periostin in the tumor microenvironment contributes to pathogenesis of MF by increasing the numbers of tissue-infiltrating macrophages.

Skin aging due to sun damage is regarded as a type of dermatoses. As mentioned above, expression of periostin is low in the papillary dermis. As skin aging continues, expression of periostin falls, resulting in less collagen production and loss of elasticity in aged skin [7].

10.7 Conclusion

Studies of the role of periostin in the pathogenesis of skin diseases provide important information about its actions on the ECM in the lesional microenvironment. Most interestingly, periostin affects not only tissue remodeling (as a component of the ECM), but also induces tissue inflammation during pathogenesis of several dermatoses. However, research into the role of periostin is still young. New findings in the near future will contribute to our understanding of its role in skin disease and clinical dermatoses.

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Conflict of Interest The authors declare no conflicts of interest.

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Periostin in the Kidney

11

Darren P. Wallace

Abstract

Periostin is a matricellular protein that is expressed in several tissues during embryonic development; however, its expression in adults is mostly restricted to collagen-rich connective tissues. Periostin is expressed only briefly during kidney development, but it is not normally detected in the adult kidney. Recent evidence has revealed that periostin is aberrantly expressed in several forms of chronic kidney disease (CKD), and that its expression correlates with the degree of interstitial fibrosis and the decline in renal function. Polycystic kidney disease (PKD), a genetic disorder, is characterized by the formation of numerous fluid-filled cysts in the kidneys. Periostin is secreted by the cyst epithelial cells and accumulates within the extracellular matrix adjacent to the cysts. In PKD mice, periostin overexpression accelerates cyst growth and contributes to structural changes in the kidneys, including interstitial fibrosis. Recent evidence suggests that periostin is a tissue repair molecule; however, its role in repair following acute kidney injury has not been investigated. It is thought that persistent expression of this protein in CKD contributes importantly to tubulointerstitial fibrosis and the progressive decline in renal function. Future studies to define the diverse actions of periostin during kidney injury may lead to effective therapies to slow PKD progression and possibly prevent the development of CKD. This chapter reviews the current literature on the expression of periostin in PKD and other forms of CKD, mechanisms for periostin stimulated cyst growth, its potential role in extracellular matrix production and renal fibrosis, and the evidence for periostin as a novel biomarker for kidney disease.

Keywords

Matricellular proteins · Polycystic kidney disease · Chronic kidney disease · Integrin signaling · Renal fibrosis

11.1 Introduction

Periostin is a 90 kDa secreted protein and the newest member of the family of matricellular proteins that includes thrombospondins, osteopontin, SPARC, tenascin-C, connective tissue growth factors and transforming growth factor-β (TGFβ)-inducible factor (βig-H3) [14, 24, 30, 74]. Periostin is expressed in multiple tissues during development; whereas in adults, expres-

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sion is restricted to collagen-rich tissues that are subjected to mechanical stress conditions, such as periodontal ligaments, periosteum, skin and cardiac valves [51]; and is highly expressed in tissue following injury [9, 32, 36, 65]. Evidence has shown that it directly interacts with components of the extracellular matrix (ECM), such as collagen, tenascin-C and BMP-1, and promotes collagen cross-linking through activation of lysyl oxidase (LOX) [33, 43]. Thus, periostin is thought to maintain the integrity of the matrix and influences the biomechanical properties of connective tissue [37, 51, 52]. On the other hand, excessive or persistent expression of periostin is associated with organ fibrosis [20, 48, 66, 67, 73, 84]. Over the last several years, periostin has been identified as a novel key factor in the progression of kidney disease. Periostin is not expressed in normal adult kidneys [26, 83], suggesting that it does not play a role in normal renal function; however, it is expressed *de novo* by injured kidneys. Ectopic periostin expression in genetic forms of polycystic kidney disease (PKD) were found to promote renal cyst growth and contribute to the development of renal fibrosis and inflammation in several forms of chronic kidney disease (CKD). This chapter will address the current understanding of the expression and functional role of periostin in kidney development and disease.

11.2 Periostin Signaling

Periostin contains an N-terminal signal sequence, an EMI domain, and four conserved FAS1 domains that have homology to fasciclin, a neural adhesion protein in insects. The C-terminus contains a heparin-binding domain [30]. The EMI and heparin-binding domains of periostin are thought to be involved in the protein's ability to bind to components in the extracellular matrix (ECM). Interestingly, the C-terminus of periostin can undergo alternative spicing [45]; however, the relevance of the splice variants remains unclear.

Periostin has high homology to βig-H3, and both proteins can bind to cell surface integrins through their highly conserved FAS1 domains [46]. Periostin binding to $\alpha_V\beta_3$- and $\alpha_V\beta_5$-integrin activates the integrin-linked kinase (ILK), a scaffold protein that forms a complex with adaptor proteins PINCH and α-parvin. Activation of ILK-PINCH-parvin (IPP) stimulates pathways involved in tissue repair, including those for epithelial-mesenchymal transition (EMT), cell adhesion, proliferation, survival, and matrix production [4, 8, 9, 27, 29, 30, 52, 86]. Periostin binding to $\alpha_V\beta_3$-integrin is also thought to promote TGFβ release from the latent TGFβ binding protein (LTBP). Increased levels of activated TGFβ, in turn, stimulate ECM production and periostin secretion through the SMAD signaling pathway, potentially contributing to a positive feedback mechanism of periostin/$\alpha_V\beta_3$-integrin/TGFβ-induced fibrosis.

The capacity for periostin to promote tissue regeneration is currently being investigated in several tissues, including bone, skin, and heart [9, 11, 27, 32, 80]. Periostin applied to cardiac tissue, following a myocardial infarction, stimulated the re-entry of differentiated cardiomyocytes into the cell cycle, leading to myocardial repair, reduced fibrosis and improved cardiac function [11, 32]. However, as with many aspects of biology, excessive or unregulated activation of a normal cellular pathway can often lead to pathological conditions. Cardiac fibrosis is associated with elevated periostin expression, and persistent exposure of recombinant periostin can lead to cardiac fibrosis [95]. Several lines of evidence have shown that aberrant expression of periostin in response to injury can have pathological consequences such as cardiac, pulmonary and renal fibrosis [14, 48, 67, 95].

Periostin is highly overexpressed in several cancers, including lung, ovarian, breast, thyroid, colon, pancreatic and glioblastoma, and it is thought to promote cancer cell proliferation and survival and stimulate angiogenesis of the tumor [1, 2, 16, 31, 44, 63, 68, 98]. This pro-tumorigenic effect of periostin may not only involve integrin-mediated activation of intracellular pathways, but also alterations in the ECM components of the tumor microenvironment that play a role in invasive and metastatic processes. Matricellular

proteins, such as periostin, and their cell signaling pathways are being considered as potential therapeutic targets for cancer treatment [53].

11.3 Periostin Expression During Renal Development

Periostin is expressed only briefly during renal development within the nephrogenic zone, a region of new nephron formation and glomerular vascularization. Ito et al. was the first to show that periostin was abundantly expressed within the nephrogenic zone of the developing rat kidney [26]. Periostin mRNA was strongly detected within the subcapsular region of 1-day-old kidney; whereas there was a very low level of periostin expression by day 2 and none detected in adult kidneys. Immunostaining demonstrated robust expression of periostin within the nephrogenic zone of embryonic porcine kidney (embryonic day 60), which shares remarkable similarities in development, anatomy and function to the human kidney (Fig. 11.1).

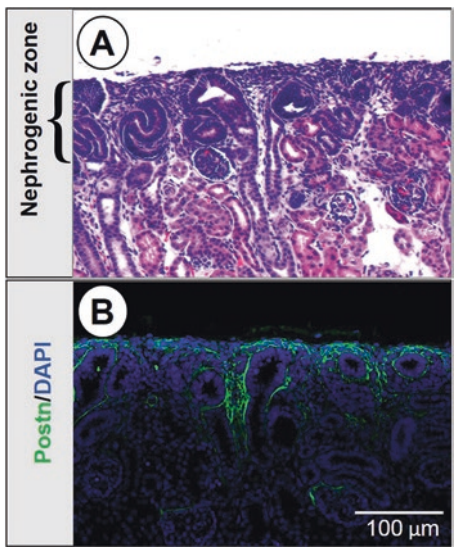

Fig. 11.1 Periostin expression within the nephrogenic zone of an embryonic porcine kidney. (**a**) Hematoxylin and eosin staining showing early nephron development in the nephrogenic zone of an embryonic day 60 pig kidney. (**b**) Staining of adjacent section with an antibody to periostin (Postn; green) and DAPI nuclear stain

Periostin (green) is localized within the interstitium adjacent to developing nephrons and glomeruli, suggesting that it plays a role in nephrogenesis and glomerular vascularization. Notably, there was very little periostin staining below the nephrogenic zone, which contains more mature structures. Periostin was not expressed in healthy adult human kidneys (Fig. 11.1b) [83], consistent with the previous study that showed a lack of periostin expression in adult rat kidneys [26].

Temporal expression and functional importance of periostin during renal development remain unclear. Early in mouse kidney development, periostin expression, determined by *in situ* hybridization, was elevated in the mesenchyme surrounding the kidney and ureter, renal stroma, metanephric mesenchyme, ureter epithelium and developing nephrons [71]. Bone morphogenetic protein (BMP)-4, which inhibits ureteral branching morphogenesis in metanephric explants, also caused upregulation of periostin mRNA. Moreover, recombinant periostin inhibited ureteral branching morphogenesis and glomerular number. While its expression in the developing kidney suggests a role for periostin in tubule formation and/or glomerular vascularization, global knockout of periostin does not appear to affect renal development in mice. Periostin knockout (*Postn*$^{-/-}$) mice are similar in general appearance, but have a modest reduction in body weight compared to age- and sex-matched wildtype littermates [62]. There was no difference in kidney weight or gross appearance between *Postn*$^{-/-}$ and wildtype littermates [84], suggesting that periostin is not required for normal renal development. It is possible that other matricellular proteins, such as βig-H3, a paralog of periostin, compensate for the loss of periostin. Considering the robust expression of periostin within the nephrogenic zone, additional studies are needed to understand a role of periostin, as well as other matricellular proteins, in kidney development. Expression of these proteins may protect the kidney from injury during nephrogenesis. To evaluate this possibility, studies could be done to impose injury or other forms of stress during renal development.

11.4 Aberrant Expression of Periostin in Kidney Disease

Chronic kidney disease (CKD) is currently an incurable medical problem that can originate from a variety of causes, such as genetic forms of renal cystic disorders, nephrotoxins, diabetes and hypertension. Renal fibrosis is the final common pathological pathway responsible for the decline in kidney function, and currently there are no effective treatments to slow renal fibrosis [13]. Renal replacement therapy with dialysis or transplantation is the only option for CKD patients who develop end-stage renal disease (ESRD). The rising incidence of CKD is not only a huge economic burden for national health systems, but also accounts for an increasing number of deaths worldwide. A major focus of kidney research is to establish a better understanding of mechanisms that govern the development and progression of CKD, allowing for the identification of useful early prognostic biomarkers and therapeutic approaches to halt disease progression.

Periostin was first identified as a contributing factor in the progression of renal disease by studies that showed high expression levels in autosomal dominant polycystic kidney disease (ADPKD), a genetic disorder characterized by the formation of numerous fluid-filled cysts within the kidneys [83]. Using an unbiased approach, periostin was identified as one of the most highly over-expressed genes in cultured human ADPKD cells compared to normal kidney cells [83]. ECM molecules, including type I and III collagen, laminins, TGFβ, MMPs and integrins were also overexpressed. Aberrant expression of periostin by cultured ADPKD cells may be a memory effect of the cystic environment, driven by excessive TGFβ production, or the result of intrinsic differences due to the cystic phenotype. Periostin was detected in media conditioned by ADPKD cells and within cyst fluid from ADPKD kidneys, suggesting that periostin is secreted by cyst-lining epithelial cells. Immunostaining revealed that periostin accumulates within the ECM adjacent to cysts of ADPKD kidney *in situ* (Fig. 11.2a). By contrast, periostin was not detected in normal kidneys (Fig. 11.2b). Elevated periostin expression was also detected in human autosomal recessive PKD (ARPKD), a juvenile form of PKD, and several animal models of PKD, including *pcy/pcy*, *cpk/cpk* and *jck/jck* mice [84], *bpk/bpk* and *Pkd1*$^{cond/-}$*: Nestin-Cre* mice [54], and PCK rats (*D. Wallace, unpublished observation*), suggesting that ectopic expression of periostin is a general feature of PKD regardless of the underlying genetic mutation.

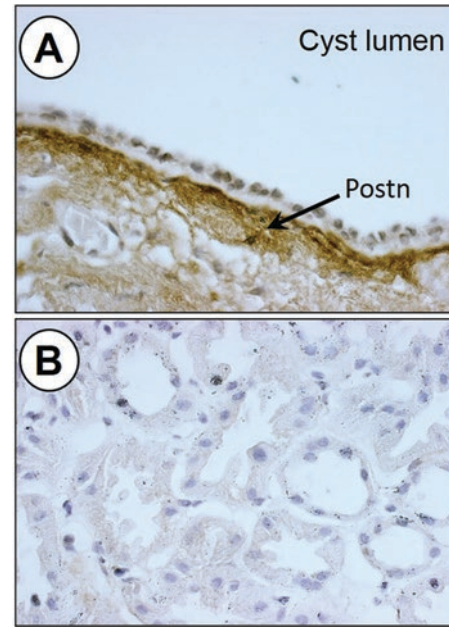

Fig. 11.2 Periostin expression in ADPKD and normal human kidney. (**a**) Representative section of a human ADPKD cyst shows robust staining for periostin in the extracellular matrix adjacent to the cyst epithelium. By contrast, periostin was not detected in normal human kidney (**b**). (Reprinted with permission from Wallace et al. [83])

Subsequent studies found that periostin was highly expressed in kidney biopsy samples from a range of kidney diseases. Sen et al. examined glomerular gene expression profiles from renal biopsies from patients with proteinuric glomerulopathies, including focal-segmental glomerulosclerosis, membranous nephropathy, proliferative lupus nephritis and IgA nephropathy [67]. Elevated gene expression was observed for

several matricellular proteins, such as SPARC, thrombospondin, and connective tissue growth factor; however, periostin showed the highest expression and had the most consistent induction in the biopsy samples. Immunohistochemistry showed that periostin localized to areas of mesangial expansion and sites of interstitial fibrosis and inflammation. The degree of periostin staining in the biopsies negatively correlated with estimated glomerular filtration rate (eGFR) in patients with progressive nephropathies. In other studies, Mael-Ainin et al. showed elevated renal periostin expression in mice that had undergone unilateral ureteral obstruction (UUO) and in a rat model of hypertensive renal injury [40]. Renal periostin was also shown to be increased after 5/6 nephrectomy in rats and streptozotocin-induced diabetic nephrectomy in mice [66]. One hypothesis for its *de novo* expression in CKD is that periostin is a repair molecule that is typically expressed during renal injury repair; however, persistent insult to the kidney causes unregulated expression leading to renal inflammation and interstitial fibrosis.

11.5 Periostin Accelerates Cyst Growth in Polycystic Kidney Disease

ADPKD is characterized by progressive damage to the kidneys caused by the presence of numerous fluid-filled cysts. It is one of the most common monogenic diseases with a prevalence of 1 in 500–1000 people, affecting more people than Down syndrome, Huntington's disease, hemophilia, sickle cell disease, myotonic dystrophy and cystic fibrosis combined. It is estimated that approximately 12.5 million people worldwide have ADPKD.

The formation and progressive enlargement of cysts involve aberrant proliferation of tubule epithelial cells and the accumulation of fluid within the cavity of the growing cyst due to Cl^--dependent fluid secretion [18, 82, 94]. Inexorable cyst growth causes a loss of functional nephrons, and progressive development of inflammation and fibrosis that lead to the loss of renal function. Approximately, one-half of these patients progress to ESRD by age 60, requiring dialysis or renal replacement therapy, making it the third leading cause of ESRD in the United States after diabetes mellitus and hypertension. ADPKD is caused by mutations in *PKD1* (~85% of cases), which encodes polycystin-1, or *PKD2*, which encodes polycystin-2. The polycystins physically interact and are thought to function as a nonselective cation channel involved in intracellular Ca^{2+} homeostasis or signaling. While several cellular pathways appear to be dysregulated in ADPKD kidneys [70], it remains unclear which of these pathways are most significant for cyst growth, fibrosis, and inflammation and ultimately the decline in organ function. The abnormal response to elevated levels of the secondary messenger cyclic AMP has been the focus of many studies and is the basis for the first FDA approved therapy for ADPKD, the vasopressin-2 receptor antagonist tolvaptan [61, 81, 85, 93, 94].

The discovery that periostin was highly overexpressed by cyst-lining cells and accumulated in the matrix of PKD kidneys led to a series of *in vitro* and *in vivo* studies to determine if periostin contributes to disease progression. TGFβ and bone morphogenetic protein (BMP)-2, both of which stimulate periostin expression in osteoblasts [24, 36], were found to be overexpressed in PKD. Treatment with TGFβ potently induced periostin expression in normal human kidney cells and led to the loss of an epithelial cell phenotype [40, 83]. Collecting duct (CD)-specific overexpression of active TGFβ causes a robust increase in renal periostin expression and interstitial fibrosis, leading to a decline in renal function (*D. Wallace, unpublished results*). These observations suggest that aberrant expression of periostin *in situ* may be due, in part, to local TFGβ expression by cystic cells. Olsan et al. also found that IL-4 and IL-13 induced periostin expression in mouse IMCD cells and that blocking the activation of transcription factor STAT6 with pyridine-6, a JAK inhibitor, reduced the effect of IL-4 and IL-13 on periostin expression [54]. These investigators also showed that gene ablation of STAT6 dramatically reduced renal periostin expression and cyst growth in *bpk/bpk* mice, a

rapidly progressive model of cystic disease. It is possible that STAT6 regulates the expression of molecules, such as periostin, that play a role in tissue repair when their activity is properly regulated; however, in PKD constitutively active STAT6, mediated through IL-13 signaling, leads to the overexpression of periostin, thus contributing to cyst growth and disease progression.

Renal periostin levels were found to be highly elevated in *pcy/pcy* mice, a slowly progressive model of PKD. This model develops massive enlarged kidneys and renal fibrosis, leading to organ failure by ~42 weeks. These cystic kidneys have elevated levels of phosphorylated S6 (P-S6) and S6 kinase (P-S6K), downstream targets of mTOR, and increased cell proliferation. Global gene ablation of periostin in *pcy/pcy: Postn*$^{-/-}$ mice significantly reduced the level of renal mTOR signaling, cell proliferation, cyst number, cystic index, and the size of the cystic kidneys. Loss of periostin also caused a reduction in renal fibrosis, preservation of renal function in *pcy/pcy* mice and significantly extended the lifespan of the mice [84]. In a subsequent study, periostin was selectively overexpressed in the renal collecting ducts, a predominant site for cyst formation [58]. Ectopic CD expression of periostin was not sufficient to induce *de novo* cyst formation in otherwise normal mice, consistent with the lack of a mitogenic effect of periostin on normal kidney cells *in vitro.* However, CD-overexpression of periostin in *pcy/pcy* mice increased mTOR signaling and cell proliferation, leading to accelerated cyst growth, increased renal fibrosis and a more rapid decline in kidney function (Fig. 11.3). These *in vivo* studies provide strong evidence that renal expression of periostin contributes importantly to cyst growth and fibrosis in PKD.

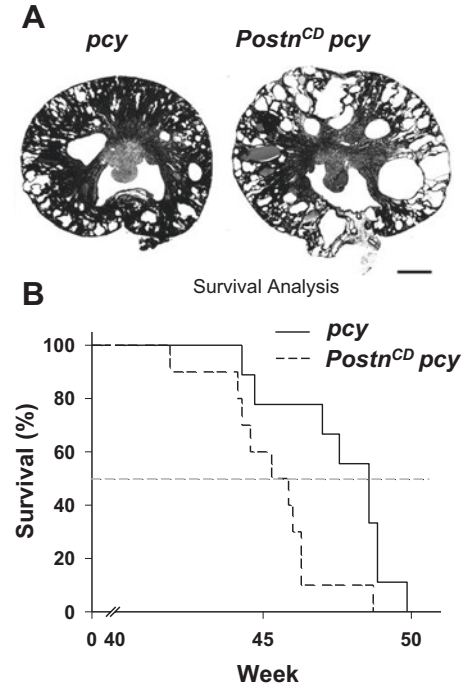

Fig. 11.3 Kidney-specific periostin expression accelerated renal cyst growth in PKD mice and reduced survival. Transgenic mice with selective overexpression of mouse periostin in renal collecting ducts (CD) were crossed with *pcy/pcy* (*pcy*) mice, a model of slowly progressive polycystic kidney disease (PKD), to generate *Postn*^{CD} *pcy* mice. (**a**) Representative kidney sections from 10-week old *pcy* mice and *Postn*^{CD} *pcy* mice. There was a significant increase in cystic area, interstitial fibrosis, and total kidney volume (not shown). (**b**) CD-specific overexpression of periostin decreased the survival of *pcy* mice. (Reprinted with permission from Raman et al. [58])

11.6 Periostin Stimulates ILK-Mediated mTOR Signaling in PKD

The mammalian target of rapamycin (mTOR) is an important target for integrin signaling. Activation of mTOR requires GTP-bound form of Rheb, a small GTPase, which is inhibited by tuberin (TSC2), a GTPase-activating protein [76, 92]. Several kinases, including Akt and ERK, phosphorylate tuberin, leading to upregulation of mTOR activity [42]. mTOR effectors include ribosomal S6 kinase (S6K; p70S6K1 and p70S6K2) and eukaryotic initiation factor 4E-binding proteins 1 (4E-BP1) and 2 (4E-BP2). mTOR activation of S6K increases phosphorylation of subunit 6 of ribosomal protein (S6), which is important for protein translation, cell growth and proliferation.

Evidence has shown that the mTOR pathway is abnormally activated in cystic cells in human and rodent models of PKD [69, 75, 77, 79];

however, the mechanism responsible for the activation of this pathway remains unclear. Recombinant human periostin caused a further increase in phosphorylated Akt (P-Akt) and downstream mTOR targets S6 (P-S6) and S6K (P-S6K) and stimulated ADPKD cell proliferation. Periostin also accelerated *in vitro* cyst growth of ADPKD cells cultured within a 3-D collagen matrix. This mitogenic effect of periostin was attenuated by an α_V-integrin blocking peptide [59]. Importantly, periostin did not stimulate mTOR or proliferation of normal human kidney (NHK) cells, a difference that is thought to be due to the higher expression levels of $\alpha_V\beta_3$-integrin by cystic cells compared to normal tubule cells [83].

In cancer cells, periostin binding to $\alpha_V\beta_3$-integrin promotes proliferation by activation of integrin-linked kinase (ILK), leading to the stimulation of Akt and mTOR signaling [96]. ILK was originally characterized as a kinase [21]; however, certain characteristics of ILK make it unclear if ILK is, indeed, a *bona fide* kinase. The kinase domain of ILK lacks conserved motifs found in conventional kinases and mutations in this domain fail to alter normal development [15, 90]. It is generally accepted that ILK serves as a scaffold protein necessary for the formation of a multiprotein complex that includes two adaptor proteins PINCH and α-parvin [57]. The ILK-PINCH-Parvin (IPP) signaling complex plays a central role in the communication between the cell and ECM. ILK physically interacts with β_1-and β_3-integrin and appears to be responsible for linking integrins to the actin cytoskeleton and recruiting focal adhesion components that mediate cell proliferation, survival and migration during tissue repair.

Periostin-induced Akt/mTOR signaling and proliferation of ADPKD cells were blocked by CPD 22, an ILK inhibitor, and by ILK shRNA knockdown [59]. In mice, conditional knockout of both alleles of ILK in CDs was lethal by ~10 weeks; however, heterozygous ($Ilk^{+/-}$) knockdown of ILK had no effect of renal morphology or function of wildtype mice. Importantly, CD-specific knockdown of only one allele of ILK in either *pcy/pcy* mice or $Pkd1^{flox/flox}$: *Pkhd1-Cre*, a rapidly progressive orthologous model of ADPKD, markedly improved renal function and survival of the mice [59], supporting the hypothesis that ILK is an important intermediate and potential therapeutic target for the mitogenic effect of periostin on renal cyst growth in PKD.

11.7 Periostin Contributes to a Futile Repair Response in ADPKD Cystic Cells

Evidence has demonstrated a link between kidney injury and cyst initiation and PKD progression [22, 58, 87, 88]. A meta-analysis of studies looking at gene pathways dysregulated in PKD, showed that many of the same gene expression changes were observed in injury repair [41]. Moreover, several research groups demonstrated that kidney injury by nephrotoxins or ischemia reperfusion accelerated cyst formation and disease progression in PKD mice [3, 22, 56, 72]. ADPKD cells are described as being incompletely differentiated with a loss of expression of epithelial-specific genes and upregulation of developmental and mitogenic pathways compared to normal kidney cells [7, 70]. ADPKD cells have abnormal activation of the mTOR pathway and increased expression of transcription factors, including c-myc, contributing to aberrant cell proliferation and cyst growth [5, 10, 41, 60, 70, 75]. There is increased TGFβ-Smad signaling and abnormal expression of structural ECM components such as type I and III collagens and laminin, soluble ECM-associated factors and surface receptors and integrins [23, 47, 55, 70, 78, 83, 91], consistent with transcriptional reprogramming found during tissue repair [6, 38, 87–89].

Selective CD overexpression of periostin in *pcy/pcy* kidneys stimulated mesenchymal markers vimentin and α-smooth muscle actin (α-SMA), indicating an EMT response in the epithelial cells and myofibroblast activation, which likely contributed to development of fibrosis [58]. The capacity for periostin to promote tissue repair pathways was examined in

human ADPKD cells. Periostin increased the expression of genes in pathways involved in repair, including integrin and growth factor signaling and ECM production [58]. There was also increased stimulation of focal adhesion kinase, Rho GTPase, cytoskeletal remodeling and migration of ADPKD cells. These data are consistent with the hypothesis that periostin is part of a maladaptive repair process in the cystic cells that contributes importantly to an incompletely differentiated cellular phenotype, increased cell proliferation and excessive matrix production in PKD kidneys.

11.8 Mechanisms of Fibrosis in Chronic Kidney Disease

Renal fibrosis is the common end-point for all forms of CKD [12]. The extent of interstitial fibrosis on kidney biopsy is regarded as the best correlation to CKD stage and is commonly used as a prognostic indicator and guide for treatment [39, 49]. Renal fibrosis involves the deposition of ECM molecules and fibrogenesis, leading to scar material within the renal interstitum. This occurs with activation of fibroblasts to α-smooth muscle actin (αSMA) positive myofibroblasts, which are recognized as the key cell type responsible for matrix production during fibrosis [97]. Tubule epithelial cells are often the site of kidney injury; however, their role in the development of fibrosis remains controversial. One hypothesis is that tubule epithelial cells undergo EMT following injury with the loss of epithelial markers and gain of mesenchymal characteristics, ultimately becoming myofibroblast-like cells and migrating into the interstitial space. However, cell lineage tracing failed to find evidence of tubule epithelial cells contributing to the myofibroblast population in fibrotic kidneys [25]. Instead myofibroblasts appear to originate from pericytes. More recent studies found that tubule epithelial cells undergo a partial EMT during renal fibrosis, expressing both epithelial and mesenchymal cell markers, and remain attached to the tubule basement membrane [17]. This partial EMT induces the expression and release of fibrogenic cytokines into the interstitium by the tubule cells that contributes to myofibroblast activation. TGFβ is the most recognized fibrotic factor and promotes both EMT and stimulates ECM synthesis by renal epithelial cells, pericytes and myofibroblasts. TGFβ is secreted as a latent complex at high concentrations, which is directly linked to the ECM. Regulation of TGFβ is based on extracellular activation of the latent complex releasing the activated TGFβ in the tissue.

Damage to the tubule epithelium may also induce fibrosis by other mechanisms. There is evidence that metabolic reprograming in tubule cells leads to decreased fatty acid oxidation and an imbalance in fatty acid synthesis, uptake and consumption, resulting in intracellular lipid accumulation [28, 34]. Restoration of fatty acid metabolism was found to protect the kidneys from tubulointerstitial fibrosis in mice undergoing renal injury [28]. Key questions that remain are (1) what are the central pathways responsible for renal fibrogenesis, (2) are there methods to target these fibrotic pathways, and (3) what are potential biomarkers to monitor the progression and regression of renal fibrosis?

11.9 Periostin Contributes to Fibrosis in Chronic Kidney Disease

Periostin has been shown to play an important role in fibrosis in several tissues, including the kidney. Periostin expression is induced by mediators of renal fibrosis and inflammation including TGFβ, angiotensin II, PDGF-B and interleukin (IL) 4 and 13 [35, 54]. Periostin is one of the most highly upregulated genes in human CKD specimens and in experimental models of renal fibrosis. It can contribute to fibrosis by three possible mechanisms. First, periostin binding to $\alpha_V\beta_3$-integrin induces an EMT response leading to increased collagen deposition involving the SMAD pathway (Fig. 11.4). Second, periostin binds collagen and fibronectin in the ECM, and it interacts with BMP-1 to promote the proteolytic cleavage of the propeptide of LOX to its active form [43]; active LOX, in turn, catalyzes intra-

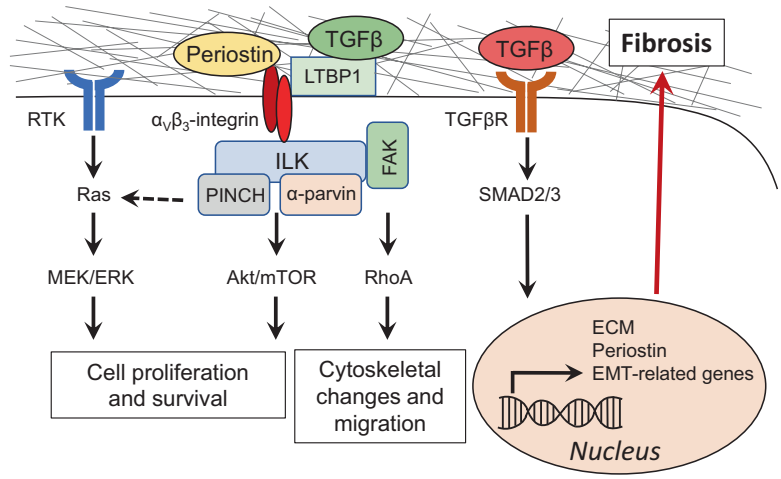

Fig. 11.4 Proposed model for periostin mediated renal pathogenesis. Periostin binding to $\alpha_V\beta_3$-integrins stimulates signaling pathways involved in tissue repair, including cell proliferation and survival, cell migration and epithelial-mesenchymal transition (EMT). Periostin binding to the integrins is also thought to release latent TGFβ causing active TGFβ to stimulate the SMAD pathway, leading to the expression of extracellular matrix (ECM) molecules, including periostin, thus causing a feed-forward mechanism for renal fibrosis. Periostin also activates lysyl oxidase (LOX) leading to cross-linking of the collagen fibrils (not shown). Receptor tyrosine kinase (RTK); Integrin-linked kinase (ILK); PINCH (particularly interesting new cysteine histidine-rich protein); Ras homology family member RhoA; Focal adhesion kinase (FAK); Transforming growth factor-β (TGFβ) and its receptor (TGFβR); Latent TGFβ binding protein 1 (LTBP1)

and intermolecular covalent cross-linking of collagen fibrils. Third, periostin binding to $\alpha_V\beta_3$-integrin increases the release of activated TGFβ. Since TGFβ stimulates periostin secretion, the two factors appear to function in a feed-forward mechanism leading to ECM deposition and interstitial fibrosis. In addition, periostin expression in CKD may recruit profibrotic cells into the kidney, contributing to the fibrotic event.

Similar to the correlation between tubulointerstitial fibrosis and CKD progression, renal fibrosis in ADPKD has been identified as the most significant manifestation associated with the progression to ESRD [19, 50]. In human ADPKD and mouse models of PKD, periostin localizes to regions of fibrosis [83]. Knockout of periostin reduced cyst growth as well as fibrosis and preserved renal function [84]. By contrast, CD-specific overexpression of periostin increased both cyst growth and fibrosis, accelerating the decline in function [58]. While these studies suggested a role of periostin in fibrosis in this genetic form of CKD, it was not clear if periostin had a direct effect on fibrosis or if changes in fibrosis were a consequence of changes in cyst burden. Subsequent studies in patient biopsies and animal models confirmed that periostin expression was elevated in different forms of CKD. Periostin was highly expressed in the tubulointerstitial and fibrotic compartments in biopsies from patients with glomerulopathies, and its expression was inversely correlated with renal function [67]. Periostin was also reported to be upregulated during disease progression and downregulated during regression in a rat model of hypertensive renal disease [20]. Immunohistochemical analysis revealed that periostin has predominantly at perivascular localization in areas where there are deposits of extracellular matrix. This study also showed intense extracellular staining for periostin in injured fibrotic regions in human biopsy specimens of kidneys with chronic allograft nephropathy. Mael-Ainin et al. investigated whether periostin was involved in the development of renal disease and whether blocking its expression improved renal function [40]. They found that periostin could be detected within the kidney 2 days after unilateral ureteral obstruction (UUO).

There was high expression of TGFβ by 15 days that was associated with induction of p-SMAD3 expression in the tubule cells. Immunostaining indicated increased staining for vimentin and a marked decrease in E-cadherin, consistent with EMT. Gene knockout of periostin prevented the increase in TGFβ, preserved the renal epithelial phenotype and lessened the extent of injury-induced fibrosis. Importantly, this group also showed that *in vivo* delivery of an anti-sense oligonucleotides to periostin, diminished periostin overexpression and protected the kidneys in a model of hypertensive nephropathy [40]. These data suggest that blocking periostin or its downstream pathways may be a therapeutic approach to slow the development of CKD.

11.10 Periostin as Biomarker of Progressive Renal Injury

CKD typically develops over many years with a long period in which the disease is clinically silent. Early identification of patients at risk for renal disease can lead to early intervention and a better long-term outcome. Diagnosis and course-of-treatment of CKD are commonly based on biomarkers that assess kidney function, such as measurements of glomerular filtration rate (GFR), blood urea nitrogen, serum creatinine and urinary protein levels; however, these measures are imprecise, change only after considerable loss of functional nephrons and do not directly measure tissue injury. The use of a combination of biomarkers for processes responsible for tubulointerstitial change may provide a more complete picture of the severity of renal disease [13]. The discovery of diagnostic and prognostic biomarkers for early CKD has been challenging. Kidney injury molecule-1 (KIM-1), neutrophil gelatinase-associated lipocalin (NGAL), cystatin C and monocyte chemoattractant protein-1 (MCP-1), have been shown to reflect renal tubule injury and are currently being investigated as biomarkers in CKD [64].

Evidence for renal periostin expression in CKD has led to investigation of periostin as a potential tissue and urinary biomarker for the progression of kidney disease. Satirapoj et al. showed that there was elevated periostin expression in 5/6 nephrectomy in rats, streptozotocin-induced diabetic nephropathy and UUO in mice, as well as biopsy specimens of patient CKD kidneys [66]. In this study, they found that periostin was predominantly in the cell cytoplasm of the distal nephron. This contrasts with observations in PKD kidneys where there was low cytoplasmic staining and strong ECM staining for periostin [83]. The authors speculate that cytoplasmic periostin accumulation in injured distal tubule cells may represent a failure of the cell secretory system. Importantly, there was a marked increase in the excretion of urinary periostin after 5/6 nephrectomy in a longitudinal experiment, demonstrating that the excretion of periostin increased over time with chronic injury. While the mechanism for periostin excretion into the urine has not been addressed; these observations are consistent with findings in PKD [83]. Firstly, periostin was robustly detected within cyst fluid of human ADPKD kidneys. Secondly, isolated ADPKD cells grown on permeable supports secreted periostin across both the basolateral and apical aspects of the cells. The potential for periostin to be a urinary biomarker for CKD was shown by its detection in urine from proteinuric and non-proteinuric CKD patients, but not from healthy individuals. More recently, urinary periostin levels were found to be significantly elevated in patients with normoalbuminuria, microalbuminuria and macroalbuminuria type 2 diabetes compared to healthy controls [65]. While several studies have confirmed that periostin is expressed within the kidney tissue and excreted into urine of CKD patients, further research is necessary to assess the potential for periostin to be a prognostic biomarker for CKD progression.

11.11 Concluding Remarks

Periostin has emerged as an important signaling molecule that modulates the ECM integrity and stimulates cellular signaling pathways involved in tissue repair, such as EMT, cell growth and proliferation. Over the past 10 years, periostin

has been identified as an important factor in kidney disease. Periostin promotes cyst growth in PKD and *in vivo* and *in vitro* studies have provided insights into the molecular mechanisms responsible for periostin-mediated cell proliferation. Recent evidence has shown that there is *de novo* expression of periostin early in kidney injury and that its expression contributes to interstitial fibrosis and progression of CKD. Although we recognize that periostin contributes to kidney disease, several questions remain. Does periostin have a role in renal development? Periostin is clearly expressed at high levels in the nephrogenic zone of the developing kidney, suggesting that it contributes to nephron development or glomerular vascularization; however, periostin knockout mice have normal-appearing kidneys. Do other signaling molecules, such as βig-H3 compensate for the loss of periostin? How is renal periostin expression regulated and what signals lead to its overexpression in kidney disease? What are the central pathways for periostin-induced tubulointerstitial fibrosis in CKD? Finally, can periostin be an effective biomarker for the progression of kidney disease? Future studies will be focused on these important questions. Since periostin is not expressed in the normal adult kidney, blocking the expression of periostin or its downstream signaling pathway in the kidney may be an effective and safe therapeutic approach to slow cyst growth in PKD and the development of CKD.

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Periostin in Eye Diseases

12

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Abstract

The transparency of the eye can be disturbed by several eye diseases. It has recently been reported that periostin plays pivotal roles in the pathogenesis of several eye disease, such as diabetic retinopathy (DR), age-related macular degeneration (AMD), glaucoma, pterygia, corneal dystrophy, and chronic ocular allergic diseases. In these diseases, formation of fibro (vascular) tissue plays an important role. Gene expression profiling of human retinal fibro (vascular) membrane revealed significant up-regulation of periostin. The expression of periostin after environmental perturbations, including IL-4 and/or IL-13 induction, can alter normal physiological interactions among fibroblasts, macrophages and ECM protein in the eye. Modulating the expression of periostin by low-molecular weight compounds, antibodies or RNAi directed against the molecule could be a novel therapeutic strategy for inhibiting the progression of those periostin-involved eye diseases.

Keywords

Vitreoretinal disease · Genome-wide gene expression profiling · Proliferative diabetic retinopathy · Proliferative vitreoretinopathy · Age-related macular degeneration · Fibrovascular membranes · Epiretinal membranes · Neovascularization · Fibrosis · Retina · Choroid · Cornea · Conjunctiva · Pterygia · Glaucoma · Corneal dystrophy · Atopic keratoconjunctivitis · IL-4 · IL-13 · Serum · Tear · Mouse model of oxygen-induced retinal neovascularization · Mouse model of laser-induced choroidal neovascularization · Single-stranded RNA interference

The eye rests in the front half of the cavity of the orbit upon a fascial hammock surrounded by fat and connective tissue. Only its anterior portion is exposed, and it is protected by the bony orbital rim. The one third of the eye exposed anteriorly consists of a central transparent portion that is the cornea, and a surrounding opaque portion that is the sclera. The sclera is covered with the bulbar conjunctiva, which is continuous with the palpebral conjunctiva lining the inner surface of the protective tissue curtains, the eyelids.

The globe has three main layers, each of which is further divided. The outer supporting coat consists of the transparent cornea, the opaque sclera, and their junction, the corneoscleral sulcus or limbus. The middle vascular

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layer, or the uvea, consists of the choroid, the ciliary body, and the iris, which contains a central opening, the pupil. The inner layer consists of the retina, which is composed of two parts, a sensory portion and a layer of pigment epithelium.

The eye encloses three chambers: the vitreous cavity, the posterior chamber, and the anterior chamber. The vitreous cavity, is located behind the lens and zonule, and is adjacent to the retina throughout.

Light entering the eye passes through the transparent cornea and then the pupil that is a hole whose size is controlled by surrounded by the iris. Behind this is the lens, which focuses light on the retina that is a light-sensitive layer lining at the rear and inside of the eye.

The transparency of the eye may be disturbed by several eye diseases. It has recently been reported that periostin plays a pivotal role in the pathogenesis of several eye diseases such as diabetic retinopathy, age-related macular degeneration, glaucoma, pterygia, corneal dystrophy, and chronic ocular allergic disease, etc.

12.1 Gene Expression Profiling of Epiretinal Membranes

Proliferative vitreoretinal diseases such as diabetic retinopathy (DR), proliferative vitreoretinopathy (PVR), and age-related macular degeneration (AMD) are a leading cause of decreased vision and blindness in developed countries [66]. In those diseases, retinal fibro (vascular) membrane (FVM) formation above and beneath the retina plays a pivotal role in the primary pathology [19, 26, 41]. The FVM formation reflects a wound healing response, but can be refractory if occurring excessively in the eye [30, 31]. Recent technological advancements in genomics have given investigators new opportunities to identify global gene expression in particular tissues in the eye [69, 70]. Therefore, we sought to develop a novel molecular targeting agent based on the gene expression profiling of human epiretinal FVMs (ERMs) [75].

In order to identify genes responsible for intraocular proliferation, we first determined the gene expression profiles of human retina, ERMs associated with proliferative diabetic retinopathy (PDR-ERMs), PVR (PVR-ERMs), or less-aggressive secondary ERMs [1, 23. 69, 70]. We next determined highly expressed genes in PDR- and PVR-ERMs by comparing the gene expression profiles between PDR-, PVR-ERMs and the retina [23], and genes that determine aggressiveness of ERMs by comparing the gene expression profiles between PVR-ERMs and less-aggressive secondary ERMs [1]. The former was subdivided by functional subsets of genes related to extracellular matrix, cell adhesion, proliferation, differentiation and other functions, and the latter was related to cell proliferation and adhesion. Subsequent analyses identified periostin as a pivotal molecule whose expression is up-regulated specifically in proliferating ERMs compared to the retina, and facilitates their proliferation, because it was identified at both comparison procedures.

Periostin, a matricellular protein belonging to the fasciclin family, plays a role in cell motility interacted with integrin αvβ1, αvβ3, and αvβ5 during tissue development and remodeling. Recent studies have demonstrated that periostin is involved in the development of heart valves, tooth, and bone [49, 57] and tumor metastasis [37]. In tissue remodeling, periostin stimulates regeneration of heart tissue after myocardial infraction [5, 52], cutaneous wound healing [46] and chronic allergic inflammation [40].

12.2 Periostin in Diabetic Retinopathy

Diabetic retinopathy (DR) is one of the leading causes of vision loss in the working-age population worldwide [55]. Retinal neovascularization (NV) arises at the advanced stage of DR leading to proliferative DR (PDR) [67]. Vision loss can result from abnormal FVM formation with subsequent intravitreal hemorrhage and tractional retinal detachment [19]. Despite recent advances in vitrectomy techniques [60, 69, 70, 72–74], usage of retinal laser photocoagulation and intravitreal injection of anti-vascular endothelial

growth factor (VEGF), the prognosis for patients with DR is sometimes poor, especially in those with PDR [54].

The mRNA of periostin was detected in all of the10 FVMs obtained from the patients with PDR. In contrast, it was barely detectable in the normal retinas. In addition, RT-PCR yielded multiple bands, indicating the existence of splice variants [42] of periostin in the FVMs. Immunohistochemical analysis exhibited co-localization of α-smooth muscle actin (α-SMA) and periostin in the cells of FVMs [71].

We next examined the amount of periostin in the vitreous samples of PDR patients collected at vitrectomy, and in those of patients during secondary ERM or macular hole (MH) surgery (control) [71]. The concentration of periostin in the vitreous was significantly elevated in the patients with PDR than in the eyes with secondary ERM or MH. The concentration of periostin was significantly associated with the presence of FVMs, suggesting that periostin is closely related to FVM formation in PDR [71].

When we examined the relationship between periostin and VEGF, there was no significant correlation between the vitreous concentrations of periostin and VEGF in the vitreous with PDR [71]. This indicates that periostin and VEGF do not behave in a directly synchronized manner during the development of FVMs. Moreover, compared to VEGF, periostin is presumed to be nonfunctional in normal retinas, because of the very low levels of periostin in the normal control retinas [71].

We also examined the mRNA/protein concentration of periostin in a mouse model of oxygen-induced retinal NV (OIR). The results showed that the level of periostin mRNA/protein in the OIR retinas was significantly elevated at P17 than that in the control retinas [43]. Immunohistochemical analyses of retinal sections exhibited that periostin-positive cells were co-localized with both α-SMA andCD31in the preretinal pathological NVs. In the retinal flatmounts, periostin was colocalized with F4/80. Moreover, periostin was colocalized in the preretinal pathological NVs with CD206 [43]. These findings indicated that the expression of periostin was enhanced in the vascular endothelial cells, pericytes, and M2 macrophages in the preretinal pathological NV of OIR retinas.

To investigate whether periostin alters the ischemia-induced retinal NV, we calculated the size of the neovascular tufts and avascular areas in the OIR retinas of wild-type (WT) mice and periostin knockout (KO) mice at P17. In the OIR retinas, the neovascular tufts represent preretinal pathological NV, whereas the avascular areas indicate the physiological revascularization [21]. The size of the neovascular tufts was significantly reduced in the OIR retinas of periostin KO mice than that in WT mice [43]. The mean avascular area was significantly greater in periostin KO mice than that in WT mice [43]. These results suggested that periostin promotes both preretinal pathological NV and physiological NV in OIR retinas.

In vitro experiments using human retinal microvascular endothelial cells (HREC) showed that periostin stimulated the ischemia-induced retinal NV by Akt phosphorylation via integrin αvβ3 [43].

12.3 M2 Macrophage as a Cellular Source of Periostin

FVMs usually contain different types of cells, such as macrophages/monocytes, hyalocytes, retinal glial cells, fibroblasts, laminocytes and vascular endothelial cells [56]. Among these cells, the macrophages/monocytes have a wide range of biological functions [59, 68]. We have demonstrated that macrophage-attracting chemokines, CCL2, CCL3 and CCL4, played important roles in retinal NV through the recruitment of macrophages/monocytes in a mouse model of OIR [20, 21, 68].

Evidence has been accumulating that macrophages consist of at least two subtypes, classically activated M1 and alternatively activated M2 [39, 53]. The M1 macrophages are proinflammatory and play a pivotal role in driving inflammation, and the M2 macrophages are involved in debris scavenging, NV and fibrosis.

We have demonstrated that there was an increase in the expression of CD163 in the vitre-

ous and FVMs from PDR patients [29]. CD163 is a M2 macrophage marker and showed a close relationship with periostin [29]. The increased expression of CD163 indicated that the M2 macrophages may play a role in the formation of FVMs.

Granulocyte-macrophage colony-stimulating factor (GM-CSF) and macrophage colony-stimulating factor (M-CSF) promote monocyte-macrophage lineage differentiation both in vivo and in vitro [39, 53]. The macrophages differentiated with GM-CSF or M-CSF can be further polarized to more specific cell-types in response to additional stimuli. For instance, when M1-like macrophages differentiated with GM-CSF are exposed to T helper (Th1) cytokines, such as interferon-γ (IFNγ), they are polarized into more activated M1 macrophages and express the M1 cell-surface marker CD80 [38]. In contrast, when human M2-like macrophages differentiated with M-CSF are stimulated by Th2 cytokines, such as interleukin (IL)-4 and/or IL-13, they are polarized into more activated M2 macrophages and express the M2 cell-surface marker CD163 [39].

We have demonstrated that the concentration of M-CSF, but not GM-CSF, was significantly higher in the vitreous of PDR patients than in control patients [73, 74]. An early upregulation of M-CSF signaling of, microglia, glia and neurons in the retinas of diabetic rodents has been reported [36], suggesting that a higher concentration of M-CSF in the vitreous of PDR patients is derived from those cells in the diabetic human retina. In addition, the concentration of M-CSFin the vitreous of patients with PDR was significantly correlated with that of a soluble form of CD163 (sCD163) [73, 74]. Recently, we demonstrated that CD163-positive M2 macrophages were clustered nearby neovascular tufts in a mouse model of OIR [77]. Along with the predominance of M-CSF over GM-CSF in the PDR vitreous, these findings suggest that diabetic retinas are a M2 macrophage-dominant microenvironment.

The concentration of IL-13 was significantly higher in the vitreous of PDR patients than in control patients, but IL-4 was hardly detectable [73, 74]. IL-13 shares many functional roles with IL-4, because both cytokines exploit the same IL-4Rα/Stat6 signaling pathways [78]. However, recent studies have shown a dominant role for IL-13 in the pathogenesis of several fibrotic diseases such as asthma, pulmonary fibrosis and systemic sclerosis [44]. Consistent with these findings, the concentration of IL-13 was significantly related to the existence of FVMs [73, 74], indicating that IL-13 is closely associated with the formation of FVM in PDR patients. Moreover, the expression of periostin in HRECs was significantly upregulated in a dose-dependent manner only by IL-13 [43]. IL-13 was also expressed by CD4-positive cells in the retinas of OIR.

In addition, a higher correlation between the vitreous levels of M-CSF, sCD163 and periostin in eyes with PDR was detected. Finally, the treatment of M-CSF-differentiated human macrophages by IL-13 resulted in a striking induction of CD163 and periostin with very little upregulations of CD80 [73, 74]. These results suggest that the recruited monocytes in diabetic retina may differentiate into M2-like macrophages by M-CSF, and further polarize to activated M2 macrophages which promote the formation of FVMs by producing periostin.

12.4 Periostin in Proliferative Vitreoretinopathy

PVR is a destructive complication of retinal detachment (RD) and vitreoretinal surgeries [33]. PVR is believed to represent a maladapted retinal wound healing process with proliferation of retinal and immune cells, resulting in the formation of scar-like fibrous membranes which may cause tractional RD.

At present, surgical removal of the fibrous membranes and restoration of the physiological conditions are the first treatment option of PVR. Although the success rates of RD surgery was considerably improved by vitrectomy combined with silicone or C3F8 gas tamponade, the surgical treatment for PVR is often unsuccessful.

The development of PVR is a multifaceted process involving cellular and humoral factors. The results of earlier studies demonstrated that

the cells that are critical for the formation of PVR-ERMs are glial cells, retinal pigment epithelial (RPE) cells, fibroblasts, and macrophages/monocytes [18].

Consistent with our global expression analysis [23], periostin mRNA expressions were detected in the PVR-ERMs, but were barely detectable in the normal retinas [22]. The vitreal concentrations of periostin in PVR patients were markedly elevated compared with those in patients with MH; and RD, while the vitreal concentrations of TGFβ2 in PVR patients were also significantly higher than those in patients with MH and RD [22]. Additionally, there was a strong association between the vitreal concentrations of periostin and TGFβ2 in PVR patients. Moreover, Spearman's rank correlation showed that the vitreal concentrations of periostin were significantly correlated with PVR grade.

Immunohistochemical analysis exhibited elongated patterns of periostin expression in PVR-ERMs. In PVR-ERMs, RPE cells expressed periostin andα-SMA as well as integrin αV. These findings suggest that most of the myofibroblasts in the PVR-ERMs are transdifferentiated RPE cells and that periostin and/or integrin αV are predominantly expressed in the RPE cells but not in the glial cells.

In vitro, periostin increased proliferation, migration, adhesion, and collagen production in RPE cells via integrin αV-mediated FAK and AKT phosphorylation [22]. Periostin inhibition suppressed migration and adhesion induced by PVR vitreous and TGFβ2. In vivo, periostin blockade had the inhibitory effect on progression of rabbit experimental PVR without affecting the viability of retinal cells [22].

Although the etiology of PVR is not fully understood, there is considerable evidence that a variety of cytokines and growth factors present in the vitreous regulate the fibrous membrane formation, and those factors promote cellular responses indispensable for PVR, including cell proliferation, adhesion and migration [2, 11, 16, 17, 27]. In a recent study, a cocktail of reagents neutralizing 8 cytokines or growth factors, including transforming growth factor-βs (TGF-βs), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), and PDGF receptor α (PDGFRα), inhibited experimental PVR [48]. In contrast, blocking solely periostin showed a equivalent inhibitory effect on PVR progression. Moreover, periostin blockade alone inhibited PVR vitreous-induced cell migration and adhesion, in spite of the presence of all the other factors in the vitreous. This may be because periostin expression is regulated by those growth factors or their receptors [6, 35]. Therefore, blockade of periostin predominates the deleterious effects of the upstream PVR-driving growth factors.

These results identified periostin as an important molecule for fibrous membrane formation and a promising therapeutic target for PVR.

12.5 Periostin in Age-Related Macular Degeneration

Age-related macular degeneration (AMD) is a leading cause of a severe vision loss in the older population of developed countries [8]. It is estimated that the prevalence of AMD will increase, which would then accelerate both the medical and social burdens of the countries. At the advanced stage of AMD that is choroidal FVMs, which is made up of choroidal neovascularization (CNV) and choroidal fibrosis, can lead to severe vision loss [51]. In this process, there is proliferation, migration and adhesion of various types of cells, including vascular endothelial cells, RPE cells, fibroblasts, glial cells and macrophages/monocytes. There is also deposition of matricellular proteins [3]. Several growth factors, such as VEGFs, placental growth factor, tenascin-C, connective tissue growth factor and TGF-βs and their receptors, are involved in this process [7], 30, 31].

To examine whether periostin is involved in the formation of choroidal FVMs, the expression of the periostin mRNA in the RPE-choroid complexes after laser injury was compared with that in normal RPE-choroid complexes in a mouse model of laser-induced CNV. The expression of periostin mRNA in a mouse CNV model group was significantly higher, compared with the control group and reached a peak on day 14 [41].

Immunohistochemical analyses exhibited periostin-positive staining in RPE65-positive RPE cells after the laser injury. In the human choroidal FVMs, periostin was enhanced in the cytokeratin-positive RPE cells [41]. These findings suggested that the periostin expression was enhanced in the RPE cells both in the FVMs of AMD patients and in the mouse CNV model.

To further examine whether periostin enhances the formation of choroidal FVMs, we quantified the volume of the CNVs at day 7 and fibrous volumes at day 21 in both periostin KO and WT mice. The average CNV volumes in the periostin KO mice were significantly smaller than those of WT mice. There was an approximately 60% reduction in the average fibrosis volume in periostin KO mice than in the WT mice [41]. These findings suggest that periostin is a promoter of choroidal FVMs formation.

12.6 Periostin in Glaucoma

Glaucoma is an optic neuropathy affecting approximately 60 million people worldwide and is the second most common cause of irreversible vision loss. Elevated intraocular pressure (IOP) is the major risk factor for developing glaucoma and is caused by disturbed aqueous humor drainage through the trabecular meshwork (TM) and Schlemm's canal (SC). In primary open angle glaucoma (POAG), the elevated IOP in turn leads to deformation at the optic nerve head (ONH) especially at the lamina cribrosa (LC) region where a deposition of extracellular matrix (ECM) molecules such as collagen and fibronectin occurs [63].

Zhao et al. [76] showed a significant role for periostin in the structural integrity of TM tissues. The collagen fibers in periostin−/− TM were significantly disrupted, with larger empty spaces, representing abnormal distribution or even absence of collagen fibers. In addition, because of lack of support from normal collagen fibers, the trabecular cytoplasm became uneven and showed severe distortions. Overall, these results substantiate a role for periostin in maintaining mechanical strength and structural integrity of TM tissue. Mutation in CYP1B1 has been reported as disease-causative for patients with congenital glaucoma. Notably, antioxidant NAC restored the expression of periostin in Cyp1b1−/− TM cells. In general, periostin expression is determined by Cyp1b1 and the cellular oxidative state, and its proper expression is indispensable for structural development and integrity of TM tissue [76]. Cyp1b1−/− TM tissue was reported to express less periostin than normal Cyp1b1+/+ TM tissue and possessed irregularly distributed collagens. Similarly, human glaucomatous TM tissues expressed decreased levels of periostin in comparison to the levels expressed in normal human TM.

In glaucoma, there are characteristic ECM changes related to optic disc cupping in ONH and subsequent visual field defects. Matricellular proteins including periostin appear to play a role in fibrosis and increased ECM deposition. Importantly, those matricellular proteins are widely expressed in tissues particularly in the ONH in addition to TM. The potential role of periostin in glaucoma is emerging as having an involvement in the pathophysiology of the TM and LC region and might thus be potential targets for treatment intervention in glaucoma [64].

12.7 Periostin in Pterygia

A pterygium is an excessive fibrovascular proliferation of degenerated bulbar conjunctival tissue on the ocular surface. At present, surgery is the only method for treating pterygia, and recurrences are a major complication of the surgical treatment of pterygia. Although several approaches have been tried to minimize recurrences, including amniotic membrane transplantation, recurrences still occur [9].

Kuo et al. [32] have demonstrated that the expression of periostin accurately differentiate primary from recurrent pterygia, and they suggested that the IL-4-induced periostin may play a significant role in the pterygia recurrence.

12.8 Periostin in Corneal Dystrophy

Corneal dystrophies are characterized by the progressive loss of corneal transparency as a result of extracellular amyloid and non-amyloid deposits, which accumulate in different layers of corneal tissues. 5q31-linked corneal dystrophies are pathologically heterogeneous, autosomal-dominant disorders caused by mutations in the TGFBI (transforming growth factor-β-induced) gene, encoding the TGFBI protein (also known as keratoepithelin or Big-H3) [58] To date, many different mutations causing corneal dystrophies have been attributed to mutations in TGFBI, the most frequent of which are mutations within exons 4 and 12, resulting in amino acid substitutions in Arg124 and Arg555, respectively [24]. The different mutations in TGFBI cause clinically different types of corneal dystrophies, which are classified according to the accumulation patterns of the deposits. Those include lattice corneal dystrophies type I and IIIA, deep stromal lattice corneal dystrophy, granular corneal dystrophies (GCDs) type I and II (also known as Avellino corneal dystrophy), Reis-Bucklers corneal dystrophy (also known as corneal dystrophy of Bowman's layer type I), or Thiel-Behnke corneal dystrophy (also known as corneal dystrophy of Bowman's layer type II) [15]. Histological examinations of corneal tissues exhibit the presence of amyloid deposits in lattice corneal dystrophies and GCD II, hyaline accumulations in GCDs, Thiel-Behnke corneal dystrophy, and subepithelial fibrous material in Reis-Bucklers corneal dystrophy.

Kim et al. [25] demonstrated that periostin specifically associates with TGFBI via the NH2-terminal cysteine-rich EMI domain and colocalizes in the trans-Golgi network of COS-7 cells and corneal fibroblasts. In addition, corneal dystrophy-linked mutations in TGFBI disrupt its subcellular localization and impair its association with periostin. Moreover, it was reported that periostin accumulates in extracellular corneal deposits in GCD II patients caused by homozygous R124H mutations in TGFBI. These findings indicate that TGFBI and periostin may play coordinate cellular roles and that periostin might be involved in the pathogenesis of 5q31-linked corneal dystrophies.

12.9 Periostin in Vernal Keratoconjunctivitis and Atopic Keratoconjunctivitis

Clinical courses and responses to treatments are different among several ocular allergic diseases. Seasonal allergic conjunctivitis (SAC) and perennial allergic conjunctivitis, which are associated with acute clinical courses, are in most cases easily treated by topical antiallergic drugs. Therefore, generally speaking, with appropriate treatment, visual disturbance does not occur [34]. In contrast, vernal keratoconjunctivitis (VKC) and atopic keratoconjunctivitis (AKC), which represent chronic and more severe clinical courses, can be associated with corneal changes, tissue remodeling, and fibrosis such as corneal ulcers and the formation of giant papilla, which could result in loss of vision [61]. When patients with VKC or AKC are refractory to first-line treatments including antihistamines, longer use of either topical or systemic corticosteroids or immune-suppressants may be required. Thus, it is very important to diagnose each particular chronic ocular allergic disease and to determine the severity of disease accurately to decide therapeutic strategies in order to prevent severe comorbidities. However, accurately determining the severity of those chronic ocular allergic diseases is sometimes difficult.

Fujishima et al. [12] showed that tears from patients with ocular allergic disease showed significantly higher periostin levels than those from allergic patients without conjunctivitis and from patients with VKC, AKC, and SAC in descending order. Tear periostin was associated with serious comorbidities including large papilla formation and corneal disturbance in AKC, although both tear IL-13 and serum periostin had little or no such abilities. Furthermore, after topical tacrolimus treatment, tear periostin tended to reduce in most patients with AKC, which was accompanied

by their clinical improvement. They concluded that periostin produced in conjunctival tissues, which are stimulated by IL-13, may be involved in the pathogenesis of ocular allergic diseases. In addition, tear periostin can be potentially applied as a biomarker for diagnosing allergic conjunctivitis and evaluating disease severity as well as the efficacy of treatments in AKC.

12.10 Drug Development of Innovative Periostin-Targeting Ribonucleic Acid for Inhibiting Proliferative Vitreoretinal Diseases

RNA interference (RNAi) is a natural mechanism of post-transcriptional silencing of gene expression that has been recently considered to be a novel type of therapeutic system [10]. Because of their high potency and selectivity, RNAi-based therapy has several advantages over conventional therapeutic options including antisense, antibody and aptamer therapy. Moreover, RNAi agents can be easily synthesized, and the processes required for identifying and optimizing them are prompt. However, previous investigations using canonical double-stranded small interfering RNAs (siRNAs) revealed several obstacles such as the adverse off-target effects through Toll-like receptor 3 (TLR3) activation, the lack of a safe drug delivery system (DDS), and the lack of stability [4, 28, 47, 65]. We developed a novel single-stranded RNAi agent, NK0144, targeting periostin, which self-anneals into a distinctive structure containing a canonical double-stranded RNA to overcome these obstacles [43].

12.10.1 In Vivo Inhibitory Effect of Single-Stranded RNAi Agent Targeting Periostin on Retinal Neovascularization

We have demonstrated that this single-stranded RNAi agent which target periostin (NK0144) significantly inhibits the migration and tube formation of HRECs driven by IL-13, and the preretinal pathological NV in OIR retinas by an intravitreal injection without any DDS [43]. In addition, the inhibitory effect of the single-stranded RNAi agent was larger than the canonical double-stranded siRNA (NI0079). Moreover, treatment with NK0144 resulted in a significant increase in the physiological revascularization compared to the treatment control.

The sequence used for periostin knockdown exists not only in human periostin but also in mouse, rat, rabbit, and rhesus macaque periostin [41]. This suggests that NK0144 can be utilized for both in vitro and in vivo experiments and would also be suitable for clinical trials in the future. The actions that determine the differences of the effect on ischemia-induced retinal NV between the single-stranded RNAi and the canonical double-stranded siRNA agent were not completely demonstrated. However, it is demonstrated that the single-stranded RNAi agent has no off-target gene silencing, better stability against nuclease, and no immunostimulatory effects via TLR3 activation [13, 14, 41, 62]. Therefore, intravitreal injection of naked single-stranded RNAi agent targeting periostin may be a safer and a more efficient therapeutic strategy for blocking preretinal pathological NV.

12.10.2 In Vivo Inhibitory Effect of Single-Stranded RNAi Agent Targeting Periostin on Progression of Choroidal FVM Formation

We have also demonstrated that naked NK0144 significantly inhibits the expression of periostin, and proliferation, adhesion and migration of RPE cells without influence on cell viability [41]. Moreover, we observed that labeled single-stranded RNA without any DDS was detectable in the RPE-choroid for at least 5 days after an intravitreal injection [41]. This indicates that it was retained within cells at the CNV site for a considerable period of time. In contrast to canonical double-stranded siRNAs, we found that naked NK0144 significantly inhibited choroidal

FVM formation (both NV and fibrosis) without serious toxicity.

These results strongly suggest that intravitreal injections of naked NK0144 may also be a safer and more efficient therapeutic option to inhibit choroidal FVM.

Although anti-VEGF therapy for PDR and AMD is now a mainstream therapy to prevent retinal and choroidal FVM formation, it was recently reported that anti-VEGF therapy may cause impairment of the normal retinal function and the maintenance of the choriocapillaris [50]. This is partly because VEGF plays a pivotal role in retinal homeostasis. Therefore, therapies that block VEGF to inhibit pathological NV could result in unexpected complications of the normal retina and should be used carefully. In contrast to VEGF, we have demonstrated that periostin was barely detected in the normal retina [23, 71]. We also reported that the correlation between the vitreous concentration of VEGF and periostin was weak in PDR patients [71]. Additionally, previous studies have demonstrated that the binding of VEGF with VEGF receptor-2 (VEGFR2) promoted NV mainly through the PLCγ/PKC/MAPK pathway [45], whereas the binding of periostin with integrin αvβ3 promotes NV mainly via the FAK/Akt pathway [22, 43]. These are good evidences of the concept that anti-periostin therapy may have independent effects on retinal and choroidal FVM formation from anti-VEGF therapy. Therefore, periostin may be an interesting therapeutic target to regulate "disease-specific" pathways involved in the formation of retinal and choroidal FVM, while minimizing the unfavorable side-effects on the normal retina. Additional preclinical studies regarding the stability, toxicity and effect of duration are underway for establishing the novel periostin-targeting RNAi agent for combating retinal and choroidal FVM formation.

12.11 Conclusions

The expression of periostin after environmental perturbations, including IL-4 and/or IL-13 induction, can alter normal physiological interactions among fibroblasts, macrophages and ECM proteins. As shown in this chapter, accumulating evidence has suggested that periostin plays central roles in the pathogenesis of fibrosis and tissue remodeling in several diseases of the eye, as well as those of other organs. Therefore, modulating the expression of periostin by low-molecular weight compounds, antibodies or RNAi directed against the molecule could be a novel therapeutic strategy for inhibiting the progression of those several periostin-involved eye diseases. Periostin can also be potentially applied as a biomarker to diagnose eye diseases and to evaluate disease severity as well as the efficacy of treatments.

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The Multiaspect Functions of Periostin in Tumor Progression

13

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Abstract

Extracellular matrix protein periostin is highly expressed in various tumors and plays a critical role in tumor development and progression. Periostin is mainly secreted by stromal cells such as cancer-associated fibroblasts, myofibroblasts, osteoblasts and bone marrow-derived mesenchymal stromal cells. But in some cases, tumor cells, especially cancer stem cells, can also produce periostin. Periostin has been shown to regulate multiple biological behaviors of tumor cells, including proliferation, survival, invasion, angiogenesis, metastasis and chemoresistance. Moreover, an excessive periostin deposition exerts a pivotal role in remodeling various tumor microenvironments, such as cancer stem cell niche, perivascular niche, premetastatic niche, immunosuppressive microenvironment, bone marrow microenvironment and other tumor growth-supportive microenvironments. In this review, we provide an update understanding of the multifaceted functions and mechanisms of periostin in tumor development and progression.

Keywords

Periostin · Tumor microenvironment · Cancer stem cell niche · Perivascular niche · Premetastatic niche · Fibrotic microenvironment · Metastatic niche · Bone marrow

13.1 Introduction

Matricellular proteins are a set of nonstructural extracellular matrix (ECM) proteins that are generally expressed at low levels in most adult tissues

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but are highly induced at sites of inflammation, injury and tumors within organs [5, 31, 42]. As a matricellular protein, periostin plays a vital role in embryonic development and tissue repair and also is involved in multiple pathological processes of various diseases such as inflammatory diseases, fibrosis and tumor progression [8, 25, 26, 31]. Current studies have demonstrated that periostin can regulate cancer cell proliferation, survival, angiogenesis, invasion and metastasis by interacting with its cell-surface receptor integrins, which will influence intracellular signal pathways [55]. Moreover, periostin acts as an important extracellular adhesion molecule to mediate communications between the cells, and the cells and their extracellular microenvironments. In most cases, periostin is mainly secreted by stromal cells, especially cancer-associated fibroblasts (CAFs), myofibroblasts, osteoblasts and bone marrow-derived mesenchymal stromal cells (BM-MSCs), but in some cases periostin can be produced by tumor cells, especially cancer stem cells (CSCs) [9, 27, 31, 35]. In addition, periostin exerts pivotal roles in establishing and remodeling various tumor microenvironments to promote cancer cell survival and proliferation, and facilitate their further progression [9]. Here, we summarize the multifaceted functions and molecular mechanisms of periostin in tumor progression.

13.2 The Expression and Biological Functions of Periostin in Tumors

Periostin has been reported to be abnormally expressed in various human tumors, including colorectal cancer (CRC), breast cancer, pancreatic cancer, glioma, gastric cancer, lung cancer, liver cancer, leukemia and other cancers. Periostin also can modulate multiaspect biological behaviors of tumor cells during the development and progression of these tumors [9, 31, 55].

13.2.1 Colorectal Cancer

We have demonstrated that periostin is overexpressed in more than 80% of human primary colon cancers, and its level is even higher in the corresponding hepatic metastatic tumors. Moreover, periostin enhances metastatic growth of colon cancer by promoting cell survival and angiogenesis via AKT pathway [1]. Another study further confirmed the upregulation of periostin expression in primary CRC and liver metastases [62]. ELISA analysis showed that the serum levels of periostin in CRC patients are significantly higher than those in healthy volunteers and patients of benign colorectal polyps or adenomas. Higher preoperative serum levels of periostin in CRC is considered to be related with distant metastasis, advanced stage disease and poor prognosis [3]. Interestingly, the levels of intratumoral stromal periostin expression, rather than epithelial periostin expression, can be used to predict unfavorable prognoses of CRC independently. Moreover, colonic fibroblasts-derived periostin significantly promotes CRC cell proliferation, invasion and chemoresistance [73].

13.2.2 Breast Cancer

Periostin is highly expressed in breast tumors and can enhance tumor growth of breast tumor cells by promoting angiogenesis through increasing the expression of VEGF2 [60]. Malanchi et al. [36] further found that periostin is highly expressed in CAFs of mouse mammary tumors and their metastatic lungs. Periostin deficiency significantly inhibits the metastatic outgrowth of mammary tumor cells in the lungs but shows no markedly inhibitory effect on the growth of primary tumors [36]. We also found that periostin overexpression promotes tumor growth and lung metastasis of breast cancer cells in mice [67]. Survival analysis of databases of breast cancer patients showed that high expression of periostin predicts a decreased distant metastasis-free survival probability in ER-negative breast cancer [68] and a significantly higher risk of recurrence specifically in basal-like breast cancer [44]. Moreover, chemotherapy upregulates the expression of tumor-specific variants of periostin in breast tumors and periostin knockdown inhibits the outgrowth and invasion of mesenchymal tumor cells upon chemotherapy [44].

Periostin expression was mainly observed in CAFs [36, 68] and its level in CAFs of invasive ductal breast carcinoma is significantly higher than that of ductal carcinoma in situ [54]. Serum periostin was found to be detectable in early breast cancer and high levels of serum periostin correlate with breast cancer-specific mortality of patients [47]. Serum periostin levels are markedly increased in mice and patients with bone metastases from breast cancer [7, 57]. Interestingly, the serum C-terminal intact form of periostin was thought to assist in detecting bone metastasis formation of breast cancer in the experimental bone metastasis model [16]. Periostin was identified as a marker that is enriched in exosomes derived from breast cancer using proteomic analysis [64].

13.2.3 Pancreatic Cancer

In invasive ductal adenocarcinoma of pancreas, periostin was mainly observed in the stromal lesion adjacent to infiltrating cancer cells, whereas in the non-neoplastic pancreas, no positive periostin can be detected in epithelial cells of the pancreas and stromal cells around normal pancreatic ducts [14]. Similarly, other studies also demonstrated that periostin is mainly expressed in tumor stroma of pancreatic cancer [12, 21]. Moreover, neoplastic stroma-derived periostin is significantly correlated with the depth of invasion as well as lymph node metastasis of pancreatic cancer [4]. Baril and colleagues found that the serum level of periostin is significantly increased in pancreatic cancer patients compared to non-cancer controls. Periostin can promote the invasion of pancreatic cancer cells and enhance the survival of pancreatic cancer cells exposed to hypoxic conditions via the activation of AKT pathway [2]. In addition, increased periostin expression in pancreatic cancer cells promotes the tubule formation of human umbilical vein endothelial cells, and ERK phosphorylation and VEGF expression are increased after recombinant periostin treatment, while opposing results were observed when silencing periostin [32]. However, a study revealed that periostin has a biphasic effect on pancreatic cancer cells: the low concentration (150 ng/ml) of periostin reduces cell migration and metastasis, whereas the high concentration (1.5 μg/ml) of periostin promotes cell migration [21]. Recently, Nielsen and colleagues demonstrated that tumor-associated macrophages (TAMs) can secrete granulin to activate hepatic stellate cells into myofibroblasts, and then myofibroblasts secrete periostin and other factors to promote the formation of fibrotic microenvironment to facilitate metastatic outgrowth of pancreatic cancer cells in the livers [45].

13.2.4 Glioma

In glioma tissues, periostin is expressed in both tumor cells and stroma, but stroma exhibits higher expression than tumor cells. Moreover, the expression of stromal periostin is positively correlated with glioma grade and contributes to a poor prognosis by enhancing cell invasion and proliferation among high-grade glioma patients [66]. Another report demonstrated that the level of periostin is directly relative to glioma grade and recurrence, and inversely correlate with survival time in adult human glioma [38]. Periostin promotes glioma cell migration and adhesion through interacting with αvβ3 and αvβ5 integrins, and knockdown of periostin remarkably impairs the survival of xenografted glioma stem cells [38]. Zhou et al. found that glioma stem cell-derived periostin can recruit M2 type of TAMs via integrin αvβ3 to enhance glioma growth. Periostin knockdown in glioma stem cells impairs TAM recruitment, inhibits tumor growth and increases survival of mice xenografted with glioma stem cells (GSCs) [77]. A recent study demonstrated that silencing periostin inhibits U-87 glioblastoma cell invasion and EMT by decreasing fibronectin and vimentin expression and partly by reducing Smad2, AKT and FAK phosphorylation [49].

13.2.5 Gastric Cancer

In comparison with normal gastric tissues, periostin is significantly overexpressed in gastric cancer tissues and metastatic lymph nodes [28]. In gastric cancer tissues, higher periostin expression was found in stromal myofibroblasts but not in carcinoma cells [24]. Kikuchi and colleagues further demonstrated that co-cultured periostin-expressing NIH3T3 cells significantly promote gastric cancer cell proliferation through ERK activation. However, periostin deficiency impairs tumor outgrowth of gastric tumor cells inoculated in the gastric wall of mice [24].

13.2.6 Lung Cancer

Morra and colleagues reported that both tumor stroma and epithelia are the source of periostin production in non-small cell lung cancer (NSCLC) and epithelial periostin expression is correlated with higher tumor grade [40]. Interestingly, five out of eight isoforms were detectable in NSCLC and the matched normal lung tissues [40]. Serum periostin protein levels were shown to be remarkably increased in NSCLC patients than those in healthy controls and benign lung diseases patients. Cox regression analysis further demonstrated that serum periostin is an independent prognostic factor of NSCLC patients [74]. We previously found that periostin expression can be induced by TGF-α and bFGF under the stress of chemical-mimic hypoxia in A549 NSCLC cells and that periostin promotes the survival of A549 cells via activation of AKT pathway [50]. Periostin also can promote epithelial-mesenchymal transition in lung cancer by activating p38/ERK pathway and repressing the expression of microRNA-381 which targets both Snail and Twist [18]. Additionally, periostin is significantly increased in cisplatin-resistant A549 cells compared with parent cells, and overexpressing periostin renders A549 cells more resistant to cisplatin-induced apoptosis via activation of STAT3 and upregulation of Survivin [17].

13.2.7 Liver Cancer

We have found that periostin is highly induced in the liver of mice with various diseases, including acute and chronic liver inflammation, liver fibrosis and non-alcoholic steatohepatitis (NASH), indicating that periostin is a critical contributor in various inflammatory liver diseases [19, 29, 70]. We also observed a high level of periostin in regenerating liver of mice after 2/3 partial hepatectomy and a significantly elevated plasma periostin level in hepatocarcinoma patients undergoing resection of liver tumors [71]. Immunohistochemical analysis of tissue microarrays revealed that the expression of periostin is up-regulated in hepatocellular carcinoma (HCC) tissues compared to corresponding normal tissues [20]. Higher periostin expression is associated with positive microvascular invasion, more frequent multiple tumors, advanced stage disease and poor prognosis [20, 34]. A study related to HCC angiogenesis demonstrated that sulfatase 2 (SULF2)-induced HCC angiogenesis is dependent on periostin and that knockdown of periostin in HCC cells reduces SULF2-induced angiogenesis and tumor growth *in vivo* [6]. Additionally, periostin expression in HCC cells is significantly increased after hypoxic treatment, and periostin is attributed to arsenic trioxide resistance in HCC cells under hypoxic condition [33].

13.2.8 Leukemia

Recently, we also found that periostin is overexpressed in non-solid tumors [35]. Periostin expression is significantly elevated in the bone marrow from B-cell acute lymphoblastic leukemia (B-ALL) patients compared to healthy controls. Similarly, both periostin mRNA and protein expressions are significantly increased in the bone marrow of mice models after injection with human or mouse B-ALL cells. Interestingly, periostin was highly expressed in BM-MSCs but not in B-ALL cells. Furthermore, periostin promotes the proliferation and colony formation of

both human and mouse leukemia cells, and periostin-deficient mice injected with B-ALL cells show much lower leukemia burdens than wild-type counterparts [35].

13.2.9 Other Cancers

High periostin expression was also detected in other cancers including ovarian cancer, melanoma, prostate cancer, clear renal cancer and oral squamous cell carcinoma [39, 46, 56, 61, 63]. In most cases, periostin plays a tumor-promoting role in the development and progression of these tumors.

13.3 The Roles of Periostin in Tumor Microenvironments

Malignant tumor formation and progression are extremely sophisticated pathological events, which can be triggered and regulated by the crosstalk between tumor cells and their surrounding tumor microenvironments [53, 58]. Tumor microenvironments are complicated dynamic microenvironments which are formed during tumor development and metastasis, and consist of many different stromal compartments including various stromal cells, immune cells and ECM components. As a non-structural ECM protein, periostin has been revealed to exert critical roles in various tumor microenvironments, including CSC niche, perivascular niche, premetastatic niche, immunosuppressive microenvironment, as well as bone marrow microenvironment and other tumor growth-supportive microenvironments, to promote the survival and proliferation and fitness of primary or disseminated tumor cells (Fig. 13.1).

13.3.1 Cancer Stem Cell Niche

CSCs are a subpopulation of tumor cells which have the abilities of self-renewal and differentiation and driving tumor initiation and progression [65]. Several studies reported that periostin can induce or sustain stem cell-like phenotype of cancer cells in tumor progression [27, 36, 67, 72]. Malanchi and collogeaues identified periostin as a novel component in breast CSC niche using the MMTV-PyMT mouse breast cancer model. Specifically breast CSC-derived TGF-β can induce CAFs to secrete periostin into the CSC niche in which periostin will recruit Wnt and augment Wnt signaling in CSCs. That is to say, cancer-associated fibroblast-derived periostin is a critical component in breast CSC niche [36]. An analysis involved with periostin expression status and CSC ratio further revealed that periostin is related to breast CSCs and can be served as an independent prognostic factor in clinical breast cancer [72]. We also found that periostin promotes a stem cell-like trait and a mesenchymal phenotype in human mammary epithelial cells and breast cancer cells, thereby enhances breast tumor growth and lung metastasis [67]. Additionally, in basal-like breast cancer, periostin secreted by tumor cells can maintain breast CSCs by regulating the expression of IL-6 and IL-8 cytokines [27]. Interestingly, a recent study found that periostin derived from activated hepatic stellate cells induces stem cell-like phenotype of heat-exposed HCC cells via activation of integrin β1/AKT/GSK-3β/β-catenin/TCF4/Nanog signaling pathway [76].

13.3.2 Perivascular Niche

The perivascular microenvironment is crucial for tumor cells to sustain survival in microvasculature of primary and distant organs and to form secondary metastatic colonization after a period of latency [37, 41]. Bissell and colleagues identified endothelial tip cell-derived periostin as one of tumor-promoting factors in perivascular microenvironment [15]. They applied the model of orthotopic injection of metastatic breast cancer cells expressing GFP and then resected the primary tumors. Several weeks later, they observed that small clusters of GFP-positive breast cancer cells at the quiescent state directly reside on microvascular endothelium of lung and bone marrow [15]. These endothelial cells express a

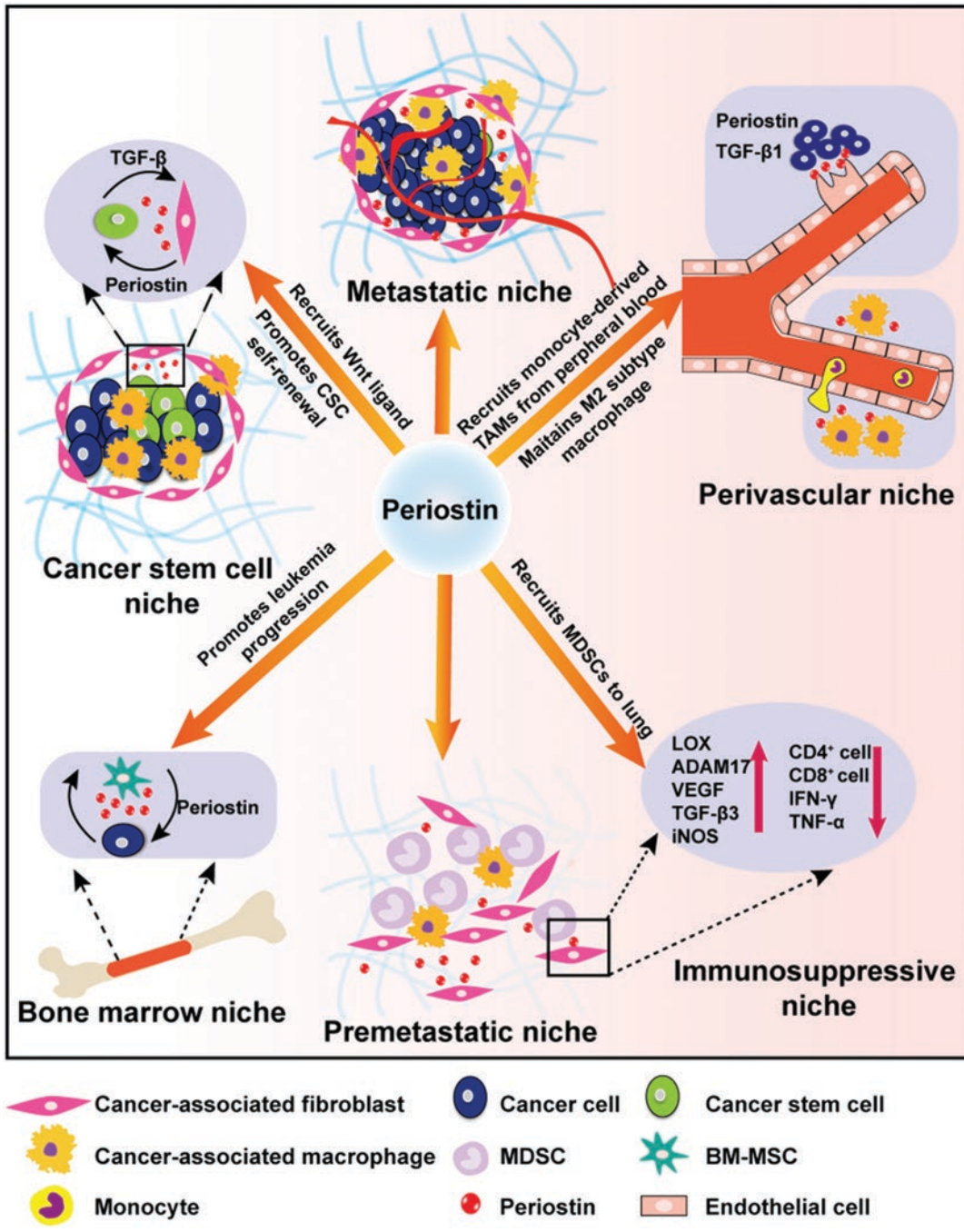

Fig. 13.1 The roles of periostin in tumor microenvironments. CAF-derived periostin promotes tumor outgrowth in metastatic niche. In cancer stem cell niche, cancer stem cells secrete TGF-β to induce periostin expression in CAFs and subsequently periostin recruits Wnt ligands to promote self-renewal of breast cancer stem cells. In the perivascular niche, sprouting neovasvular-derived periostin and TGF-β promote micrometastatic outgrowth of disseminated breast cancer cells, and glioblastoma stem cell-derived periostin augments tumor outgrowth by recruiting monocytes-derived TAMs from peripheral blood. Moreover, periostin promotes the formation of premetastatic niche in the lungs to facilitate subsequent breast tumor growth in metastatic sites. Periostin also recruits MDSCs to the lungs to create an immunosuppressive microenvironment for breast cancer cells. In bone marrow microenvironment, BM-MSC-derived periostin contributes to leukemia progression

high level of TSP-1 and form a perivascular niche to induce and sustain breast cancer cell quiescence. However, neovascular tips cells in sprouting neovasculature secret periostin, TGF-β1 and other factors to form a distinct perivascular niche to accelerate tumor growth of breast cancer cells. These data demonstrate that the mature microvascular niche provides inhibitory cues to sustain quiescence of disseminated tumor cells, whereas the sprouting neovascular niche provides stimuli such as periostin and TGF-β1 to promote metastatic outgrowth of disseminated breast tumor cells [15]. Interestingly, perivascular niche is also one of important resident sites for CSCs. Bao and colleagues demonstrated that GSCs in perivascular niche secret periostin to recruit TAMs for accelerating tumor growth [77].

13.3.3 Premetastatic Niche

Systemic cues from primary tumors and tumor-mobilized bone marrow-derived cells, and their induced stromal factors in predetermined metastatic sites contribute to the formation of premetastatic niche prior to the arrival of tumor cells [30, 51, 69]. We previously found that periostin is not only highly expressed in metastatic lungs but also in premetastatic lungs of MMTV-PyMT mice. CAF-derived periostin can promote the formation of premetastatic niche in the distant lung tissues to facilitate pulmonary metastasis of breast cancer [68]. The orthotopical model further shows that periostin is highly induced in the premetastatic lungs of mice after orthotopically injected with breast tumor cells for 5 weeks, at which point the disseminated breast tumor cells are not detectable in the lungs. We further intraperitoneally injected the conditional media from human breast tumor cells into tumor-free nude mice for 14 days and found that ectopic periostin overexpression or recombinant periostin treatment promotes the formation of premetastatic niche in the lungs. Moreover, periostin deficiency inhibits the formation of premetastatic niche in the lung of MMTV-PyMT mice and suppresses lung metastasis of breast tumor cells [68]. Therefore, our data provide direct evidence that periostin is a novel component of premetastatic niche [68] rather than only a contributing factor in metastatic niche in previous studies [36, 65]. Interestingly, similar to the function of the high level of periostin in premetastatic niche induced by primary tumors, tissue injury-induced periostin also contributes to tumor metastasis. Periostin and other ECM proteins such as type 1 collagen and fibronectin which are significantly increased at the wound site are involved in attracting remotely transplanted melanoma cells to come and form metastatic lesions [13]. Another recent study found that periostin is widely expressed in bleomycin-induced lung fibrotic lesions and that bleomycin-induced lung fibrosis markedly promotes lung metastatic colonization of B16 melanoma cells. Periostin antisense oligonucleotide significantly reduces bleomycin-induced lung fibrosis and lung metastatic colonization of melanoma cells [59]. These data suggest that both the high level of periostin in premetastatic niche induced by primary tumors and the induced periostin in injured tissues promote metastatic outgrowth in distant locations.

13.3.4 Tumor Immunosuppressive Microenvironment

Myeloid-derived suppressor cells (MDSCs) are a kind of critical component that plays an immune suppressive role in facilitating tumor progression. Recent report demonstrated that neutrophils not only contribute to the formation of metastatic niche but also can be found in premetastatic niche [69]. We revealed that both MDSCs and periostin are critical components in premetastatic niche and that periostin promotes the formation of immunosuppressive premetastatic niche and immunosuppressive metastatic niche. Periostin deficiency suppresses the accumulation of MDSCs in the lungs and decreases their immunosuppressive functions, which impairs the formation of premetastatic niche and subsequently reduces pulmonary metastatic colonization of breast cancer cells [68]. Our findings demonstrated that periostin contributes to immunosuppressive niche formation in pulmonary

metastasis of breast cancer by promoting the accumulation of MDSCs in the lungs and increasing immunosuppressive functions of MDSCs through activation of ERK, AKT and STAT3 [68].

13.3.5 Bone Marrow Microenvironment

The bone marrow microenvironment is composed of various types of hematopoietic cells and their progeny cells, stromal cells, and abundant blood vessel and various ECM components. Bone marrow microenvironment is also an important reservoir for disseminated solid tumor cells. Current evidence supports that the interplay between tumor cells and surrounding bone marrow microenvironment is critical for leukemia progression and bone metastasis of solid tumors [11]. As an ECM protein, periostin plays a critical role in maintaining hematopoietic stem cells [22]. As mentioned above, our recent study demonstrated that the bone marrow of both human B-ALL patients and B-ALL mouse models exhibits a high level of periostin and that periostin is mainly expressed by BM-MSCs [35]. Additionally, periostin is highly expressed by stromal cells in metastatic bone of breast tumor [7]. These data suggest that bone marrow-derived periostin might contribute to remodeling bone marrow microenvironment to help leukemia cells or disseminated solid tumor cells, which survive and subsequently outgrow and metastasize.

13.3.6 Other Tumor Growth-Supportive Microenvironments

Periostin is mostly secreted by stromal cells in above mentioned tumor microenvironments. CAF-derived periostin contributes to constitute a tumor growth-supportive microenvironment in tumor progression. Another kind of stromal cell, myofibroblast-like stellate cells are specifically present in the liver and pancreas [48]. Pancreatic stellate cells (PSCs) interplay with cancer cells and other stromal cells to form a tumor growth permissive microenvironment during pancreatic cancer progression [52]. Interestingly, several studies reported that PSCs exhibit much higher periostin expression, and cancer cells can induce periostin secretion from PSCs [12, 21]. Moreover, periostin secreted by PSCs can increase the expression of various ECM proteins, including collagen, fibronectin and TGF-β1, which create a tumor-supportive microenvironment and contribute to accelerating tumor growth and increasing resistance of cancer cells to hypoxia and starvation [12]. However, currently, there are few or no reports about the functions and mechanisms of periostin in hypoxic microenvironment, acidified microenvironment, invasion front niche, fluid microenvironment and stiff tissue microenvironment.

13.4 Conclusion

Tumor initiation and progression is an extremely complex biological process which is involved with aberrant expression and functional dysregulation of numerous genes. As an ECM protein, periostin is highly expressed in various tumors. Periostin expression in tumor tissues was usually found to be correlated with tumor grade, clinical stage and prognosis of patients. Many studies also elucidate the relationship of serum periostin levels and clinical pathological parameters in various cancers [47, 7, 10, 74]. These findings suggest that periostin might be a potential valuable biomarker for diagnosis and prognosis of tumors. Therefore, accurate quantification of periostin levels in tissues or in sera and standardization of technically detective procedure should be further specified and defined. In addition, stringently-controlled clinical trials in large cohort of patients treated homogenously should also be necessary to consolidate previous findings and to confirm whether periostin could be as a reliable biomarker for diagnosis and prognosis. The sensitivity and specificity of periostin used as a clinical biomarker is another issue which needs to be considered and clarified. It is important to note that full-length periostin and eight

splice variants have been investigated in humans [26]. A few reports described different splice variants patterns of periostin in different diseases [16, 39, 40, 43, 75]. However, a series of questions related to periostin splice variants, including their expression patterns, tissue specificity and their different biological functions, have not been well clarified. The sensitivity and specificity of periostin antibodies to different periostin isoforms also require further investigation.

Considering the multifaceted roles of periostin in tumor cell proliferation, survival, angiogenesis, invasion, metastasis and chemoresistance, periostin might be a promising therapeutic targeted molecule in future clinical application. Several studies showed that periostin secreted from stromal cells or tumor cells exerts biological effects on tumor cells by interacting with integrins of tumor cells and subsequently activating intracellular downstream signal pathways [9, 31]. Thus, it is possible for us to design and synthesize small chemical molecules or inhibitors of antigen epitope domains and integrin receptors, which can target periostin function so as to inhibit tumorigenesis and metastasis or reverse drug resistance. Again, while designing targeted molecules, the issue related to periostin isoforms should be concerned. Understanding different isoforms should be rather essential and meaningful for probing molecules targeting periostin.

As a secretory protein, periostin can directly exert its functions on tumor cells in paracrine or autocrine mode. Moreover, periostin actively contributes to establishing and remodeling various tumor-supportive microenvironments for tumor cell progression. Nevertheless, the specific molecular mechanisms about how periostin remodels distinct tumor microenvironments still have not fully clarified. Similarly, the role of different periostin isoforms in remodeling tumor microenvironments also needs to be further investigated. Periostin functions as a scaffold for the assembly of highly sophisticated extracellular architectures via interacting with other ECM proteins including fibronectin, tenascin, collagen and itself [23, 26, 31]. Thus, identification of novel interacting proteins of periostin by high-throughput techniques such as proteomic analysis will be an interesting topic. Targeting these protein-protein interactions is also one of promising approaches for drug screen.

Taken together, current studies have revealed that periostin is aberrantly expressed in various tumors and can regulate multiaspect biological behaviors of tumor cells during tumor development and progression. Future studies will further uncover the underlying molecular mechanisms of periostin in tumor progression and will provide potential drug targets against tumors.

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Part V

Other Types of Fibrosis and Tissue Repair

Liver, Stroke, Rhinosinusitis

14

Akira Kudo

Abstract

In addition to main types of fibrosis, other types of fibrosis in incredible diseases have been reported. Among them, liver fibrosis, stroke, and rhinosinusitis are described as new cases of periostin action. To understand periostin function in a new area of fibrosis linked with inflammation, periostin can be used as a new tool to elucidate the mechanisms of unknown diseases.

Keywords

Liver fibrosis · Stroke · Rhinosinusitis · Uterine fibroid · Muscular dystrophy

14.1 Liver Fibrosis

Liver fibrosis occurs due to chronic liver diseases, the leading causes of death, are derived from viral hepatitis, alcoholism, toxins, and autoimmune diseases [1]. Liver fibrosis is characterized by deposition of a collagen-rich extracellular matrix that alters tissue architecture by forming a fibrous scar [9]. In this fibrosis, inflammation is the essential initial event, inducing apoptotic death of hepatocytes and recruitment of inflammatory leukocytes and macrophages that produce cytokines and pro-fibrogenic factors, which activate collagen-producing hepatic stellate cells.

In this fashion, periostin promotes hepatic fibrosis by activating hepatic stellate cells through αv integrin [26] and TGF-β1 [8], and the synergistic effect of pro-inflammatory TNFα and IL-17 induces periostin expression [1]. Furthermore, granulin secreted from macrophages activates hepatic stellate cells into myofibroblasts that secret periostin [20]. During liver fibrosis development, the interaction between periostin and angiotensin II plays a roll, because the renin-angiotensin system is a key mediator of arterial blood pressure and body fluid homeostasis and serves an important role in the regulation of local hemodynamics in organs [27]. In liver fibrogenesis, periostin promotes collagen cross-linking by activating LOX and LOXL for enhancement of matrix stiffness [14]. Interestingly, in a recent observation, periostin was shown to be involved in liver regeneration [28].

14.2 Stroke

Periostin is expressed in neurons in normal brain. During cerebral ischemia, its expression is decreased temporarily in the ischemic region

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and increased in astrocytes and surrounding endothelial cells [23]. During intracerebral hemorrhage, which occurs in about 10% of all strokes, serum periostin concentrations are positively related to National Institutes of Health Stroke Scale score and hepatoma volume [12]. This is consistent with the observation that periostin is increased in the serum of patients after large-artery atherosclerotic stroke [7]. During subarachnoid hemorrhage, periostin is induced in brain capillary endothelial cells and neurons. Furthermore, periostin antibody treatment was found to improve neurobehavior, brain edema and blood-brain barrier disruption [15].

Spinal cord injury, another type of CNS injury, causes persistent, severe motor and sensory dysfunction, in which astrocytes that are resident cells in CNS play crucial roles in astrocytic scar formation [6]. In injured spinal cords, periostin expression was observed in addition to a high expression of type I collagen [6]. Similar to myocardial infarction, astrocytic scar formation competed with axonal regeneration, indicating that astrocytic scars inhibit axonal regeneration. After spinal cord injury, phagocytic activity of microglia and macrophages is enhanced, and TGF-β signaling contributes to neuronal survival and axonal regeneration. In this case, periostin expression is induced to repair tissues, and then excessive function of periostin accelerates fibrogenesis to induce astrocytic scar formation coupled with type I collagen production, and N-cadherin-mediated cell-cell adhesion in astrocytes through integrin signaling is essential. During axonal regeneration, BMP-induced astrocytes derived from embryonic glial-restricted precursors, in which periostin is expressed, promote extensive axonal growth and motor function recovery after spinal cord injury, indicating that periostin plays an essential role [22]. Furthermore, periostin significantly enhances proliferation and differentiation of neural stem cells after hypoxic-ischemic injury [17], and exhibits neurite outgrowth activity in cortical neurons, since periostin shows prolonged and increased expression in the primate cerebral cortex [18].

14.3 Rhinosinusitis

Sinusitis is one of the most commonly diagnosed diseases in United States (US), affecting 37 million people every year [25]. A severe phenotype of chronic rhinosinusitis (CRS)—CRS with nasal polyp—greatly impairs the quality of life and occurs in 1–4% of the general US population [29]. Nasal polyp formation in chronic rhinosinusitis is associated with upregulation in Th-2 immune response along with IL-4, IL-5 and IL-13, [19]. Periostin is induced with IL-4 and IL-13, suggesting that periostin is possibly a factor for polyp formation as well as a good marker for CRS. Firstly, gene expression profiling was performed to reveal the genes involved in CRS and aspirin-sensitive asthma (ASA), indicating enhancement of periostin gene expression in patients together with the *met* proto-oncogene and phosphatase 1 regulatory subunit 9B [25]. In other marker surveys of CRS, periostin coupled with pendrin [10] or osteopontin [5] was found. Periostin can be further developed as a marker for CRS in patients, since serum periostin has been shown to be a novel biomarker for the presence of nasal polyps [19] and another marker for CRS patients with asthma [2]. Moreover, serum periostin coupled with IgE and SE-IgE is used as a biomarker to identify moderate to severe CRS with nasal polyps [13]. The histological analyses revealed that periostin expression was observed in nasal polyps, with the diffuse type as the major one (85%), in which periostin was expressed throughout the lamina propria. In the superficial one which is a minor one (12.5%), it was detected in the subepithelial layers [24]. Furthermore, functional analyses of periostin for poly formation in CRS indicated that periostin plays an important role in the occurrence and progression of eosinophilic nasal polyp, because periostin accelerates eosinophil recruitment and activation in inflammation [30].

14.4 Other Fibrotic Diseases

Uterine fibroids are the most prevalent tumors in woman, occurring in over 70% of pre-menopausal women. In all sizes of human uterine fibroid

(small, medium, and large), periostin expression is preferentially up-regulated along with tenascin and interestingly TGFB1, indicating that periostin is a hallmark of uterine fibroids [11]. In acute cruciate ligament (ACL) injury, a high level of periostin in the first 3 months after injury is shown, and extracellular matrix organization is affected after more than 12 months post-injury [3]. This delayed ACL reconstruction increases the risk of meniscus and cartilage injuries [4]. Oral lichen planus (OLP) is an inflammatory disease affecting oral mucosa that occurs in 0.5–2.0% of the general population. Periostin expression is increased in the oral mucosa and serum of OLP patients, indicating that periostin is a good biomarker for early diagnosis and treatment of OLP [31].

Muscular dystrophy (MD) broadly encompasses a diverse group of genetic disorders, resulting in loss of muscle fiber. In MD, TGFβ leads to increased fibrosis in human dystrophic muscle. Therapeutic manipulation of periostin may offer alternative MD disease progression when TGFβ responsiveness is targeted, since deletion of periostin improves muscular dystrophy [16]. Similarly, deletion of periostin improves atherosclerosis, indicating that periostin is a regulator of atherosclerotic lesion formation and progression [21].

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Part VI

Inflammation

Roles of Periostin in Asthma

15

Hisako Matsumoto

Abstract

Periostin is a matricellular protein that is deeply involved in type-2/eosinophilic airway inflammation and remodeling in asthma. While its expression in airway epithelial cells is correlated with the thickness of airway basement membrane, more importantly, periostin can be detected stably in blood with little variability, reflecting airway type-2 inflammation and remodeling. As for a result, serum periostin can serve as a valuable marker to identify patients with type-2 severe asthma who are insensitive to inhaled corticosteroids, and consequently have the excess decline of pulmonary function with asthma exacerbations. Serum periostin may significantly help to improve management of patients with severe asthma.

Keywords

Serum periostin · Biomarker · Severe asthma · Type-2 inflammation · Airway remodeling · Airflow limitation · Eosinophilic chronic rhinosinusitis · *IL4RA*

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15.1 Introduction

Asthma is a heterogeneous disease, with several different subtypes, each of which may be caused by different pathophysiologic mechanisms. Nonetheless, a majority of asthma cases are driven by type-2 airway inflammation, which is often defined as sputum or tissue eosinophilia coupled with the gene-expressions upregulated by interleukin (IL)-4 and IL-13 signaling. Chronic type-2 airway inflammation is often accompanied by airway remodeling in a physiological consequence of significant airflow limitation.

Owing to the widespread use of inhaled corticosteroids (ICS) and related medications, most asthmatics have been well controlled now for several decades, but nonetheless 5–10% of patients remain outliers in this context and need intensive care, such as the use of biologics against type-2 inflammation [1]. Although such medications opened a new era for the management of severe asthma, not all patients respond equally to them. Therefore, it is necessary for the effective management of severe asthma to accurately identify type-2 inflammatory subtypes and patients who can successfully respond to these new biologics. Periostin, which is a matricellular protein, is a key molecule that notably links type-2 airway inflammation and airway remodeling. From a clinical standpoint, it is very fortunate that periostin can be readily detected in the blood, as it

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reflects airway type-2 inflammation and remodeling [2–4]. In this chapter, we first describe the several important roles of periostin in the asthmatic airways, before examining the way how serum periostin can be useful in treatment of severe asthma.

15.2 Roles of Periostin in Asthma

15.2.1 Periostin in Airway Inflammation and Remodeling in Patients with Asthma

Airway inflammation and remodeling are the well-known cardinal features of asthma, and periostin is deeply involved in both of these effects. Yuyama et al. first demonstrated that the expression of *the POSTN* gene, which encodes periostin, is upregulated by IL-4 and IL-13 in human bronchial epithelial cells, both being key type-2 cytokines in asthma [5]. Subsequently Woodruff et al. showed that *POSTN* is one of the most upregulated genes in airway epithelial cells collected from patients with asthma, finding a 4.4-fold increase compared with healthy control subjects [6]. Similarly, higher periostin expression (3.7-fold) has been observed in airway epithelial cells from children with asthma than in cells from atopic non-asthmatic or healthy children, irrespective of ICS use [7]. Sputum cells from patients with asthma also showed higher *POSTN* gene expression than those from healthy subjects [8]. Importantly, cellular sources of periostin are not limited to airway epithelial cells, because fibroblasts [9, 10], airway smooth muscle cells [11], endothelial cells [12] and inflammatory cells [13] can produce this specific protein.

Clearly reflecting the matricellular nature of periostin, it is deposited in the airway subepithelial layer in patients with asthma [9]. In addition, its expression in airway epithelial cells is correlated with the thickness of airway basement membranes ($r = 0.56$, $p = 0.0002$) [14]. In an interesting study, Kanemitsu et al. showed in a small sample during a long-term investigation, that the deposition of periostin in the airway subepithelium of patients with asthma was associated with a decline in forced expiratory volume in 1 s (FEV_1) over 20 years [15] (Fig. 15.1).

The presence of considerable heterogeneity in its molecular, cellular, and clinical aspects is another important dimension of asthma. The type-2 high subtype is different from the type-2 low subtype in various aspects, including the IL-13 and IL-5 levels in bronchial tissues, airway

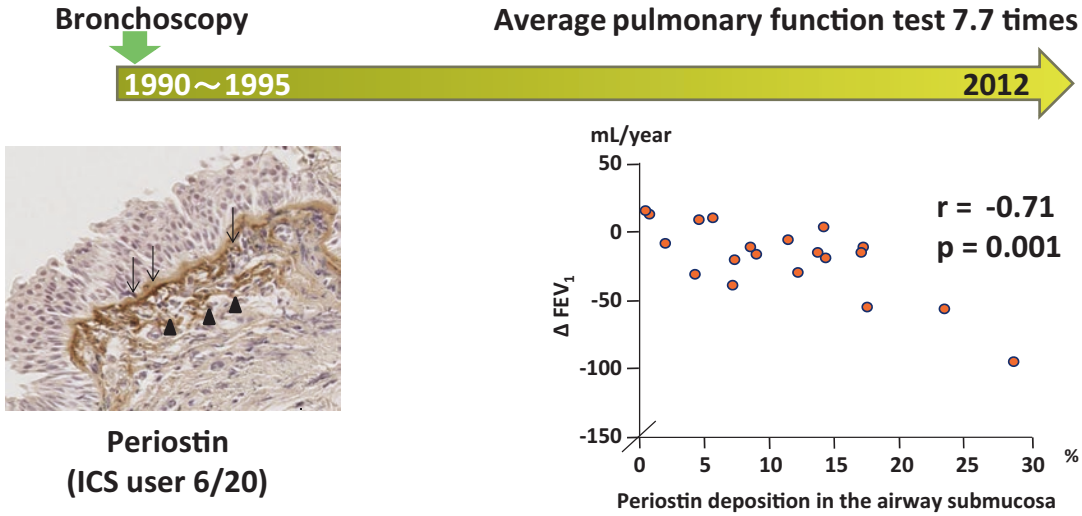

Fig. 15.1 Periostin deposition and pulmonary function decline over 20 years

fibrosis, and blood and airway eosinophilia. The expression of *POSTN* in airway epithelial cells, together with *CLCA1* and *serpinB2,* could distinguish asthmatic patients into type-2 high or type-2 low subtypes [16]. Thus, higher *POSTN* expression in airway epithelial or sputum cells serve nicely as a signature of airway type-2 inflammation.

15.2.2 Periostin in Asthma Models

Two studies performed by using periostin-deficient mice suggest that periostin may have protective effects in the setting of an allergen challenge. Both of these studies showed that periostin-deficient mice have increased airway hyperresponsiveness to methacholine after ovalbumin sensitization and challenge [17], or after challenge with the *Aspergillus fumigatus* antigen [18]. In one of these studies, periostin-deficient mice also had less mucus metaplasia [17], but in the other they did not [18]. Neither study found that periostin deficiency altered the inflammatory cell populations in the lung after allergen challenge, but both reports did suggest modest increases in peripheral inflammation with periostin deficiency (e.g. increased peripheral T-cell cytokine production and serum IgE). These two studies appear to stand in contrast to another investigation that identified a specific defect in allergen-induced eosinophil recruitment to the lungs in periostin-deficient mice [19], as well as to a fourth study demonstrating that periostin is required for house dust mite-induced allergic airway disease in mice [20]. This finding suggests that periostin may contribute to type-2 inflammation in the lung, rather than that periostin restrains it.

15.2.3 Roles of Periostin in Eosinophil Recruitment

Several *in vitro* studies have demonstrated a close relationship between periostin and eosinophilic inflammation. Johansson et al. reported that IL-5, IL-3, or granulocyte macrophage colony-stimulating factor (GM-CSF)-stimulated eosinophils to adhere to periostin through the αMβ2 integrin – however IL-4 and IL-13 did not have this effect [21]. They also found that IL-5-stimulated eosinophils that are adherent to periostin form podosomes and show random motility on periostin. The same research group recently demonstrated that ADAM8 might be involved in the migration of eosinophils on periostin-rich extracellular matrices [22]. Noguchi et al. demonstrated that periostin induces the generation of a superoxide anion ($O2^-$) from eosinophils through the αMβ2 integrin, while it also increases the production of several cytokines and mediators, including IL-6, IL-8, transforming growth factor (TGF)-β1, TGF-β2, cysteinyl leukotrienes, and prostaglandin E2 [23].

15.3 Roles of Serum Periostin in Asthma

15.3.1 Serum Periostin Levels in Asthma

Periostin is secreted basolaterally from airway epithelial cells stimulated by IL-13 [14], and is readily detected in the blood of patients with asthma. Although some influencing factors exist, the serum periostin level is considered to largely reflect its level of production in the airways. When compared with healthy subjects, higher serum periostin levels for asthma have been observed in several studies [24, 25], however, a large population-based cohort GA (2) LEN investigation did not find this upward shift [26]. The lack of a clear difference in this study might be due to that asthma is a heterogeneous disease comprising several different subtypes, and the ratios of theses subtypes may matter in serum periostin levels across the overall asthma population.

In children, bone turnover may significantly affect serum periostin levels, an important reality considering that these levels in children are two to three-fold higher than in adults [27]. Indeed the highest levels were found in children of 2-year old, and readings of 150 ng/mL or higher

(Elecsys®) were associated with increased risk of asthma at age 6. In this regard, two related studies examined children aged 6 years or older. In one of these, serum periostin levels (Shino-test) clearly distinguished children with asthma from them without it [28]. In the other study, serum periostin levels (Shino-test) were associated with airway hyper-responsiveness to methacholine and mannitol in children with asthma aged 6 years or older [29]. Thus, determining the level of serum periostin in children aged 6 years or older may help in the diagnosis of asthma.

However, when interpreting serum periostin levels caution should be paid to the specific enzyme-linked immunosorbent assay (ELISA) kits used, because the potencies to discriminate type-2 high subtype differ among several commercial or non-commercial ELISA kits [30]. In this chapter, we discuss the origin of any serum periostin assays, which is specified as much as possible. Most of the studies cited in this chapter used either an ELISA kit from Shino-test (Kanagawa, Japan) [31] or the Elecsys® periostin immunoassay (Roche Diagnostics) [32]. Fortunately, both assay kits have been shown to correlate strongly with each other ($r = 0.92$, $p < 0.001$), but nonetheless higher levels have been systematically observed for serum periostin levels measured by using the kit from Shino-test, compared to the Elecsys® periostin assay [26].

Fortunately, serum periostin levels are quite different from levels of exhaled nitric oxide (FeNO) and blood eosinophil counts, as they are relatively stable and have little variability. Thus serum periostin meets a key requirement for a reliable biomarker. In the phase 2b study of lebrikizumab, the average CV for FeNO, blood eosinophil counts, and serum periostin (Elecsys®), which were assessed 1 week apart during the run-in period, was 19.8%, 21.3%, and 5.0%, respectively [33]. Consistently, another study showed that the CV for FeNO and serum periostin assessed at 2–3 weeks apart was 8.2% and 2.2%, respectively [34].

A recent investigation by the Periostin Study Team reported on the longitudinal variation of serum periostin levels (Elecsys®) in patients with stable asthma (n = 60, aged between 18 and 75 years, who were taking a median ICS of 500 μg, equivalent to fluticasone propionate per day) [35]. In this study, the within-participant variance of periostin readings over an 8-week period were 15.3 with a 95% confidence interval of ±7.7 ng/mL, and the CV for serum periostin was 6.3%. They found no seasonal variation in these results. This team also examined daytime variation of serum periostin in patients with asthma on ICS treatment and healthy subjects [36]. Serum periostin levels, blood eosinophil counts, and FeNO levels all showed daytime variation – higher in the morning and lower in the evening. However, the degree of variation was small, suggesting that the daytime variation of serum periostin may be negligible and therefore useful in helping to identify type-2 inflammatory subtypes.

15.3.2 Serum Periostin as a Biomarker of Type-2 Airway Inflammation

The utility of serum periostin as a biomarker of type-2 airway inflammation in severe asthma has been established. Jia et al. showed that elevated periostin was the best single predictor of airway eosinophilia in patients with severe asthma that was uncontrolled despite maximal ICS treatment ($P = 0.007$) by using a logistic regression model, which included FeNO, serum IgE, and blood eosinophil counts as independent variables [34]. More recently, the U-BIOPRED Study Group conducted a cluster analysis of sputum samples from 104 moderate to severe asthmatic subjects, using their transcriptomic features. Serum periostin effectively distinguished a transcriptome-associated cluster (TAC) 1 subtype that was enriched for IL-13/Th2, as well as innate lymphoid cell (ILC)2 signatures from a TAC3 subtype that was enriched for ILC3, Th17, oxidative phosphorylation and aging signatures [37]. Therefore, serum periostin may serve as a marker of type-2 airway inflammation in severe asthma, similar to the expression of *POSTN* in airway epithelial cells from patients with steroid naïve mild to moderate asthma.

15.3.3 Serum Periostin as a Biomarker of Airway Remodeling

Based on the findings reported by Jia and colleagues, the authors determined serum periostin levels (Shino-test) in patients with asthma receiving long-term ICS treatment (n = 224; average age: 62.3 years; 171 females) who were recruited for the Kinki Hokuriku Airway disease Conference (KiHAC) multicenter study [24]. Patients with a smoking history of more than 10 pack-years were excluded from this study. This particular cohort was assembled to identify serum markers and genetic factors associated with pulmonary function decline in patients with asthma. In this study, annual changes in FEV_1 were assessed from at least 1 year after initiating ICS treatment to the time of enrolment, or later (this produced an average of 16.2 measurements for 8 years per individual).

Among several serum markers that included blood eosinophil counts, serum eosinophil cationic protein, high sensitivity C reactive protein, IL-6, and IL-17, it turned out that serum periostin levels were the sole reliable biomarker for a greater annual decline in FEV_1. When a cutoff of 95 ng/mL of serum periostin measured with an ELISA kit (Shino-test) was used for dichotomous data, high serum periostin (≥95 ng/mL) was associated with a decline in FEV_1 of 30 mL · $year^{-1}$ greater, independently of the most intensive treatment step and previous smoking (≤10 pack-years) history. The cutoff value of 95 ng/mL was determined because this value had a very high specificity (0.985) to discriminate patients with asthma on long-term ICS treatment from healthy subjects, strictly reflecting their type-2 predominant condition. Thus high serum periostin can identify patients with a rapid decline in pulmonary function, despite their receiving long-term ICS treatment for asthma. Thereafter, associations between serum periostin levels and airflow limitations have been confirmed in several cross-sectional studies [25, 26, 38–40]. Serum periostin levels (R&D Systems) in steroid naïve patients with asthma were also associated with airway wall thickening, as assessed by quantitative computed tomography [41].

15.3.4 Role of Serum Periostin in Comparison or in Combination with Other Type-2 Biomarkers

Serum periostin levels correlate with other biomarkers of type-2 inflammation, such as serum IgE, FeNO, and blood eosinophil counts, though they are modest [24, 26, 38, 39, 42]. Weak correlations among type-2 biomarkers may originate from various factors, including different sources of the samples, i.e. blood or airways, regulatory cytokines, i.e. IL-4/IL-13 or IL-5, several influencing factors, and different responses to medications. Thus, serum periostin levels, FeNO, and blood eosinophil counts may be compensatory or composite markers of type-2 inflammation. Nonetheless, the serum periostin level is distinct from other conventional biomarkers, because it integrates the information regarding chronic inflammation and remodeling in the type-2 inflammatory airways.

Patients with airflow limitations in severe asthma had higher blood eosinophil counts, compared with those without such limitations [43]. Meanwhile, blood eosinophilia alone is not a marker of airflow limitation. Nagasaki et al. performed cluster analyses on the KiHAC population and identified four clusters (Fig. 15.2a): one was characterized by late-onset, eosinophilic, and moderate decline in FEV_1; another smaller cluster was characterized by a mixed type of inflammation (eosinophilic and neutrophilic) with the lowest FEV_1, greatest decline in FEV_1, and poorest asthma control among four clusters [44]. Serum periostin levels were the highest in the late-onset, eosinophilic cluster, followed by the cluster with mixed type inflammation. Most of the patients with a rapid FEV_1 decline, defined as a reduction of ≥30 mL · $year^{-1}$, were included in these two clusters. The author proposes that the measurement of serum periostin may efficiently identify patients with blood eosinophilia who may develop a rapid decline in pulmonary

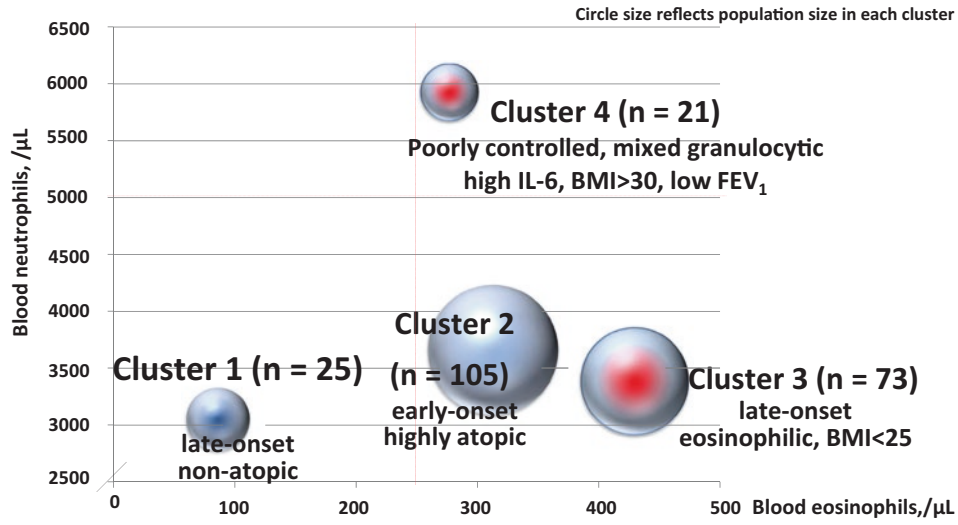

Fig. 15.2a Cluster analysis of the KiHAC population

function, despite receiving long-term ICS treatment for asthma (Fig. 15.2b). In addition, the late-onset, eosinophilic cluster in the KiHAC population may overlap with a cluster that was characterized by late-onset eosinophilic asthma with few symptoms [45, 46]. Consequently, serum periostin measurements may lead to better management of patients who are at risk of undertreatment regarding their pulmonary function, when only symptom-based strategies are implemented.

To find a better marker to identify patients who are insensitive to ICS treatment, the utility of a composite marker of high FeNO and high serum periostin levels was examined in a subanalysis of the KiHAC population [47]. A total of 121 patients were enrolled, including 88 females with a mean age of 59 years, who were taking daily ICS doses of 525 μg, equivalent to fluticasone propionate. The included subjects were stratified into four groups, according to FeNO levels (cutoff value, 25 ppb) and serum periostin levels (cutoff value, 95 ng/mL). The patient group with high levels of both FeNO and periostin (n = 23) received more intensive treatment. This group had a history of asthma admission, and had experienced an annual decline in FEV_1 of ≥30 mL, which is more frequently than those in the patient group with only high levels of FeNO (Fig. 15.3). Despite receiving intensive treatment, patient group with high levels of both FeNO and periostin had an odds ratio of approximately 3 for asthma exacerbations that required systemic corticosteroid treatment over the next 2 years, compared with the remaining patients, even after adjustment for airflow limitation and recent episodes of asthma exacerbation. Since FeNO is more likely to predict future exacerbations of asthma [48] and serum periostin may be a predictor of reduced pulmonary function under type-2 inflammation [49], the measurement of both FeNO and serum periostin may efficiently identify patients who need intensive treatment for severe refractory asthma [50].

15.3.5 Geno-Endo-Phenotypes of Patients with High Serum Periostin

Matsusaka et al. showed that serum periostin levels (Shino-test) were unrelated to asthma severity, but delineated a phenotype of patients with high serum periostin characterized by later asthma onset (aged 40 years or older), blood

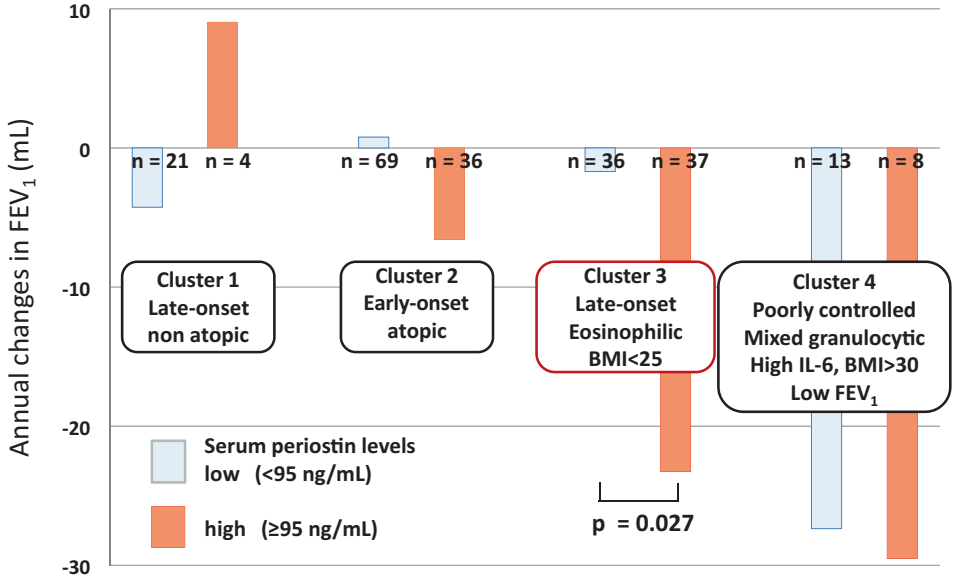

Fig. 15.2b Superiority of serum periostin over blood eosinophil to identify patients who may develop a rapid decline in pulmonary function

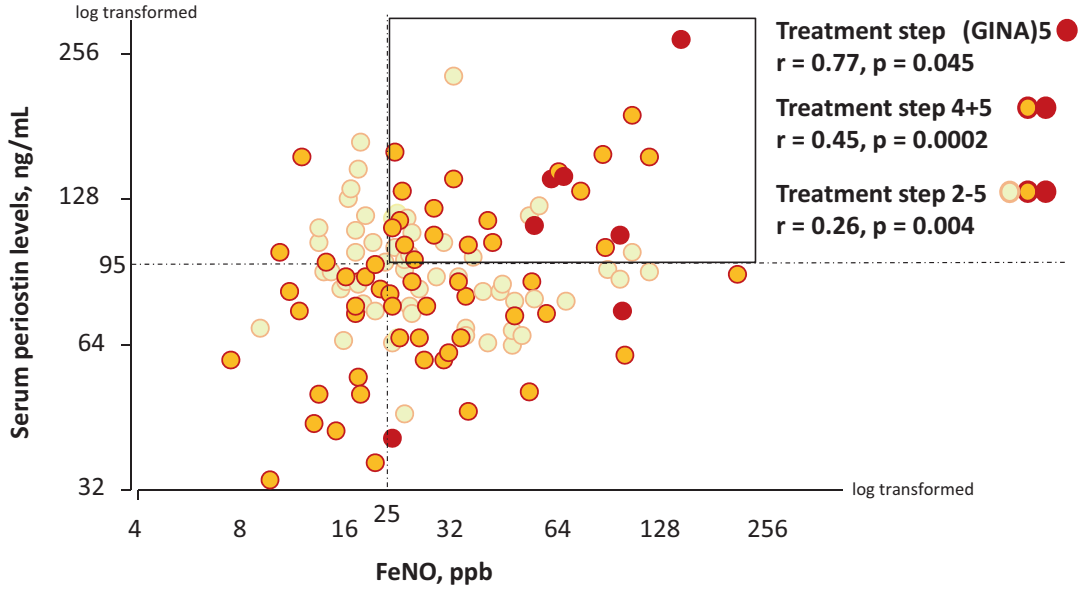

Fig. 15.3 Utility of serum periostin and exhaled nitric oxide (FeNO) as a composite marker

eosinophilia having airflow limitations, aspirin intolerance, and comorbidity of chronic sinusitis with olfactory disturbance [25]. Hinks et al. endotyped patients with severe asthma using topological analysis, determining three endotypes with high periostin (Shino-test) [51]. One was an endotype characterized by high sputum eosinophils and neutrophils having severe airflow obstruction, nasal popylposis, and aspirin intolerance. The second was characterized by sinonasal disease, and the third was identifiable by early onset asthma, high sputum IL-5, and high FeNO

levels. Thus, high serum periostin is closely associated with later asthma onset, concomitant sinonasal disease, possibly eosinophilic chronic rhinosinusitis (ECRS), and airflow limitation. However, patients with early onset asthma with eosinophilic disease may also present high serum periostin levels.

In the KiHAC population, serum periostin levels were associated with the GG genotype of rs3829365, one of the 3 tag SNPs of the *POSTN* gene: GG 98.7 ng/mL *vs.* GC/CC 86.1 ng/mL (p = 0.003) [24]. Of note, in the absence of *POSTN* rs3829365 GG, serum periostin levels were elevated in the GG genotype of *IL4RA* rs8832 [52]. This variant of *IL4RA* rs8832 was identified as a genetic marker of good responses to pitrakinra in a pharmacogenetics study of pitrakinra, an inhibitor of the IL-4 receptor α that is a common sub-chain for both IL-4 and IL-13 signaling [53]. Patients with the *IL4RA* rs8832 GG genotype showed high serum periostin levels (≥95 ng/mL) more frequently than those with the *IL4RA* rs8832 AA/AG genotypes (54% vs 35%, *P* = 0.03) [52]. In addition, the aforementioned patient group with high levels of both FeNO and periostin had a higher frequency of the GG genotype of *IL4RA* rs8832 (35%) compared to the remaining patients (15%) [50]. Greater frequency of the GG genotype of *IL4RA* rs8832 in patients with high levels of FeNO, and serum periostin is plausible when considering that both periostin and inducible nitric oxide synthase that synthesizes NO in airway epithelial cells are upregulated by stimulation with IL-4 and IL-13, which potentially pass the signal *via* the important IL-4 receptor α.

15.4 Influencing Factors of Serum Periostin

15.4.1 ECRS and Allergic Rhinitis

Periostin is overexpressed in the nasal tissues of patients with ECRS [54], a subtype of CRS with eosinophil-enriched nasal polyps, which is often considered as a synonym of CRS with nasal polyps (CRSwNP). ECRS is an intractable comorbidity of severe asthma, which produces a risk of asthma exacerbations and poor asthma control. Earlier studies demonstrated that elevated serum periostin levels in patients with asthma were associated with comorbidity of CRS, as assessed by self-completed questionnaires (Fig. 15.4A) [24] [25]. Two recent studies showed that serum periostin levels (Shino-test) in patients with asthma and the co-morbidity of CRS were associated with the severity of sinusitis, as assessed by the Lund-Mackay score on computed tomography (r = 0.44, p = 0.04) [39] (Fig. 15.4A), irre-

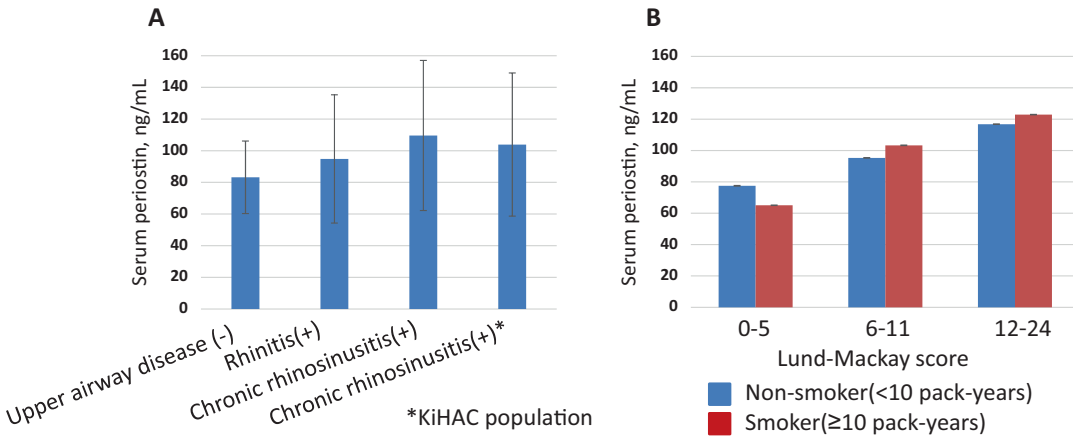

Fig. 15.4 Association of serum periostin levels with chronic rhinosinusitis in patients with asthma: (**A**) generated from data of Kanemitsu et al. and Asano et al. [24, 39], (**B**) generated from data of Kimura et al. [55]

spective of smoking status (adjusted $\beta = 0.362$, $p < 0.0001$ for non-smokers with asthma, and an adjusted $\beta = 0.499$, $p < 0.0001$ for smokers with asthma) [55] (Fig. 15.4B). Patients with aspirin intolerance, a situation that is often complicated with ECRS and severe eosinophilic asthma, also show elevated serum periostin levels [56].

Tajiri et al. determined serum periostin levels (Shino-test) in patients with ECRS concomitant having severe asthma who were subsequently treated with omalizumab, a monoclonal anti-IgE antibody. These patients showed significant improvements in Lund-Mackay scores as well as total and rhinological symptom scores using the SNOT-20 instrument, and they improved scores for nasal blockage and dysosmia after a 16-week treatment with omalizumab [57]. Although this was a small preliminary study, the changes in Lund-Mackay scores were strongly correlated with baseline serum periostin levels ($r = -0.92$; $p = 0.029$) (Fig. 15.5), suggesting that the serum periostin level may be a predictor of the responses to omalizumab treatment for ECRS.

Serum periostin is also elevated in the presence of allergic rhinitis, in both non-asthmatic and asthmatic subjects [42]. However, because the degree of *POSTN* expression in the nasal tissues in allergic rhinitis is weaker than that in ECRS [54], the contribution of allergic rhinitis to serum periostin levels may be milder [39].

15.4.2 Cigarette Smoke and Chronic Obstructive Pulmonary Disease (COPD)

Mertens et al. demonstrated that exposure to cigarette smoke (CS) suppressed the expression of *POSTN* and *CLCA1*, but not of *serpinB2*, in IL-13-stimulated airway epithelial cells [58]. This suppression persisted after cessation of CS exposure. In the *in vitro* study, serum periostin levels are lower in current smokers than those in never smokers consisting of both the general population and asthma [26, 36, 42, 59]. Further, serum periostin levels (Elecsys®) are lower in patients with COPD than those in patients with asthma, particularly severe asthma, or asthma with COPD overlap [60].

When compared with healthy smokers, patients with COPD irrespective of current smoking status showed higher serum periostin levels (Elecsys®) [61]. However, in patients with COPD these levels were not associated with pulmonary function, inflammatory cells in blood, sputum, or airway tissues. Of interest, Shirai et al. showed a positive association between serum periostin (Elecsys®) and serum YKL-40 levels in patients with COPD alone, although the mechanisms underlying the positive association remain unknown [60]. In 155 patients hospitalized for COPD exacerbations, serum periostin levels

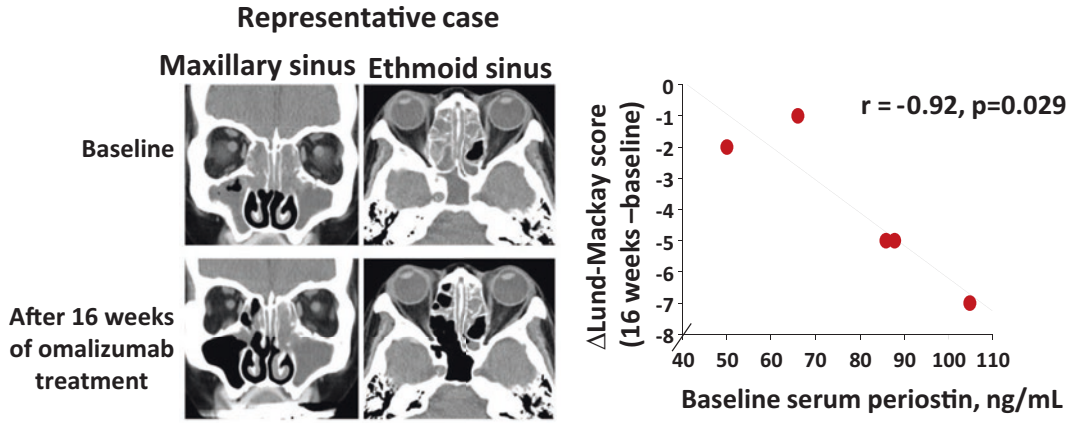

Fig. 15.5 Baseline serum periostin levels and changes in Lund-Mackay score after 16 weeks of omalizumab treatment in patients with severe asthma comorbid eosinophilic chronic rhinosinusitis

(R&D) were significantly higher on admission than those on discharge, without any differences among the severities of COPD exacerbations [62]. Frequent exacerbators generated higher serum periostin levels on discharge, compared with non-frequent exacerbators.

15.4.3 Body Mass Index (BMI)

Several studies revealed that serum periostin levels (Shino-test, Elecsys®) are negatively associated with BMI in both the general population and asthma [26, 42] [63]. A positive association of serum periostin (Shino-test) with adiponectin and a negative association with leptin were observed in patients with asthma [42].

15.4.4 Age

Serum periostin is positively associated with aging in one study [26], but not in the other [36]. The effect of age on serum periostin levels is likely more important in pediatric asthma.

15.5 Serum Periostin in the Management of Patients with Asthma

15.5.1 Responses of Serum Periostin to Steroid Treatment

In vitro studies demonstrate that steroid treatment efficiently decreases periostin production by IL-4/IL-13-stimulated airway epithelial cells [6] and fibroblasts [9], but does not inhibit periostin production by TGF-β-stimulated fibroblasts or IL-4/IL-13-stimulated microvascular endothelial cells [12]. Systemic steroids may decrease serum periostin levels, although the effects of ICS on serum periostin levels appear to be minimal. However, these levels were significantly decreased with an average of 17.2% (placebo corrected) from the baseline levels after 14 days treatment with oral prednisolone (0.5 mg/kg/day) in the BIOAIR study (n = 118) [64]. A recent investigation found changes in biomarkers of type-2 inflammation, including periostin, following severe exacerbations of asthma [65]. During these episodes, patients (n = 34) needed an average dose of 36 mg of prednisone per day (range 20–60 mg per day), for an average of 10.9 days (range 1–28 days). Blood eosinophil counts immediately fell to a mean level of 70/μL 1 day after steroid treatment, but returned to 330/μL at 12 weeks after treatment ($p < 0.001$). Median serum periostin levels reached a minimum level of 45.9 ng/mL at 1 week after treatment and returned to 50.9 ng/mL at 12 weeks after treatment ($p < 0.001$). Although the value is reduced, the decline in serum periostin seems smaller, compared with blood eosinophil counts and FeNO levels.

In steroid-naïve patients with asthma, small reductions (~10% of the baseline value) in serum periostin after ICS induction (budesonide 400 μg *via* turbuhaler twice daily) have been reported [38]. We also observed that introducing ICS (equivalent to fluticasone propionate at 400 μg daily) significantly decreased serum periostin levels at 24 weeks, but this level decreased remarkably by 5.4% only from the baseline (n = 37; mean age 55 years, 10 males). This was in contrast to the changes in FeNO levels that decreased significantly by 27.4% at 12 weeks from the baseline levels in the same steroid naïve patients (n = 37) [2] (Fig. 15.6). Perhaps this is because FeNO levels may reflect relatively acute inflammation in type-2 high airways, whereas

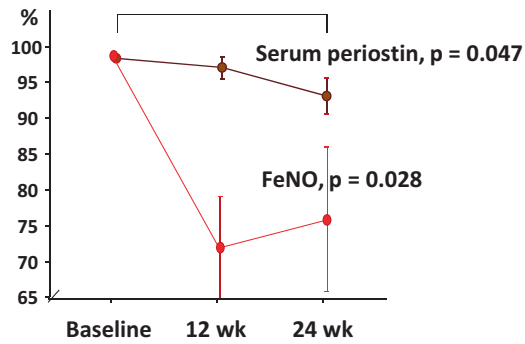

Fig. 15.6 Changes of serum periostin and exhaled nitric oxide (FeNO) levels after the initiation of inhaled corticosteroid treatment

changes of serum periostin levels are small and may reflect chronic or latent inflammation and remodeling of the asthmatic airways with ICS treatment.

15.5.2 Serum Periostin as a Companion Diagnostic and Monitoring Marker for Clinical Treatments

15.5.2.1 ICS

Kato et al. looked at the possibility of serum periostin as a marker for a step-down of ICS treatment [66]. Although this was a preliminary 12-week, single-arm and open-label study, the failure of ICS reduction was associated with elevated serum periostin (Shino-test) at baseline, having higher blood eosinophil counts and greater diurnal variation of peak expiratory flow at baseline as well. Izuhara et al. demonstrated that the impact of the genotype of rs37973 of *GLCCI1*, which is a candidate gene that may predict response to ICS on the excessive decline in FEV_1 and blood eosinophilia, was more distinct when subjects had high serum periostin levels (≥95 ng/mL) in the KiHAC population [67]. Thus, patients with high serum periostin may be relatively insensitive to ICS treatment and may need careful follow-up.

15.5.2.2 Omalizumab

The EXTRA omalizumab study had reported on the utility of type-2 biomarkers using the reduction in asthma exacerbations during the first year of treatment to predict the efficacy of omalizumab. Accordingly, patients with higher levels of FeNO, blood eosinophil counts and serum periostin at baseline were more responsive to omalizumab treatment, compared with lower levels of these type-2 biomarkers [68]. When patients had high serum periostin levels (n = 255), the rate of severe exacerbations declined by 30% when treated with omalizumab, whereas the decrease using this treatment was only 3% in the low serum periostin group (n = 279). This was confirmed by Tajiri et al. who showed that higher FeNO levels were the best predictive marker of improvement in asthma control 16 weeks after treatment and that higher serum periostin levels were the best predictive marker of a reduction in severe exacerbations 1 year after omalizumab treatment [69]. In addition, baseline serum periostin levels alone were negatively associated with free serum IgE levels after 16 or 32 weeks of treatment (Fig. 15.7).

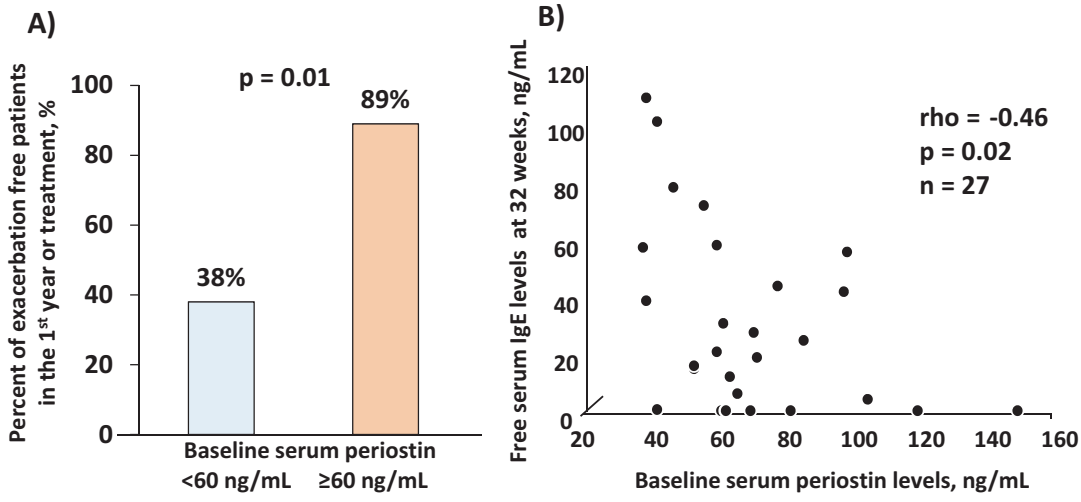

Fig. 15.7 Baseline serum periostin levels and (**A**) percent of exacerbation free patients 1 year after omalizumab treatment; (**B**) free serum IgE levels at 32 weeks of omalizumab treatment in patients with severe asthma

One recent study showed the possibility of using serum periostin as a monitoring marker for omalizumab treatment in asthma. After 16 weeks of omalizumab treatment, serum periostin (BioVendor) had decreased by 37% from its baseline level (mean baseline value 28.6 ng/mL, 16 weeks: 18.1 ng/mL, 32 weeks: 16.1 ng/mL, 48 weeks: 18.7 ng/mL). In contrast, FeNO fluctuated substantially (mean baseline value 48.9 ppb, 16 weeks: 25.5 ppb, 32 weeks: 74.0 ppb, 48 weeks: 37.2 ppb) during the treatment period [70].

15.5.2.3 Lebrikizumab and Dupilumab

In a phase 2b study of lebrikizumab (n = 219), which is an anti-IL-13 antibody, serum periostin was a good predictor of the response to lebrikizumab in patients with unstable asthma, despite ICS therapy [33]. The high serum periostin group (n = 110) showed an 8.2% increase in FEV_1 in response to lebrikizumab, compared with the placebo group, whereas there was only a 1.6% increase in FEV_1 for the low serum periostin group (n = 101). Lebrikizumab failed to produce its expected effects on asthma exacerbations in phase 3 studies involving patients with uncontrolled asthma, despite the use of both ICS and second controller therapies. However, when patients were stratified according to serum periostin alone, and not combined with blood eosinophil counts, the high serum periostin group showed a significantly greater reduction in exacerbations when treated with lebrikizumab, compared with a placebo [71]. A recent meta-analysis of five studies confirmed that a high serum periostin group showed significantly greater reduction in exacerbations with lebrikizumab treatment, compared with placebo-treatment [72]. Although these particular lebrikizumab trials have been discontinued, they revealed that serum periostin is a clear marker reflecting inflammation related to IL-13.

In a phase 3 study of dupilumab, which is an anti-IL-4R-α antibody, patients with moderate to severe asthma, uncontrolled with medium to high dose ICS plus up to two additional controller medications, received two doses of dupilumab or a placebo treatment for 52 weeks [73]. Baseline levels of serum periostin, as well as blood eosinophil counts and FeNO, showed significant improvement regarding FEV_1 at 12 weeks with both dupilumab treatment arms, while serum periostin levels at baseline were not associated with a significant reduction in exacerbations with dupilumab treatment. During the treatment period, serum periostin, as well as FeNO and serum total IgE levels, significantly decreased from the baseline values.

15.6 Sputum and Exhaled Periostin in Asthma

Although measurement of serum periostin is more useful in clinical practice, information from the airways is necessary for the proper understanding of the pathobiology of severe asthma. Several research groups have examined periostin levels in sputum and exhaled breath condensate (EBC) in asthma. Sputum levels of periostin measured by the Elecsys® periostin assay were associated with proportion of sputum eosinophils in poorly controlled asthma ($r = 0.364$, $p = 0.005$) [74]. Sputum levels of periostin that were determined by an ELISA kit (R&D Systems) were higher in an eosinophilic subtype (sputum eosinophil >2%) than those in a mixed granulocytic subtype (sputum eosinophil >2% and neutrophil >40%), and also higher in patients with persistent airflow limitations compared to those without airflow limitations. Carpagnano et al. showed that periostin levels (R&D Systems) in sputum or EBC are the highest in severe asthma, mild to moderate asthma, and control subjects, in this order [75]. In addition, periostin levels in sputum or EBC were higher in the type-2 endotype (sputum eosinophil >3% and/or blood eosinophil count ≥400/μL on at least two occasions) compared to the non-type-2 endotype. Meanwhile, periostin levels in EBC from another research group determined by the same ELISA kit (R&D Systems) were not associated with asthma severity. EBC periostin levels were higher in patients with asthma concomitant with CRS symptoms than those without such symptoms [76].

15.7 Conclusion

The role of serum periostin as a biomarker of type-2 airway inflammation and remodeling has now been elucidated. The advantages of measuring serum periostin compared to conventional type-2 biomarkers include its easy accessibility, since no specific equipment is required. When compared with blood eosinophil counts, serum periostin values are stable, with little variability. Although some factors outside the airways should be considered, serum periostin is a useful biomarker for the management of severe asthma and can serve as a predictive marker of responses to treatment.

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Part VII

Periostin in Development

Periostin and Integrin Signaling in Stem Cell Regulation

16

Athira Suresh, Atreyi Biswas, Saravana Perumal, and Satish Khurana

Abstract

Stem cell function is regulated by a huge repertoire of external cues along with stem cell intrinsic genetic and epigenetic factors. These interactions come through a variety of cell adhesion receptors, of which integrins are one of the most important classes. They interact with extracellular matrix (ECM) components and various bound proteins. Apart from inside-out signaling through which integrins ensure that the cells are stably bound to the ECM, outside-in integrin signaling, through binding to a variety of ligands, play important roles in cell fate decisions. Periostin is one such ligand whose role in functional regulation of stem cells is emerging due to its wide expression profile. In this review, we discuss the recent advancements made in the field.

Keywords

Periostin · Integrins · Stem cells · Proliferation · Stemness · Differentiation

16.1 Introduction

Stem cells can be defined as the cells that possess the potential to self-renew and differentiate into more committed progenitors or terminally differentiated cells. This is achieved by means of asymmetrical division in which the stem cells give rise to a stem cell and a relatively more lineage-committed cell. During physiological stress or developmental requirement, the stem cells can undergo symmetrical division into two differentiated cells or otherwise, they result in stem cell expansion. In all known systems, the stem cell compartment shows heterogeneity and functional hierarchy, wherein the most primitive stem cells show long-term survival and function. These cells divide rather slowly, each time giving rise to a more differentiated stem or progenitor cell with faster proliferation rate but shorter lifespan [1]. Therefore, most primitive stem cells in a given tissue are largely quiescent and enter cell cycle only rarely, upon induction [2]. There are extrinsic pathways, triggered by the cellular and physical components of the microenvironment in which the stem cells reside, that regulate their function [3]. These cellular/physico-chemical extrinsic factors with which stem cells interact to maintain their function cumulatively, make up the stem cell niche. The property of stem cell quiescence makes sure that long-surviving stem cells are largely protected from replication related DNA damages. It has also been shown that in

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hematopoietic stem cells (HSCs), occasional proliferative events repair any genomic alterations that may have accumulated during the course of their life [4]. Evidently, the fate decisions leading to symmetrical versus asymmetrical stem cell divisions play an important role in maintaining the stem cell pool as well as providing continuous supply of differentiated cells needed for regeneration of the tissue. This makes the knowledge on external stem cell regulatory cues crucial for their optimum application in clinic and industry. A number of factors important for maintaining quiescence of adult stem cells have been identified as non-cellular components [5]. These factors work in coherence with the intrinsic genetic and epigenetic factors that regulate fate decisions [2]. The intrinsic pathways make the genetic makeup of the stem cells unique, so that they can withstand the accumulated stress for a long time while dividing only rarely. As DNA damage repair pathways are preferentially coupled with replicative processes, non-proliferative stem cells tend to accumulate more non-replication related DNA damages compared with other cells [6]. Therefore, sensing DNA damage and other response pathways play crucial role in stem cell function. The function of FOXO family of transcription factors has been well described for the aforementioned reason whereby, through PI3K-Akt, it maintains the stem cell quiescence along with mediating DNA damage repair [7]. Maintenance of quiescence is crucial for the long-term survival of adult stem cells as excessive entry into cell cycle in several transgenic mouse models has been reported to negatively impact stem cell function [8]. In muscle stem cell, Notch signaling has been shown to play an important role in maintaining quiescence and is important for lineage progression of the progeny upon stem cell proliferation [9]. Apart from external biochemical factors, physical attributes of the micro-niche, where the stem cell resides, play crucial role in their functional regulation. Adult stem cells from various systems depend upon hypoxic condition for their long-term survival [10]. Anaerobic glycolytic pathways are aimed at reducing oxidative stress in these cells and are crucial for their function. In fact, a number of studies indicate that clearing oxidative stress using scavengers of reactive oxygen species (ROS) improved stem cell function [11]. Unequivocally, it can be said that stem cell function depends upon several extrinsic factors. Undoubtedly, molecular pathways responding to extrinsic signals play important role in stem cell function. Cell surface receptors play crucial role in receiving the biochemical signal emanating through paracrine or endocrine mechanisms. Some of these receptors are also exploited in identifying the lineage hierarchy within the stem cell population, and are employed as markers. Integrins make a particularly interesting class of such receptors, which not only mediate cell-cell or cell-ECM adhesion but also relay biochemical messages from the outside to inside of the cells [12]. A great number of ligands that bind to various heterodimeric integrin receptors have been identified. These factors make sure that the stem cells, as several terminally differentiated cell types, maintain themselves in a given tissue physically. In addition, these interactions regulate cell fate decisions taken by the stem cells. Periostin (Postn) is one of such ligands that elicit signals, which play an important role in the regulation of stem cell function. Here, we review the recent advancements made in our understanding of its role on a variety of stem cell populations.

16.2 Periostin and Integrin Signaling

Periostin (Postn) is a 90 kDa ECM associated protein expressed by a wide variety of cell types and plays a key role in maintenance of tissue architecture [13]. It is structurally similar to the insect neuronal adhesion protein fasciclin I [13] and is composed of 836 amino acids. It was first identified as a cell adhesion protein in mouse osteoblast cell lines in 1993 and was termed as osteoblast-specific factor-2 (OSF2) [14]. The protein was renamed as Postn due to its preferential expression in the periostium of long bones [15]. It is a secretory protein and regulates critical intracellular functions by interacting with specific receptors and cell surface localized enzymes

[16]. Postn, a secreted protein, consists of the signal sequence, EMI domain, 4 fasciclin domains and heparin-binding domain in the C-terminal [17]. It belongs to the group of matricellular proteins and interacts with ECM components like fibronectin and tenascin C via EMI domain and Fas1 domain, respectively [18]. The gene that encodes Postn is located at 13q.3 in human and at 3C in mouse genome [15]. According to ensemble database, 10 isoforms of Postn are reported in human and 9 isoforms are reported in mice. Sequence analysis shows 89.2% sequence homology between human and mouse Postn for entire translated polypeptide and 90.0% sequence identity for the mature form. Although Postn shows overall sequence similarity in mouse and human, the C-terminal of the protein is less conserved than the remaining portion [19]. It is expressed in tissues like periodontal ligaments [20], tendons and cardiomyocytes to support mechanical loading of the cells [21]. It is also known to help the systems of collagen crosslinking and acquiring bone mass where cleavage of C-terminal region was required for establishing interaction between Postn and the ECM proteins [22].

Being important in maintaining tissue architecture and its remodeling during morphogenesis, Postn has been found to be important in several processes during ontogeny such as development of arterioventricular valves [23], formation and maintenance of bones and teeth [24]. Postn and its paralog BIGH3 (transforming growth-factor-β inducible gene h3), an ECM protein differentially expressed during bone marrow regeneration, can form homodimers via disulfide bonds in the EMI domains [25]. It has also been shown that treatment of Postn can lead to ectopic osteogenesis and upregulation of collagen 1 in mice [26]. Mice lacking Postn expression showed reduction in osteoblast-specific differentiation markers such as type I collagen, osteocalcin, osteopontin, and alkaline phosphatase [27]. These mice also showed lower bone mass due to increased sclerostin (Sost) expression. Postn expression is required for the normal physiological function of a wide range of tissues such as lungs, thyroid gland, placenta, ovary, skin, aorta, stomach, and uterus [28]. Probably, the most crucial role of Postn is shown in cancer progression where it has been described as one of the important markers in several types of cancers. Recent studies suggest its role in stem cell function; demonstrating its role in regulating stem cell homing, niche formation and regulation of cell fate choices via binding to a variety of integrin receptors. It is important to note that it can bind to multiple integrins, and it has been shown that Fas1 domains of the protein can interact with integrin-αvβ3, αvβ5 [28]. It has also been shown to bind to integrin-α6β4, which is important for survival and differentiation of bone mesenchymal stem cells (MSCs) [15].

Integrins are a major group of cell surface receptors involved in cell adhesion and signaling [29]. They contain extracellular, transmembrane and cytoplasmic domains and mediate inside-out as well as outside-in signaling. Integrin mediated signaling is important in a wide range of cellular functions such as apoptosis, proliferation and differentiation by influencing cell fate decisions [30]. Till date, 18 type α- and 8 type β-subunits have been reported to make 24 different heterodimeric integrin receptors in vertebrates [31]. Most of these integrins are shown to be involved in various functions without showing significant redundancy [32]. The α-subunit determines the ligand specificity, while the β-subunit is connected to the cytoskeleton, and affects signaling pathways [33]. Among these integrins, αvβ1, αvβ3 and αvβ5 have been shown to act as binding partners for Postn in a variety of cell types, where it controls physiology in various tissues [34, 35].

16.3 Bone and Mesenchymal Stem Cells

The skeletal system starts to form in the first few weeks of conception in humans. The process of bone formation or ossification takes place via intramembranous or endochondral ossification [24]. Intramembranous ossification takes place via differentiation of mesenchymal stem cell (MSCs) to osteocytes. On the other hand, intermediate cartilage template formation followed by bone and bone marrow formation takes place

through endochondral ossification [36]. Distribution of osteoblast precursors into endosteum, trabeculae and periosteum occurs in concomitance to the vascularization of bone marrow and formation of HSC niche leading to the establishment of hematopoietic stem cells (HSCs) in bone marrow [37]. The fact that Postn was originally identified in the osteoblastic cell line, and its expression was prominently found in the periosteum of bone, already indicated its involvement in bone tissue development/function. It has been found that Postn expression is regulated in association with osteoblast differentiation and bone formation in the early stages of bone development [15]. In the mouse model for bone repair, Postn was implicated in mesenchymal stem cell differentiation towards bone forming cells aiding in repair. It was one of the six novel transcripts identified as upregulated in fractured bone. Its expression was identified in MSCs as well as in pre-osteoblastic cells but not in mature osteoblasts [38]. Postn has been reported to inhibit Sost that inhibits bone formation by Wnt and BMP signaling, probably via PTH and mechanical loading. Postn interacts with Notch receptor and has anti-apoptotic effect on osteocytes upon mechanical stress [39]. In corroboration, further studies using E16.5 fetuses showed that *Postn* isoforms are expressed in pre-osteoblasts, cartilage primordia of ribs, upper and lower jaws, mesenchymal cells, undifferentiated taste bud precursor cells, ameloblasts and odontoblast during the development of tooth [40]. Knockdown of *Postn* resulted in decreased expression of osteoblast differentiation markers like collagen type 1, osteocalcin, osteopontin, alkaline phosphatase, and cbfa1. Postn knockout mice have decreased bone mass and strength with decreased collagen crosslinking, demineralization and increased microdamage [41].

It was observed that Postn enhances the differentiation of MSCs to osteo-lineage cells [42]. In these cells, Postn was shown to bind with Itgav-b3 and activate canonical Wnt signaling. Treatment of human MSCs with soluble Postn led to inhibition of glycogen synthase kinase-3β, increasing β-catenin levels through the phosphorylation of lipoprotein related protein-6 (LRP-6). Differentiation of MSCs towards osteo-lineage was confirmed by alkaline phosphatase expression and calcium nodule formation [42]. Furthermore, Postn has been implicated in the migration and differentiation processes ensuing epithelial-mesenchymal transition (EMT), during embryonic development as well as tumorigenesis [19]. For example, during cardiac development, Postn was shown to enhance mesenchyme matrix invasion [43]. Postn, being an ECM protein, seems to be a natural candidate in the process of heart valve development that showed binding to Itgavb3 and Itgavb1 in vitro, through which the mesenchymal cells adhered and spread in a dose-dependent manner. It was also clearly shown that these effects were mediated by a PI3K dependent signaling cascade initiated by the outside-in integrin signaling. Interestingly, while Postn was shown to mediate mesenchyme invasion in various biological processes, there were several cancer types where its effects were inhibitory. Postn suppressed invasion and metastasis during the progression of bladder cancer in humans [44]. Varied response towards Postn in different cancers has been well reported and is attributed to different heterodimeric integrin receptors as binding partners in addition to differently spliced isoforms [19].

In the case of human periodontal ligament derived MSCs, Postn was found to promote differentiation, proliferation and migration [45]. In the presence of soluble recombinant human Postn, the MSCs showed increased proliferation and migration in an in-vitro scratch assay. Interestingly, these effects could be mediated at least by autocrine regulation as knockdown of Postn expression using lentiviral vectors showed significant decrease in these processes. Apart from proliferation and migration, osteogenic differentiation of these stem cells was also positively affected by Postn treatment as examined by alkaline phosphatase assay and Runx2, osteocalcin, osteopontin expression. Almost parallel reports showed that these effects were mediated by JNK mediated signal transduction pathways, such that inhibition of its phosphorylation led to neutralization of all effects of Postn on periodontal ligament derived MSCs [46]. Using human

adipose tissue derived MSCs, it was shown that Postn might be at least one of the mediators for the tumor supporting potential of these cells [47]. The function of cancer stem cells in several models has been shown to be affected by Postn, especially, the metastatic properties of several cancers such as breast cancer, pulmonary and gastric carcinoma.

Apart from MSCs, Postn has been shown to promote differentiation of myogenic progenitor cells [48]. While it did not affect their proliferation, Postn stimulated fusion of myocytes to form myotubes in a dose dependent manner (Fig. 16.1). In addition, it was observed that Postn expression was much higher in a regenerating skeletal muscle corroborating its function in myogenesis along with other effectors such as apelin and oncostatin M. Postn has been shown to mediate TGF-β1 induced activation of hepatic stellate cells, which leads to fibrotic events in liver. Silencing Postn mRNA led to significant decrease in the expression of α-SMA and collagen I in response to TGF-β1 [49].

16.4 Postn in Hematopoietic System

The hematopoietic system is maintained by a rare population of stem cells called hematopoietic stem cells (HSCs) that reside in the BM in adult mammals and remain largely quiescent [50]. As in other adult stem cell systems, HSC population presents itself in a hierarchical manner with the most primitive HSCs at the top of the pyramid. As we come down the hierarchy, the commitment

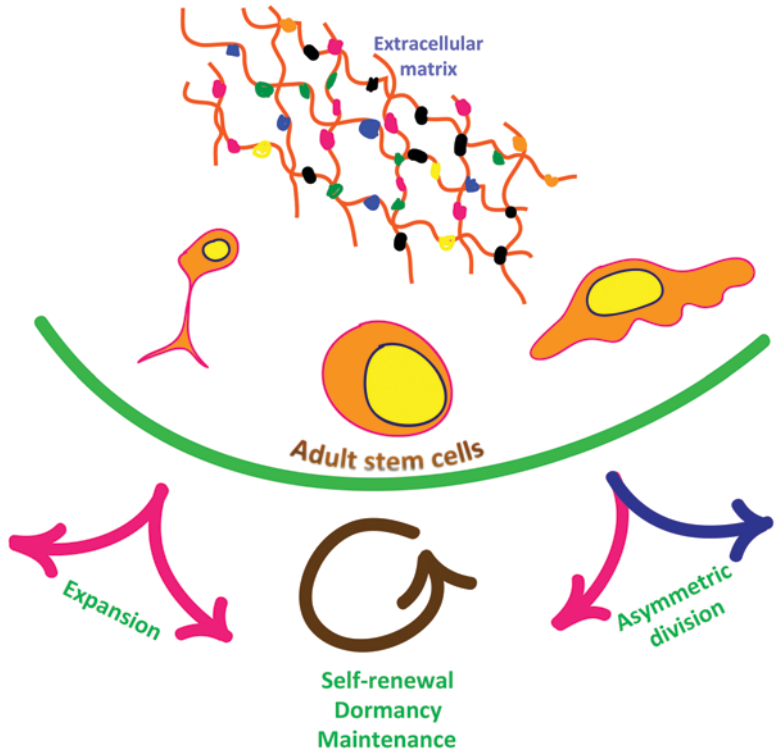

Fig. 16.1 Graphical representation of the studies done to show the importance of Postn-Itgav interaction in adult hematopoiesis
(**a**) In vitro experiments show that Postn inhibits culture-induced HSC proliferation, thereby enhancing HSC function. (**b**) Interruption of Postn-Itgav interaction in *Postn*$^{-/-}$ and *Vav-Itgav*$^{-/-}$ mice leads to pre-mature ageing of the hematopoietic system and loss of HSC stemness. (**c**) Studies on signaling pathway involved show the up-regulation of *p27kip1* expression through manipulation PI3K/Akt pathway in HSCs cultured with Postn

to a particular lineage becomes more firm, proliferation rate increases and lifespan of the stem cell decreases. These progenitor cell populations maintain the flow of required number of terminally differentiated blood cells. Adult HSCs undergo asymmetrical divisions to maintain their numbers and to produce less primitive stem cells. These fate decisions are regulated by a variety of extrinsic factor along with intrinsic properties of the cells [51]. The niche as well as the ECM proteins maintains the stemness of HSCs by keeping them in a balanced oscillation between quiescence, self-renewal and differentiation. Integrins are one of the most important class of cell surface receptors that mediate the effects of niche to HSCs and regulate hematopoiesis in a very significant way [52]. The expression of a wide variety of heterodimeric integrin receptors such as α2β1, α4β1, α5β1, α6β1, α9β1, αLβ2 and αvβ3 has been reported in HSCs [53]. The importance of inside-out integrin signaling for HSC attachment to niche components is widely reported. For example, α4β1 (VLA4) has been shown to play a crucial role in HSC homing and maintenance in the BM, and its inhibition results in HSC mobilization into the peripheral blood [54]. Similarly, α5β1 (VLA5) is also expressed on HSCs at high levels and is of importance [55]. Integrin β1 chain is key to the establishment of developing BM as the hematopoietic site and subsequent maintenance of HSCs [56]. Integrin-α4 is found to be important in retention of HSPCs in the BM niche [57]. Postn binding partners have been shown to mediate integrin signaling in an inside-out as well as outside-in manner and affect hematopoietic function. Integrin β3 (Itgb3 or CD61), perhaps the most important integrin chain that mediates the effects of Postn, has been suggested as a positive marker for HSC enrichment [58]. More than 60% of the $CD34^{-}$LSK cells in mouse BM showed the expression of Itgb3 and they also showed a higher number of long term repopulating hematopoietic cells than $CD61^{low/-}$ $CD34^{-}$LSKs. This was true both in the case of side-population and the non-side-population cells within the hematopoietic progenitor population. Expression of Itgb3 makes this population amenable for Postn action as it acts as one of the most common partners for its heterodimeric receptor. Expression pattern of β3-integrin was correlated with the quiescence of primitive HSC population [59]. Moreover, HSC functional attributes such as side-population phenotype, HSC markers, dormancy as well as the LTR-potential were positively correlated with Itgb3 expression. Importantly, the expression of β3 integrin was associated with αv- chain in HSCs. Taking account of Postn expression in bone forming cells and of the fact that these cells are important component of the HSC niche, our group performed extensive studies to reveal the effect of Postn-integrin interaction in hematopoiesis. We reported the expression of Itgav and Itgb3 on primitive HSCs in the BM, and our studies showed that interaction between Postn and Itgav was crucial for maintenance of HSC quiescence in BM [60]. Systemic deletion of Postn as well as hematopoietic system specific deletion of Itgav led to loss of quiescence in primitive HSCs, ultimately resulting in functional decline and premature aging of hematopoietic system. Aging of hematopoietic system is associated with loss of lymphopoietic activity in the BM and elevated myelopoietic differentiation, which in turn is associated with deregulation of stem cell proliferation [8]. Interestingly, the expression of Postn in rabbit decreased with age and was found to correlate with B-lymphopoiesis [61]. OP9 cells that can support lymphopoiesis in co-cultured hematopoietic progenitors, failed to do so upon Postn deletion. In young mice that lacked Postn, lymphopoietic activity was compensated, most probably by other integrin ligands eliciting outside-in signaling. *Postn* deficient mice that were 16 weeks old started showing defective HSC function due to higher proliferation rate leading to premature aging that included lower lymphopoietic activity, which reflected in number of lymphocytes in peripheral blood [60]. This was also reflected in the results obtained from long-term competitive repopulation assays, wherein poorly engrafting HSCs also showed decreased lymphopoiesis (Fig. 16.2). Using rabbit as a model system wherein lymphopoietic activity occurs only in the early stages of postnatal life, the importance of Postn in lymphopoiesis was

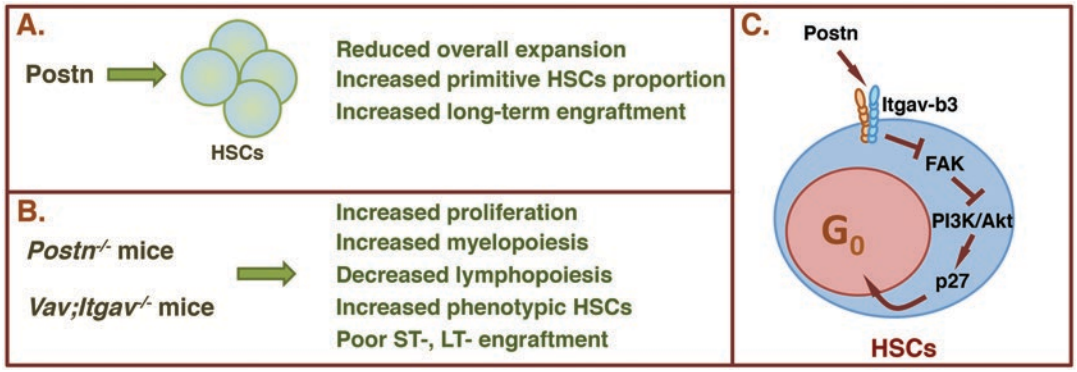

Fig. 16.2 Postn affects stemness in a cell-type and context dependent manner
Postn is an extracellular matrix associated protein expressed and secreted by a variety of cell types. It is known to affect the function of a number of adult stem cells. However, its affect remains context dependent. It has been shown to have an effect on quiescence in hematopoietic stem cells, while in the case of mesenchymal and neural stem cells, Postn affects the differentiation potential of the stem cells. Furthermore, it has been shown to regulate the function in an age dependent manner as well the level of expression determines the stem cell function (**a**) In vitro experiments show that treatment of HSCs with Postn led to decrease in the culture-induced proliferation resulting in increased frequency of long-term HSCs. (**b**) Disruption in Postn-Itgav interaction through systemic deltion of Postn or hematopoietic tissue specific deletion of Itgav leads to phenotype similar to pre-mature ageing. In both the cases, animals show increase myelopoieis with concomintant decrease in lymphopoiesis. (**c**) Postn signals through binding mainly with Itgav. Downstream signaling events lead to increase in p27 expression resulting in increased quiescence

confirmed [61]. The expression of Postn in BM stromal cells dramatically reduced as lymphopoietic activity in the BM ceased within the first 4 months of life. OP9 cells commonly used as HSC supporting cells for ex vivo experiments. Upon Postn deletion, no longer supported the lymphopoietic activity of rabbit or murine HSCs. Postn deficiency also led to increased cell death and reduced proliferation in B-cell progenitors. Other reports also showed that OP9 cells deficient in *Postn* expression showed decreased expression of several factors important for HSC function, showing a possible autocrine regulation in hematopoietic niche. Although Postn was shown to exert its function through integrin-αvβ3, wherein αv was found to be crucial for Postn binding, studies also suggested the involvement of the heterodimer in HSC function through inside-out signaling [62]. It was clearly shown that ligation of αvβ3-integrin by an antibody 2C9.G2 positively impacted the long-term repopulation (LTR) activity of HSCs in thrombopoietin (TPO) dependent manner, suggesting a role of inside-out signaling. Apart from this, the report confirmed the involvement of outside-in integrin signaling played via binding of ECM proteins in the maintenance of LTR-potential of HSCs. More recently, integrin-αvβ3 was found to have a synergistic negative effect on HSC function along with proinflammatory cytokine IFNγ [63]. Therefore, it can be concluded that integrin signaling through an inside-out or outside-in manner will largely be context driven. In this case, where IFN-γ stimulation has a negative impact on hematopoietic function, integrin signaling further reduced the function of HSCs, which lost LTR potential rapidly. Loss of β3-integrin mitigated the effect of IFN-γ.

16.5 Developmental Hematopoiesis

During embryonic development, hematopoietic events start in the yolk sac, proceed in multiple transient sites wherein hemogenic endothelium gives rise to hematopoietic progenitors that expand and mature mainly in the embryonic tissue [64]. Although direct evidence of involvement of Postn in these events is not reported, recent studies point

towards the involvement of integrin signaling in the processes involved. Itgb1 have been shown to be important in the colonization of HSCs in fetal liver, spleen, and bone marrow [65]. The embryonic site where definitive hematopoietic progenitors appear is aorta-gonad-mesonephros (AGM) where hematopoietic clusters appear from the endothelial lining of ventral surface of dorsal aorta. In these hematopoietic clusters, the expression of β3 as well as αv integrins along with αIIb was shown [66]. The expression of αvβ3 heterodimer was shown on all AGM and placenta derived HSCs. Recently, both of the integrin chains were found to be expressed in at least a fraction of VE-Cad$^+$ endothelial population sorted from E11 mouse AGM [67]. This was the case with both in vivo AGM-derived as well as pluripotent stem cells-derived hematopoietic progenitors. In an elegant study, through directed differentiation model using the GATA2w/eGFP embryonic stem cell line, Huang et al. showed that Itgb3 precisely labeled hemogenic endothelial cells [68]. This was then tested in the E10 AGM derived cells as well, and only Itgb3 expressing CD31$^+$CD41$^-$CD45$^-$Ter119$^-$ cells showed hemogenic potential. The sorted CD61$^+$ cells from AGM showed distinct hematopoietic potential in myeloid colony forming assays. These cells upon co-culture with OP9 stromal cells gave rise to putative HSCs that expressed CD45. Some of the CD61$^-$ cells on co-culture with OP9 for a day started expressing CD61, and were found to express markers for hematopoietic progenitor cells [68]. Together, these studies suggest that integrin αv as well as β3 are important in the emergence of hematopoietic cells in the AGM and point towards a possible role of Postn in this process.

16.6 Postn in Neural Stem Cells

The first clear evidence of proliferative activity in postnatal rat brain was obtained in the 1960s as mitotic cells were identified in dentate gyrus [69]. It was revealed that in young rats, ependymal and subependymal layers of third and lateral ventricles have proliferative activity, which rapidly declines with age. Like the other adult stem cells, NSC population was shown to possess functional heterogeneity [70] and were shown to differentiate into neurons, asterocytes and oligodendrocytes [71]. Different levels of lineage commitment and differentiation into a variety of neuronal sub-types are a hallmark feature of these cells. More recent studies have identified two different niches for neurogenic activity in adult mammalian brain. These neurogenic sites have been identified in the subventricular zone (SVZ) and the subgranular zone (SGZ) of the hippocampus dentate gyrus [72]. Further studies using fatemapping techniques showed that the NSCs in adult hipocampus can only give rise to neurons and asterocytes [73]. Similar studies in NSCs from adult SVZ region showed that their differentiation is biased towards neurons and oligodendrocytes [74]. Diferentiation into neuronal lineages can be demonstrated in vitro as well [75], therefore, an extrinsic regulation of their function can therefore be expected. The endothelial cells from the vasculature within the NSC niche secrete certain factors like VEGF and NT-3 that help in the maintanance of stemness [76]. Extracellular matrix and its associated proteins mediate the interaction between NSCs and their niche where laminins, proteoglycans and tenascin C are found to be crucial in the adult neurogenic niche [77]. Again in NSCs, as in the case of other adult stem cells, ECM components, through integrin signaling, support regenerative potential through stem cell maintenance or activation. A variety of integrins, some of which that can act as receptor for Postn, have been shown to express in neuronal tissue [78]. In fact, the expression of Postn itself in the CNS tissue was reported in developing fetus [79]. While Periostin-like factor (PLF), an alternatively spliced form was expressed in the developing neuronal tissue during early development around E12.5. Expression of Postn starts only in later stages that too transiently. Later, Postn mRNA and protein were detected in the neurons of adult mouse brain also [80] and thus, it has a neuroprotective role in vitro and in vivo during cerebral ischemia. Studies using the transient middle cerebral artery occlusion (tMCAo)-induced ischemia model, demonstrated the involvement of Postn in neuronal

regeneration. Expression of both full length (Pn1) as well as a splice variant, lacking exon 17 (Pn2), was examined, and it was found that Pn2 expression transiently decreased in the ischemic core site within initial 3 h of the procedure before increasing again in the ischemic as well as the peri-ischemic region, 24 h after MCAo. Kinetics of Postn expression indicated a role in neuroprotection or neuroregeneration. In fact, infusion of recombinant Postn (Pn2) intracerebroventricularly 1.5 hrs after ischemic injury led to better recovery from ischemic injury. Moreover, Postn prevented hypoxia-induced cell death in primary adult neuronal cultures as well. Interestingly, in the neuronal tissue as well, Akt pathway mediated the effects of Postn in neuronal cells. In support of the involvement of ECM-mediated integrin signaling in NSC function, the importance of Postn receptors such as Itgav, in neuroprotection, was demonstrated [81]. Expression of αv-integrin was found to be widespread among mesencephalic neurons. Along with NCAM, Itgav was found to mediate the effects of GDNF on neuronal survival and growth during development and adulthood. Furthermore, the effects of downstream pathways of integrin signaling such as Akt pathways in neuronal survival were confirmed [82]. Integrin signaling through Akt pathway prevented apoptosis in rat embryo-derived hippocampal neurons. Treatment with an integrin activating peptide EIKLLIS prevented apoptotic cell death in neurons in vitro. Effects of integrin activation on neuronal survival were mediated by PI3K activation that acted upstream of Akt phosphorylation. Another evidence of involvement of Postn in NSC functional regulation came through a report showing positive effects of Postn on NSC proliferation and differentiation [83]. In proliferation versus differentiation cultures using embryonic NSCs, it was revealed that expression of Postn might be crucial for differentiation of NSCs. Expression of Postn in NSCs was confirmed, and it was clearly shown that the number of proliferating neurons ($BrdU^+$ $NeuN^+$ cells) was significantly decreased in Postn null mice postnatally. In addition, these Postn null mice showed a decreased number of NSCs ($BrdU^+$ $Nestin^+$ cells) in dentate gyrus of hippocampus. Treatment of differentiation cultures of NSCs with recombinant Postn led to increased proliferation rate resulting in enhanced numbers of astrocytes and mature neurons in the culture within 7 days. Perhaps the most crucial evidence for the involvement of Postn in neuroprotection and function of NSCs came from the experiments showing improved neuronal regeneration following hypoxic-ischemia (HI) encephalopathy. Recombinant Postn was injected in the lateral ventricles of rat neonates. Quantification of proliferating and differentiating NSCs showed increase in stem cell numbers in Postn injected animals. Importantly, the Morris water maze test showed significant improvement in cognitive function in Postn injected animals. However, in all of these experiments, Postn injection without HI injury did not show any effect on their number of stem cells or cognitive function. These results clearly showed the importance of Postn in neuroregeneration through stem cell activation.

16.7 Cancer Stem Cells and Niche Modulation

In the past couple of decades, the concept, that tumors are initiated, maintained and propagated via their own stem cell population known as cancer stem cells has gained popularity. Starting with the observation that within the tumor cell populations, there is heterogeneity in the tumor initiating potential, there have been seminal studies done to identify the cells responsible for tumor initiation as well as metastasis. Cancer stem cells have also been shown to be at the foundation of relapse in a variety of cancers [84]. As the associated processes involve a lot of architectural change in the tissue of origin or destination, ECM components and cellular interaction with them has been shown to play a crucial role. It was observed that Postn is secreted by the ovarian cancer cells, and in this experiment, αvβ3 and αvβ5 integrins were shown to be its key receptors [28]. In addition, Postn induced adhesion and migration of ovarian epithelial cells. Postn appears to be important for niche creation and maintenance that leads to establishment of tumor. It was observed that Postn was critical for creation of metastatic niche for the CSCs in mouse breast cancer model [85]. In nor-

mal tissue, fibroblasts expressed Postn, and the infiltrating tumor cells responsible for initiation of tumors in the metastatic site induced the stromal cells to express higher levels of Postn. A significant induction of Postn in lymph node metastasis was observed in human breast cancer patients. Postn was required for colonization of the secondary target site and blocking its function prevented metastasis. In *Postn*$^{-/-}$ mice, a decrease in number and size of pulmonary metastasis was observed. Furthermore, in vitro assays showed that Postn deficient CSCs did not form tumorspheres, and this effect could be rescued by the addition of Postn. Therefore, the importance of Postn not only in the metastatic process and niche modulation but also in CSC functional maintenance could be concluded. Interestingly, in this case, Postn recruited Wnt proteins that have been implicated immensely in cancer progression.

Major involvement of Postn in a variety of other cancers have also been reported, and in several of these cases, Postn is proposed to be one of the possible prognostic markers of cancers such as breast cancer [86]. Involvement of Postn in migration and invasiveness in mesenchymal tissue has been reported. Invasiveness in breast cancer makes it a major cause of mortality with varying survival rate. It has been shown that CSCs play a major role in the invasion and metastasis of breast cancer as in several other systems. CSCs identified as $CD44^{+}CD24^{-}Lin^{-}$ tumor cells from clinical samples, expressed higher levels of Postn protein than the bulk population. Of the 1086 breast cases studies, 334 showed expression of Postn, which was positively correlated with the chemotherapy resistance in CSCs [86]. This study suggested that Postn expression was related to the CSC ratio in breast cancer samples. $CD44^{+}$/$CD24^{-}$ CSCs separated from a solid tumor injected to mammary fat pad gave rise to a tumor. After 7 days of culture, single cell suspensions of CSCs separated from solid tumors produced viable mammospheres. Postn down-regulation using siRNA in CSCs made them sensitive to chemotherapy. Using basal-like breast cancer, an aggressive subtype of breast cancer, Lambert et al. showed that both Postn and its heterodimeric receptor αvβ3-integrin were highly expressed in CSC enriched population [87]. In this case, Postn elicited Erk signaling pathway that regulated the expression of key cytokines through NF-κB transcription factor. siRNA knockdown of Postn or Itgb3 in cancer cell line model with defined CSC population (SUM159) showed a significant reduction in mammosphere formation compared to the control. Importantly, when MIII cells were sorted for cells that express αvβ3, only the cells expressing high levels of the Postn receptor could show mammosphere formation. *Postn* knockdown in these cells failed to form tumors when injected in mice in limiting dilution assays. Genome-wide expression analysis in response to *Postn* knockdown indicated that Postn affects cytokine expression and is associated with reduced STAT3 signaling. Gene network analysis following *Itgb3* or *Postn* knockdown showed defective Erk pathway-associated genes in CSCs which might affect proliferation potential of the cells [87]. Having shown the pro-metastatic role of Postn in breast cancer, the involvement of tissue resident MSCs in the process was also demonstrated [88]. It was shown that Postn expression induced a mesenchymal-like phenotype in the human mammary epithelial cells as well as the breast cancer cells. In addition to that, Postn promoted stem cell-like phenotype in these cells as they acquired multi-lineage differentiation capacity as in the case of MSCs. This could be done through overexpression of Postn or through treatment with recombinant Postn, which resulted in mesenchymal transition of the normal or cancerous epithelial cells. Through this modulation, Postn promoted breast cancer progression and metastasis in xenograft model [88]. In addition to breast cancer, Postn expression is seen to be induced in the niche of metastatic cancers such as pulmonary carcinoma [88], gastric carcinoma [89] and glioma malignancy [90]. In the case of MLL-AF9 acute myeloid leukemia (AML) model, expression of β3-integrin receptor was found to be crucial for survival of leukemic stem cells (LSCs), and deletion of the receptor led to impaired homing, LSC related transcriptional programs and induced differentiation (Fig. 16.3) [91].

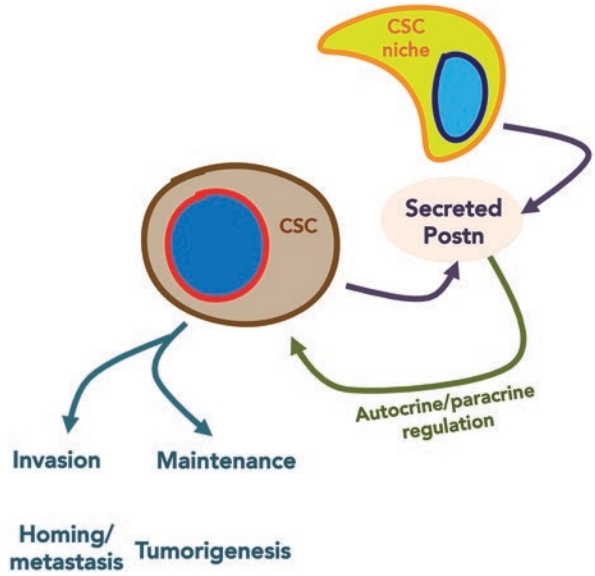

Fig. 16.3 Postn is involved in modulating cancer stem cell niche
Studies have shown the involvement of Postn in modulation of CSC niche. Both cancer stem cells as well as the mesenchymal cells in the secondary target tissues have shown to express Postn. In models, such as leukemia CSCs have been shown to express Postn receptors indicating autocrine or paracrine regulation of CSC function. Secreted Postn increases invasiveness of CSCs with enhanced metastatic properties. Expression of Postn and its receptors have been shown to play crucial role in tumorigenesis in a number of cancer models

16.8 Conclusion

Being an extracellular matrix associated protein; Postn has shown to be tremendously important in a variety of systems during development and pathogenesis. Initially implicated in mesenchymal and bone forming tissues, the field is progressing in an astonishing manner implicating Postn in the fundamental processes involved in stem cells and regeneration in a variety of tissue types. It has been observed that the effects of Postn are context dependent in a major way. A variety of cell types or the same cell types in different developmental or pathological context show differential involvement of Postn. Moreover, the signaling cascades involving Postn are shown to elicit varied molecular responses in a context dependent manner. While integrin signaling remains the most crucial mediator of its effects, both inside out and outside-in signaling events could be involved in different cell types. Involvement of multiple receptors and variable signaling cascades, careful observations with specific case studies will be crucial. Overall, Postn presents itself as an important target for several clinical applications ranging from regeneration to cancer therapy with several questions yet to be answered.

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Role of Periostin in Cardiac Valve Development

17

Roger R. Markwald, Ricardo A. Moreno-Rodriguez, Sibnath Ghatak, Suniti Misra, Russell A. Norris, and Yukiko Sugi

Abstract

Although periostin plays a significant role in adult cardiac remodeling diseases, the focus of this review is on periostin as a valvulogenic gene. Periostin is expressed throughout valvular development, initially being expressed in endocardial endothelial cells that have been activated to transform into prevalvular mesenchyme termed "cushion tissues" that sustain expression of periostin throughout their morphogenesis into mature (compacted) valve leaflets. The phenotype of periostin null indicates that periostin is not required for endocardial transformation nor the proliferation of its mesenchymal progeny but rather promotes cellular behaviors that promote migration, survival (anti-apoptotic), differentiation into fibroblastic lineages, collagen secretion and postnatal remodeling/maturation. These morphogenetic activities are promoted or coordinated by periostin signaling through integrin receptors activating downstream kinases in cushion cells that activate hyaluronan synthetase II (Akt/PI3K), collagen synthesis (Erk/MapK) and changes in cytoskeletal organization (Pak1) which regulate postnatal remodeling of cells and associated collagenous matrix into a trilaminar (zonal) histoarchitecture. Pak1 binding to filamin A is proposed as one mechanism by which periostin supports remodeling. The failure to properly remodel cushions sets up a trajectory of degenerative (myxomatous-like) changes that over time reduce biomechanical properties and increase chances for prolapse, regurgitation or calcification of the leaflets. Included in the review are considerations of lineage diversity and the role of periostin as a determinant of mesenchymal cell fate.

Keywords

Periostin · Fasciclins · Cell signaling · Lineage · Differentiation · Tissue remodeling · Extraceular matrix · Cytoskeletal organization · Valve disease

17.1 Introduction

Congenital heart defects represent the most common form of birth defects in humans, afflicting nearly 1% of all individuals. Of these, valvular abnormalities are the most prevalent and include valve structural (anatomical) defects such as Ebstein's anomaly, bicuspid aortic valve leaflets, calcific aortic valve disease, mitral valve stenosis, mitral valve arcade, parachute asymmetric

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valves, myxomatous valves, and polyvalvular disease. The two most prevalent forms of postnatal valvular diseases are mitral valve prolapse (MVP) and calcific aortic valve disease (CAVD), collectively affecting 5–7% of the population, and representing a significant burden on human morbidity and mortality [32]. Although, usually considered to be adult heart valve diseases, recent genetic findings for MVP and CAVD (with or without associated bicuspid aortic valves) have established that these two diseases have developmental origins, i.e. their roots lie in embryonic valve development [1, 10, 28, 37, 41]. After birth, these diseases open the door to progressive degenerative changes over the life of affected individuals [25], often leading to secondary complications such as arrhythmias, aneurysms, heart failure, and sudden cardiac death.

Discoveries in the identification of transcription factors, growth factors and extracellular signaling molecules have led to discoveries in downstream targets that regulate spatial and temporal changes in valvular tissue organization and biomechanical properties that occur during development, aging or disease. [25]. Among these is the 90KD matricellular protein, periostin, that is highly expressed in developing heart valves [9, 34]. Like other matricellular proteins, periostin is a multifunctional, modular protein having four repeated domains that are highly homologous to the ancient adhesion protein, midline fasciclin-1 (FAS 1) found in both animal and plant phyla [22, 44]. These modular motifs enable periostin to engage in multiple (positive or negative) binding interactions with different extracellular ligands and structural proteins as well as cell surface receptors in which signals change cell behaviors that affect organization of the extracellular matrix, adhesion, migration, determination of cell fate, differentiation, intracellular trafficking, endocytosis and apoptosis [23, 34]. Mammalian FAS1 family members are associated with multiple aspects of health and disease including valve diseases [5]. Understanding the roles of periostin and how it affects morphogenetic processes leading to proper valve formation and function (biomechanics) is the focus of this review.

17.2 Valvular Primordia Are Established by an Epithelial to Mesenchyme Transformation

In vertebrate hearts, valves develop in junctional segments of the looped tubular heart (Fig. 17.1). The atrioventricular (AV) canal or inlet connects atria and ventricles, and the outflow tract (OFT) connects the ventricular outlets to the great arteries. In vivo lineage tracing studies and culture assays have indicated that the valve leaflets and any related, supporting tissues originate from the endocardium of these junctional segments through an epithelial mesenchymal transformation (EMT). The mesenchymal progeny derived from this EMT is called cushion tissue [8, 9]. Paracrine signals uniquely secreted by the adjacent myocardium activate AV and OFT endocardial endothelium to undergo changes in cell adhesion leading to their transformation into migratory mesenchyme that seeds the primitive extracellular matrix (cardiac jelly) separating endocardium and myocardium [2, 11, 33]**.** The complex cascade of sequential changes in gene and protein expression which drive endocardial EMT has been extensively studied using 3D collagen gels and gene knockout strategies which indicate that the endocardial EMT is triggered by TGFβ and BMP2,4 signaling whose expression is spatially restricted to the valve forming regions of the primary heart tube by coordinated interactions between Notch ligands and receptors with specific T-box gene transcriptional regulation [7, 39, 49].

As a result of EMT progression, the original acellular cardiac jelly between AV and OFT endocardium of the adjacent myocardium of primary heart tube becomes circumferentially mesenchymalized (Fig. 17.1). Expansions (“buds”) of cushion tissue emerge from these mesenchymalized walls progressively grow and extend into the lumen of the AV canal and the OFT as the embryonic anlagen of future valve leaflets of the AV inlet (mitral and tricuspid valves) and the ventricular outlet (aortic and pulmonary semilunar valves). The fusion of cushion across the midline of the AV canal and proximal OFT also

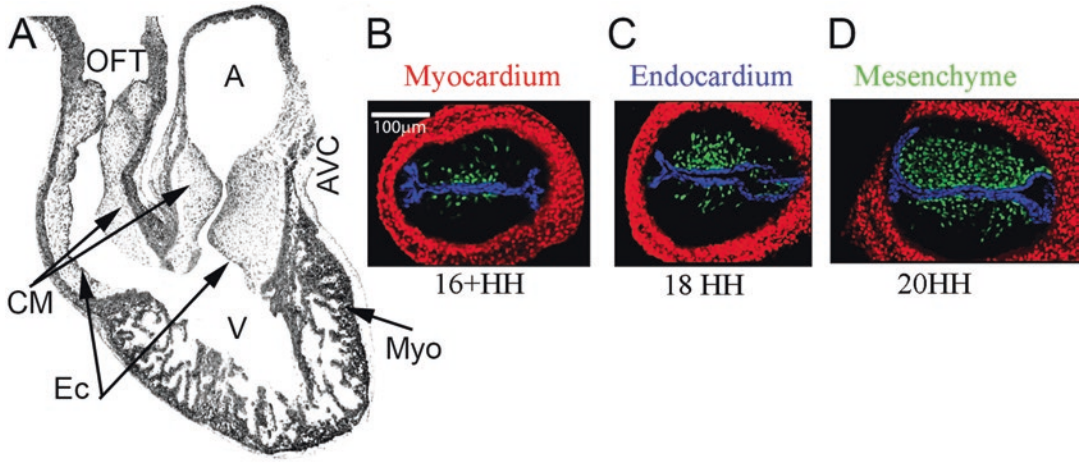

Fig. 17.1 Anatomical location of the valve forming regions in the primary heart tube. (**a**) a sagittal section of a late stage (HH32) embryonic chick heart stained with hematoxylin and eosin to show that the segmental origin of valves is restricted to the atrioventricular canal (AVC) and outflow track (OFT). Atrium (**a**), Ventricle (**b**). The heart at this stage is histologically similar to the human. Epithelial mesenchymal transformation is completed and the cushions (arrows) are fully mesenchymalized and elongated into the narrow lumen of the AVC and OFT; cushion mesenchyme (CM), endocardium (Ec) and myocardium (Myo). Epicardial derived mesenchymal cells can be seen on the external surface of the myocardium at the level of the AVC sulcus. (**b**, **c** and **d**), are cross-sectional scanning confocal images of the AV canal from stage 16, 18 and HH20 chick embryo hearts that have been digitally colorized to show the temporal and spatial progression of the endocardial EMT process that gives origin to cushion mesenchyme. Endocardium (blue), Myocardium (red) and cushion mesenchyme (green)

give origin to septal partitions that anatomically divide these junctional segments into right and left or dorsal-ventral channels or outlets [6].

17.3 Post-EMT Valvuloseptal Morphogenesis

While the primitive endocardial cushion swellings can coapt to restrict retrograde blood flow, they are still several morphogenetic steps away from becoming a biomechanically competent, mature, sculpted valve leaflet [9, 19, 21]. These steps are conceptually summarized in Fig. 17.2 and include: (i) elongation/growth of the cushion swellings into the lumen of the AV canal and OFT; (ii) differentiation of cushion cells into fibroblastic cell lineages that secrete collagenous extracellular matrix; (iii) collectively, as a tissue, cushion cells and their secreted extracellular matrix are progressively remodeled into compacted leaflets whose final maturation as sculpted leaflets or cusps is completed postnatally. For each of these post-EMT, morphogenetic periods, there is robust expression of periostin (both mRNA and protein) in all valve primordia, peaking during final maturation in the first weeks of neonatal life [4, 34, 45]. The significance of periostin expression during these developmental time periods is considered below:

17.3.1 Cushion Elongation and Growth

The initial expression of periostin during mammalian valvulogenesis occurs after the onset of EMT primarily in AV or OFT endocardial endothelial cells that have been induced (activated) to initiate EMT. Thus, as shown in Fig. 17.3a, periostin protein expression appears to be an early marker that valve endocardial cells have been activated to initiate EMT. However, genetically deleting or inhibiting periostin expression does not block EMT indicating that – unlike EMTs that occur in cancer [5] – periostin is not a trigger for EMT in valvulogenesis but rather a response to it. Once EMT is initiated, expression of

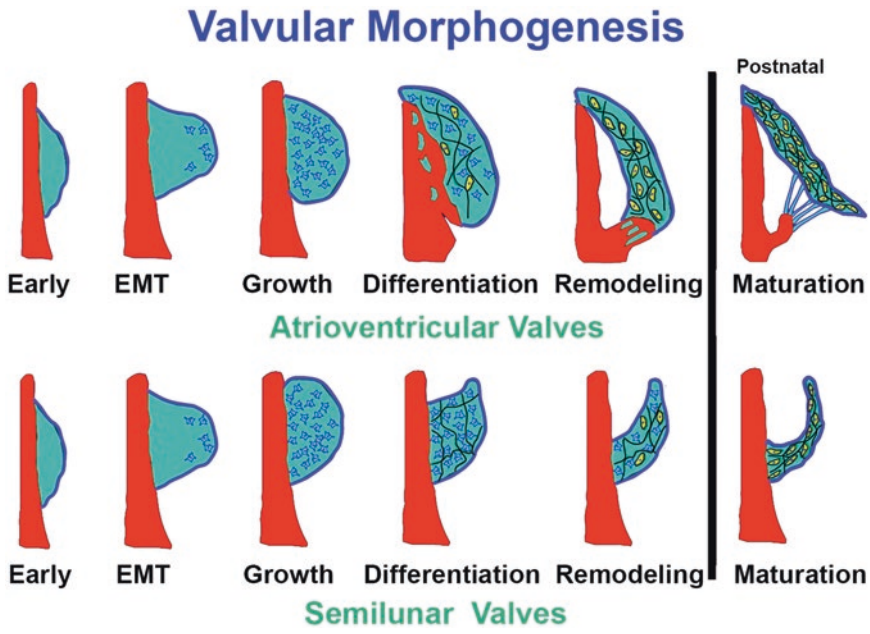

Fig. 17.2 Morphogenetic steps in valvulogenesis. The developmental progression is shown by which the prevalvular cushions of atrioventricular canal and outflow track become mature valve leaflets and cusps after birth. Blue cells are newly seeded mesenchyme derived by EMT; Yellow colorized cushion cells represent cells that have differentiated into a fibroblastic lineage as indicated by their secretion of collagen (black, wavy fibers). Remodeling involves both the cells and extracellular fibers and their compaction and alignment over time into linear tissue arrays that characterize mature valves. Periostin expression during each of these developmental stages is shown below in Figs. 17.3 and 17.4

periostin is sustained in the migrating cushion cells (Fig. 17.3b–h).

In situ, the elongation of cushion swellings into the inlet and outlet cardiac cavities occurs by proliferation and migration of its resident cells which, over time, forms a mesenchymal "model" of a future leaflet or cusp. The expression of periostin does not appear to be required for proliferation of cushions based on the valve phenotype of periostin null mice [35, 36]. Rather, other factors like epidermal growth factor or fibroblast growth factor (FGF) appear to be involved in regulating cushion proliferation. For example, injecting retroviruses expressing FGF into the outflow cushion swellings [46] resulted in the ventricular outlet becoming filled with an undifferentiated, misshaped mass of cushion tissue. Similarly, in an atrial ligation model, FGF promoted the proliferative expansion of the AV cushions but delayed their overt differentiation into migratory cells that secreted extracellular matrix [43]. This suggested that the signaling pathways which regulate cushion cell proliferation were not linked directly to those requiring periostin expression as described below.

Hanging drop cultures or a novel 3D culture system termed "cardiotubes" [16, 36, 57] have been employed to determine what function, if any, periostin played in the elongation phase of valvulogenesis. Cardiotubes are made by the extrusion of uniform-sized collagen fibrils into hollow, linear tubes of any length desired with a wall thickness of 4 mm and lumen diameter of 1 mm. To simulate an in vivo microenvironment, the collagenous walls of cardiotubes are seeded with contractile embryonic cardiomyocytes and medium is continuously circulated at physiological pressures using bioreactor technology.

When HH22-25 chick AV cushion buds were attached to the luminal (internal) side of a collagen tube that had previously been seeded with spontaneously contracting cardiomyocytes, the

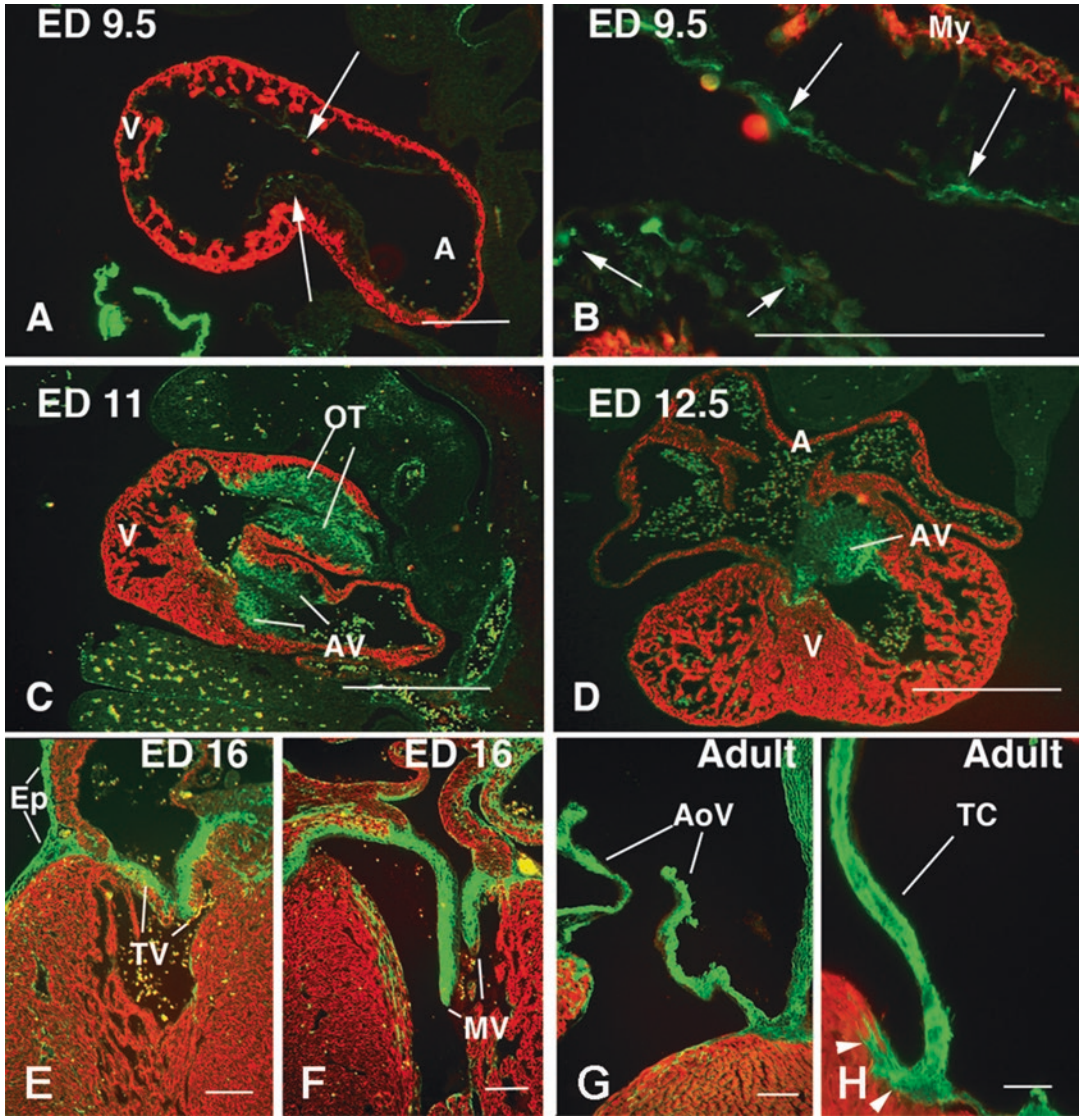

Fig. 17.3 Periostin localization during early heart development and valvulogenesis in mice. Sagittal section of an ED 9.5 mouse embryo shows onset of periostin expression (arrows) in the atrioventricular (AV) canal (**a**). Higher magnification view of the AV canal shows intermittent, endocardial expression of periostin (arrows in **b**). Sagittal section of an ED 11 mouse embryo shows periostin expression in the AV and outflow tract (OT) cushions (**c**). Frontal section of an ED 12.5 mouse heart shows intense periostin expression in the fused AV cushions (**d**). Frontal section of an ED 16 mouse embryo heart shows intense periostin immunostaining within the developing tricuspid valves (TV), epicardium (Ep) (**e**) and mitral valves (MV) (**f**). Images of an adult mouse heart show intense periostin immunostaining in the aortic valves (AoV) (**g**) and tendinous cord (TC) (**h**). Arrowheads in H show attachment of the tendinous cord to the papillary muscle. *A* atrium, *My* myocardium, *V* ventricle. Scale Bars: **a**, **b** = 50 μm; **c**, **d**, **e**–**h**= 100 μm

cushions progressively elongated into the lumen as attenuated, leaflet-like structures over a 7-day period [12, 16]. However, if pretreated prior to implantation into cardiotubes with adenoviral vectors that inhibited periostin expression, elongation of the cushions as well as their ability to express collagen or other extracellular matrix components (ECM) were inhibited [34, 36].

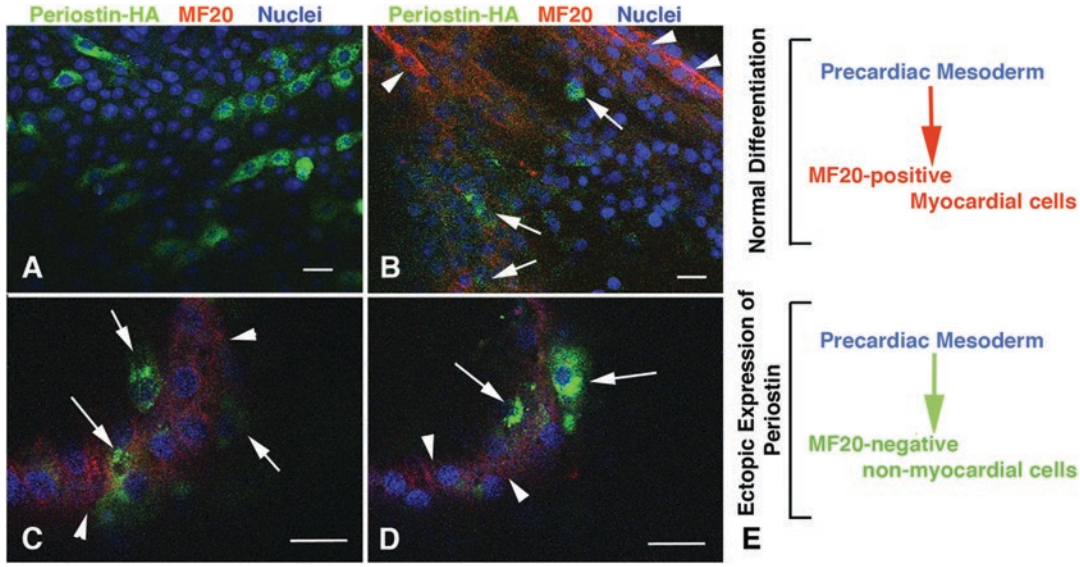

Fig. 17.4 Ectopic expression of periostin inhibits myocardial cell differentiation from precardiac mesoderm. Stage-5 chick axial (non-cardiogenic) mesoderm (**a**) and lateral plate precardiac mesoderm **b**–**d** were treated with adenovirus encoding HA(hemagglutin)-tagged full length periostin and cultured for 24 hours. Periostin expression was localized by immunohistochemistry with anti-HA antibodies. MF20 was used to localize myosin heavy chain expression to identify myocardial cells. TO-PRO-3 was used for the nuclear staining. Precardiac mesoderm differentiates into MF20-positive myocardial cells (arrow heads in **b**, **c** and **d**), whereas axial mesoderm does not (**a**). Periostin-HA positive cells (arrows in **b**, **c** and **d**) do not express myocardial marker, MF20. E illustrates normal differentiation of precardiac mesoderm and the outcome of the ectopic expression of periostin in precardiac mesodermal cells. Ectopic expression of periostin inhibits differentiation of precardiac mesodermal cells into MF20-positive myocardial cells. **a** and **b** are low magnification views and **c** and **d** are higher magnification views. Scale Bars = 10 μm

Surprisingly, inhibiting periostin expression was found to correlate with the presence of cushion cells that expressed markers (MF20) for sarcomeric myosin indicating that their normal differentiation into collagen producing cells (fibroblasts) had been diverted into a myogenic lineage. Conversely, cushion explants, which had been transfected with vectors that continually expressed periostin, were significantly elongated along a proximal to distal axis and displayed parallel tracks of collagen I. Other culture studies using pre and postnatal valve progenitor cushion cells also confirmed that periostin had only a minimal effect upon proliferation [15]. Thus, it seems unlikely that that periostin drives cushion growth and elongation by a direct effect upon proliferation. An alternative mechanism to proliferation would be if periostin promoted cushion growth by inhibiting apoptosis. Transfection E15.5 mouse AV cushion cells with periostin knockdown vectors substantially increased caspase-3 levels whereas over-expression of periostin kept the level of the apoptotic marker at a basal level. Similarly, treatment with blocking antibodies to β1 and β3-integrins also increased the level of caspase-3 [15]. This suggests that interactions of periostin with its integrin receptors in mouse valvulogenic cushion cells may contribute to the growth and elongation of cushion swellings by inhibiting apoptotic cell death.

As shown in Fig. 17.3, the in situ expression of periostin in cushion cells continues as these cells migrate to colonize the acellular, hyaluronan-rich, cardiac jelly of the primitive AV and OFT cushion swellings [35]. This suggests that periostin may also promote elongation by stimulating the synthesis of hyaluronan during the period of elongation and/or by promoting the migratory behavior of prevalvular mesenchyme cells as it did in cardiotube cultures [36]. Regarding

hyaluronan synthesis, we have found that periostin- induced kinase activities promoted phosphorylation of hyaluronan synthetase2 (HAS2), the enzyme responsible for secreting hyaluronan into the extracellular matrix [15], and it did so in a time frame that correlated with the elongation of AV cushions. Interestingly, an unexpected finding was that by inhibiting PI3K the expression of periostin itself was inhibited, indicating that a positive feedback loop may exist between periostin, PI3K, hyaluronan and its receptor, CD44, to sustain periostin expression and increase phosphoserine-HAS2 expression and hyaluronan production.

In addition to promoting hyaluronan synthesis, periostin also promoted cell migration in 3D culture assays. Transfection of embryonic day 15.5 mice AV cushion cells with periostin full length cDNA enhanced fourfold their migratory behavior whereas migration was blocked or inhibited by transfection with periostin or FAK silencing vectors or by adding inhibitors of PI3K/AKT kinase activity or βintegrin -blocking antibodies to the medium. However, silencing Erk/Map kinase did not affect migration but did reduce collagen expression [14, 15]. This indicates that periostin activation of a β-integrin/FAK/PI3k/AKT signaling pathway can promote or accelerate the migration of mice AV cushion cells harvested at the ED 15.5 when morphogenetic growth and elongation are actively occurring in vivo, [3, 15, 33]. The lack of engagement of Erk/Map kinase in cell migration is consistent with recent evidence that Erk/MapK pathways may be involved in other biological functions associated with valvulogenesis, e.g. regulating collagen synthesis, valve matrix remodeling or cytoskeletal organization [9, 41, 52].

17.3.2 Valvulogenic Cell Differentiation

Periostin Hypothesis: Once initiated in post-EMT cushion mesenchymal cells, periostin expression continues during differentiation until remodeling/maturation is completed after birth. Normally cushion mesenchyme differentiates into interstitial fibroblasts characterized by their secretion of collagen, hyaluronan and proteoglycans [15, 34]. As first noted in cardiotube and hanging drop cultures, genetically deleting the periostin gene in mice resulted in cushion cells differentiating into non-fibroblastic, mesodermal lineages, e.g. cardiomyocytes in AV cushions or bone and cartilage in the OFT cushions [36, 45]. Also, the formation of the connective tissue structures that provide anchorage for the leaflets (annulus fibrosa, mitroaortic continuity) or connect them to the papillary muscles (chordae tendineae) were affected in periostin nulls. For example, the tendinous cords of the inlet valves were truncated or absent resulting in the papillary muscles directly inserting into the valve leaflets. Adding or rescuing periostin secretion both in vivo and in vitro, restored fibrogenic differentiation in cushion cells and inhibited abnormal differentiation into other mesodermal lineages, e.g. cardiomyocytes, chondrocytes or osteocytes [34, 36, 45]. We interpreted the above findings in terms of a "periostin hypothesis" that postulated that periostin functions as a hierarchical molecular switch that can promote the differentiation of multipotential cushion cells into a fibroblastic lineage while repressing their transformation into other mesodermal cell lineages (e.g. myocytes or osteocytes) [35, 36, 47].

To further test the hypothesis that periostin has a role in determining cell lineages, we used precardiac mesoderm as a testbed to assess the effect of periostin on cell fate. Normally precardiac mesoderm is fated to differentiate into the embryonic myocardium of the primary heart tube [48]. Precardiac mesodermal cells at Hamburger-Hamilton (H-H) stage-5 in chick embryos do not as yet express the myocardial marker, MF20. When the precardiac mesoderm was microdissected out from the H-H stage-5 chick embryos and cultured *in vitro* in serum containing medium for 24 hrs, it differentiated into contractile, MF20-positive myocardial cells. However, ectopic expression of periostin in precardiac mesoderm by viral gene transfer with adenovirus encoding HA-tagged full length periostin inhibited the differentiation of transfected precardiac mesodermal cells into a MF20-positive

myocardial cell phenotype (Fig. 17.4). Neighboring cells that were not transfected with HA-tagged full length periostin cDNA expressed MF 20. These data provide evidence that ectopic expression of periostin inhibits myocardial cell differentiation from precardiac mesoderm. They are also consistent with those [31] who found that that blockage of rhythmic contractile myocyte differentiation from the heart-forming region of the mouse embryo (i.e. precardiac mesoderm) is associated with strong up-regulation of periostin expression in the serum response factor (SRF) conditional knockout mice. These results, together with the cardiotube and other culture assays [16, 36], suggest that periostin directs normal valvulogenic lineage differentiation, in part, by inhibiting the aberrant formation of inappropriate mesodermal cell lineages, e.g. a myocardial lineage.

Because periostin can affect cushion cell migration during growth and elongation of cushion swellings by an interaction with β3-integrin followed by activation of FAK/PI3/AKT signaling pathway, it raised the question as to whether a periostin-induced signaling pathway could also be involved in differentiation of cushion cells as indicated by increased synthesis of type 1 α1 collagen (COL1α1). Expression of COL1α1 is considered to be one indicator of fibrogenic differentiation [13, 40]. The cardiotube experiments [16] and the valve phenotype of the periostin null mice would suggest that these morphogenetic events are closely linked [33, 34, 36, 45]. Transfection of cushion tissues with full length cDNA periostin expression vectors promoted COL1α1 synthesis [33, 34, 36]. Conversely, COL1α1 expression was downregulated if the cells were silenced for the periostin gene. Similar findings were obtained using inhibitors for intracellular kinases and blocking antibodies, indicating that periostin induction of COL1α1 protein expression in cushion cells was mediated by the binding of periostin to β1 or β3 integrin through activation of downstream PI3K/Akt or Erk pathways in cushion cells [15].

While this indicates that periostin signaling can promote fibroblastic differentiation of prevalvular mesenchyme, it does not provide a mechanism by which periostin can act as a hierarchal switch for inhibiting differentiation of cushion cells into non-fibroblastic lineages e.g. cardiomyocyte, osteocyte [45]. One possible mechanism by which periostin can act as a gatekeeper for the differentiation of mesodermally derived, multipotential cells is suggested by studies that indicate that periostin can directly interact with EGF or notch ligands [23, 50, 51] which are known to play important cardiac developmental roles in determining cell fate and differentiation [5, 7]. Genetic deletion of the periostin gene in mice leads to ectopic expression in the embryonic OFT cushions (and to a lesser degree in the AV cushions) of the pro-osteogenic growth factor, pleiotrophin (Ptn) and delta-like 1 homolog (Dlk1), a negative regulator of Notch1 [51]. This resulted in suppression of Notch1 signaling, strong induction of the central transcriptional regulator of osteoblast cell fate Runx2, upregulation of osteopontin and osteocalcin expression, and subsequent calcification of the aortic valve. Loss of Notch signaling in the endocardial cushions of ED 12.5 periostin-null mice hearts is strongly supported by the down-regulation of Hes1, Hey1 and Hey2, all downstream targets of Notch1 signalings. These data suggest that periostin through an interaction with notch ligands or receptors represses a default osteogenic program in OFT cushion mesenchyme while promoting differentiation along a fibrogenic lineage.

Lineage heterogeneity: Why deletion of periostin abnormally affects differentiation of AV cushions (myogenic) differently from that of the outflow cushions (chondrogenic, osteogenic) is not clear. One possibility may be lineage differences in the origin of the AV canal vs. the OFT from different heart forming fields: the AV being derived from the primary (first) heart forming field vs. the second heart field origin of the outflow track [29]. Thus, conditions or signals within the heart that influence levels of periostin, e.g. genetic mutations, alterations in hemodynamic loading, teratogens, carcinogens, etc.) may differentially shift the balance of differentiation cues in prevalvular cushion cells depending upon their heart field of origin.

In addition to differences in heart field origin, lineage tracing studies indicate that cushion tissues may contain cells from lineages other than those that are derived by endothelial EMT. In the AV cushions, epicardial derived cells or EPDC also contribute to valve morphogenesis during their growth and differentiation. EPDCs like endothelial derived mesenchyme, are also derived through an EMT process which in this instance involves the epicardial epithelium (reviewed by [27]). The latter is formed by an epithelial outgrowth from the (pro) epicardial organ derived from the coelomic mesothelium that covers the external surface of the heart tube [26]. EPDCs are most abundant in an external sulcus or groove associated with the AV canal where they directly contact the AV myocardium from the external surface while endocardial derived, cushion mesenchyme accumulates along the internal border of the myocardium (see Fig. 17.1) Over time, the continuity of the myocardium in both the AV and OFT valve forming regions is not maintained and the two populations of endocardial and epicardial derived mesenchyme intermingle. Both populations express periostin and differentiate into fibrous tissues that form within the leaflet and their anchorage sites: i.e. the mitroaortic continuity and the AV annulus. The latter provides not only anchorage for the mitral and tricuspid valve leaflets but also provides insulation from electrical activity of the atria from directly entering the ventricles [26]. The absence of a fibrous annulus and persistence of the AV myocardium in periostin null mice hearts associated with an abnormal EKG is consistent with this suggestion [36, 45]. Impeding the epicardial contribution to the AV cushions resulted in decreased cardiac periostin levels and hampered the development of fibrous tissue, particularly in the atrioventricular region, resulting in heart defects reminiscent of Wolff, Parkinson White syndrome [20, 26].

Hematopoietic derived cells along with neural crest cells are examples of other lineages that have been identified in developing valves, particularly in the OFT cushions [30]. The hematopoietic cells are observed even in the absence of circulation and thus appear to be derived from endogenous hemogenic activity that arises from a subset of endocardial cells in the outflow track. These cells would be expected to be surrounded by a matrix containing periostin secreted by cushion mesenchyme/fibroblasts. In bone, moderate levels of periostin were proposed to support the clonal expansion of hematopoietic cells in myelofibrosis by interaction with integrins and remodeling of the bone marrow stromal ECM [38]. The fate(s) of these myeloid cells within the developing OFT has not been established but some that are derived by the engraftment of circulating CD45 + bone marrow cells into mature leaflets after birth, differentiate into fibroblasts based on their expression of periostin, collagen and other fibroblast markers [18, 55, 56]. Thus, there is heterogeneity in the origin of prevalvular mesenchyme which may explain the different responses of AV and OFT valves to signaling molecules like periostin or to factors that regulate expression of periostin as seen in the Smad6-null mouse [47]. Potential subset interactions have not as yet been considered in the context of valvulogenesis but differences in the pathologies of prolapsed mitral vs. aortic valves would suggest that such consideration is merited.

17.3.3 Cushion Remodeling: Prenatal Compaction to Postnatal Maturation

The progression from a randomized pattern of cushion mesenchyme/fibroblasts and extracellular matrix in early valve primordia to one that is compacted into mature leaflets requires remodeling processes that are initiated during differentiation and completed after birth [1, 9, 52]. Mature valve leaflets are characterized by a trilaminar, zonal histoarchitecture of cells and matrix consisting of (i) an “atrialis” (fibrosa) in which collagen is densely packed and fibrillary and circumferentially distributed beneath endocardium; (ii) a “zona ventricularis” is a region of less dense collagen that is intermingled with elastin along the ventricular surface of the leaflets; and (iii) a “zona spongiosa” that lies between the atrialis and ventricularis that is enriched in glycans like hyaluronan and proteoglycans that can bind

to collagen or cell surfaces [25]. Developmental remodeling errors result in the failure of the cushions to compact their cells and collagenous matrix into this zonal pattern, and may not be immediately recognized as defects at birth but over time set up a trajectory towards degenerative changes leading to pathological phenotypes (e.g. myxomatous valves) that diminish mechanical properties, e.g. blood flow can be reversed as in mitral or aortic regurgitation, ultimately leading to heart failure [25, 41].

Thus, a key question for both normal development and postnatal disease is "how is morphogenetic remodeling accomplished"? Although a multifaceted question involving multiple levels of genetic and molecular regulation (e.g. FGF4/Erk/scleraxis; [24]), periostin is one key for remodeling. It is secreted throughout all phases of valve remodeling, peaking postnatally as maturation is completed [45] where it co-distributes with collagen. In general, the distribution of periostin and collagen go "hand in hand" as shown in whole mounts of isolated mitral valves (Fig. 17.5). Periostin interaction with collagen promotes bundling and alignment by either directly binding to collagen fibrils through its N-terminal cysteine EMI domain or through its ability to promote intermolecular cross-linking of collagen fibrils by activating lysyl oxidase [23]. The ability of periostin to change the stiffness/viscosity of the embryonic prevalvular cushions over time was determined by tensiometric measurements [33]. The phenotype of periostin null valves matches what might be anticipated if a gene encoding a protein was deleted which normally functions in promoting collagen secretion, organization and compaction. Atomic force microscopic studies on periostin null valve leaflets indicated an increase in compliance compared to wildtype heart valves, reflecting a decrease in stiffness/elastic modulus and biomechanical properties [36].

Evidence from 3D collagen gel contraction assays also support a morphogenetic role for periostin in valve compaction and maturation. Three dimensional culture models are important tools for studying cell-matrix interactions because of the differences in the conformation of protein networks that are not mimicked in two dimensional culture. Grinnell [17] has developed and compared models of tissue development and found that free floating gels best represent fibrous tissue development while mechanically constrained (stressed) gels develop activated fibroblast/myofibroblast phenotypes more commonly associated with wound healing and scar formation. We applied this model to simulate valvular morphogenetic remodeling [3]: undifferentiated cushion mesenchyme was seeded into a highly porous, low density, free-floating collagenous gel and assayed for the potential of periostin to enhance cell-mediated compaction (condensation) of the collagen gel lattice. Normal (wildtype) seeded cushion cells retained their potential to progressively promote gel compaction, and conversely this potential was completely inhibited by transducing the cells with periostin silencing vectors or with silencing vectors that inhibited beta integrins or the downstream signaling targets of periostin-integrin binding (e.g. FAK, AKT/PI3K, Pak1). Adding exogenous periostin to the medium of 3D gel cultures seeded with wildtype cushion cells accelerated compaction [3, 15] and, importantly, restored the potential of periostin null cushion cells to promote gel compaction.

However, periostin was effective in promoting collagen compaction only when cushion cells were seeded (entombed) within the gels and only when periostin activated these cells (as periostin added without cells did not compact the gel). Thus, in some way, the downstream targets of periostin/integrin signaling activated effector proteins that enabled cushion cells to exert forces sufficient to pull or draw (i.e. "compact") collagen into tightly condensed foci in the center of the gels. We have suggested that one mechanism by which a periostin activated, effector protein could promote compaction would be to modify cytoskeletal microfilament dynamics. One such effector protein candidate could be Pak 1 (p21 activated kinase) which is phosphorylated by periostin induced signaling through an integrin/Fak/mTOR/p70S6K pathway [14].

Pak 1 is one of six distinct isoforms of Pak encoded in mammalian cells, which historically

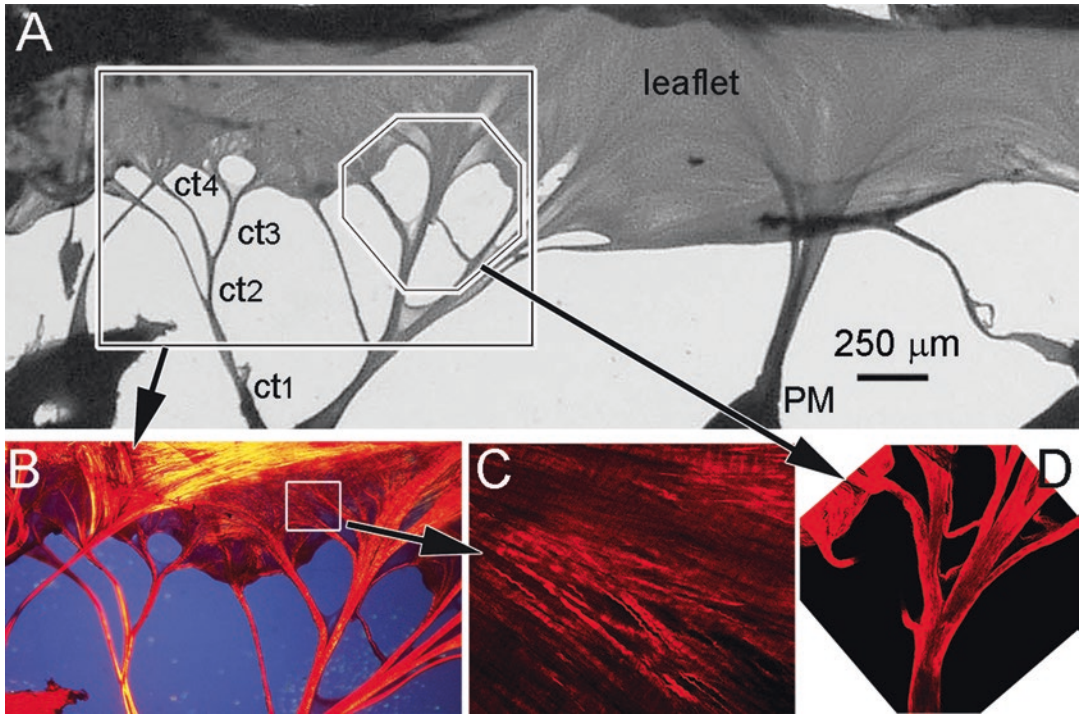

Fig. 17.5 Periostin and collagen co-expression in an adult mouse mitral valve. The entire left AV valve leaflet was microdissected from a 2–3 month old mouse heart and prepared for collagen and periostin staining as a whole mount to show that collagen and periostin go "hand in hand". (**a**) the original unstained, whole mount leaflet visualized for orientation by a dissection microscope. Note that the mitral leaflet like all AV valve leaflets has a tendinous supporting apparatus – the chordae tendineae – which connect to the tip of a papillary muscle and then undergo generational branching (ct2, ct3, ct4) before ramifying throughout the leaflet (as part of the zona atrialis and zona fibrosa). (**b**) corresponds to the rectangular insert in **a**. The global distribution of picosirius stained collagen fibers visualized by polarized microscopy can be seen. Note that collagen is seen throughout the valve leaflet, the chordae tendineae and annulus fibrosae (i.e. the base of the leaflet where it attaches to the wall of the heart). The intense red staining indicates mature collagen fibers whereas the yellow stained collagen reflects newly synthesized collagen that is less highly cross-linked. (**c**) corresponds to the small rectangle insert in **b** and shows at higher magnification the parallel alignment of the densely packed collagen fibers in this mature (fully remodeled) leaflet. (**d**) corresponds to the tissue inside the polygon shown in **a** that was subsequently stained for periostin expression. Note that periostin expression is intense and closely parallels (co-localizes) with that shown for collagen in **b**

are known to be activated by small GTPases, e.g. Cdc42 and Rac1. Periostin activation of Pak1, however, can occur independent of these GTPases and, thus, may constitute a second and separate pathway for activating Pak1 [14]. The significance of activating Pak1 is that once phosphorylated, it can interact directly or indirectly with the actin cytoskeleton and promote actin polymerization. One importance of changing the organization of the actin cytoskeleton is that it can modify the organization of the extracellular matrix through membrane proteins (e.g. integrins) that link cytoskeletal microfilaments to collagen and other extracellular matrix fibers [22, 33, 40, 44]. Such a mechanism could provide the cytoskeletal forces required for remodeling and compaction of cushion primordia into mature valve leaflets or cusps.

Yeast 2-hyrbid studies suggest that the binding of Pak1 to the actin cytoskeleton may be indirect i.e. mediated through the actin binding protein, filamin-A (FLNA) [54]. FLNA is expressed in cushion prevalvular cells during remodeling and compaction [41, 42, 52].

Expression of FLNA in cushion cells and ventricular interstitial cells is so specific as to be a marker for these fibroblastic-like cells [37]. In cushion cells, genetic analyses indicate that FLNA functions normally to promote the expression and transport of β-integrins to the cell surface and regulate changes in Ras-Mek-Erk pathway that balance secretion and degradation of the extracellular matrix. Genetically inhibiting FLNA expression in cushion cells resulted in enlarged but poorly remodeled valve leaflets that were prone to myxomatous degeneration over time [52]. These findings have added significance for valve morphogenesis because genetic analyses of patients with non-syndromic mitral valve prolapse revealed mutations in FLNA.

Thus, collectively these findings suggest a central regulatory axis by which periostin can function in valve morphogenetic remodeling, compaction and maturation. A conceptual diagram for this putative regulatory axis is presented in Fig. 17.6. It is based on our published understanding of how periostin signaling can modify cellular functions in valve cushion progenitor cells [14, 15, 33, 52]. A key pathway for regulating assembly and organization of the extracellular matrix would be triggered by periostin binding to integrins to initiate a signaling pathway (β-integrin/Fac/Src) that activates (phosphorylates) Pak1 and its potential to bind FLNA and effect changes in actin cytoskeletal organization.

Summation: Clearly such a regulatory pathway is just a starting point for understanding the role of periostin in valve morphogenesis. There is still much to learn about its potential for interacting with other regulatory networks that participate

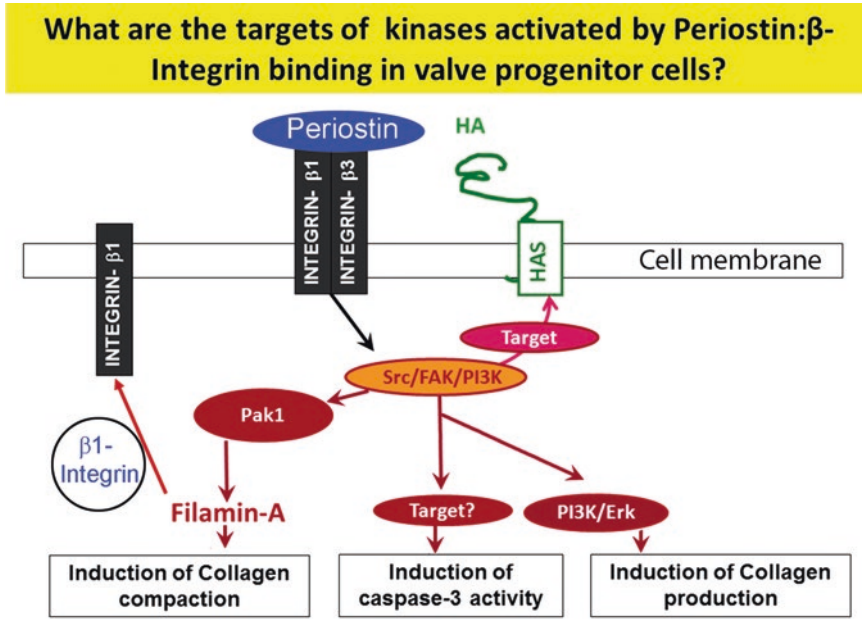

Fig. 17.6 Conceptual model of periostin signaling during valvulogenesis. This model is based on Ghatak et al. [14, 15] and Toomer et al. [53] and is intended to highlight some of the kinase targets known to be activated by periostin-integrin signaling. These include AKT/PI3K which phosphorylates hyaluronan synthetase (HAS2), the enzyme that produces hyaluronan, a potential ligand for CD44 during the elongation phase of valve development. Erk whose activation has been linked to differentiation of cushion cells and synthesis of collagen. Activation (phosphorylation) of Pak1 in cushion cells has been shown to promote cytoskeletal actin assembly. It is further proposed in the model that Pak1 can also bind to and phosphorylate filamin A in cushion cells as it does in other cell types. Filamin A is intensely expressed in cushion cells where genetic analyses indicate that it functions in the transport of integrins to the cell surface and as a regulator of remodeling and compaction (possibly through its role as a major actin binding protein). Most recently [14] Pak1 has also been shown to inhibit apoptosis in cushion cells by inhibiting synthesis of caspases

in cushion mesenchymal cell migration, matrix synthesis, matrix deposition, matrix organization, as well as the role of distinct cell populations (lineages) that integrate within the developing and adult valve. For example, each cushion cell has a primary cilium whose temporal presence correlates with the establishment of the histoarchitecture of mature leaflets [53]. How or if ciliary genes interact with periostin or its signaling intermediates presents new opportunities for mechanistic discoveries. Time may prove that understanding how a valve is built (i.e. develops) may ultimately shed light on valve diseases or engineering their replacements.

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Part VIII

Biomarker

18 Practical Application of Periostin as a Biomarker for Pathological Conditions

Isao Kii

Abstract

In physiological condition, periostin is expressed in limited tissues such as periodontal ligament, periosteum, and heart valves. Periostin protein is mainly localized on extracellular collagen bundles and in matricellular space. On the other hand, in pathological condition, expression of periostin is induced in disordered tissues of human patients. In tumor development and progression, periostin is elevated mainly in its microenvironment and stromal tissue rich in extracellular matrix. Tumor stromal fibroblasts highly express periostin and organize the tumor-surrounding extracellular matrix architecture. In fibrosis in lung, liver, and kidney, proliferating activated fibroblasts express periostin and replace normal functional tissues with dense connective tissues. In inflammation and allergy, inflammatory cytokines such as IL-4 and IL-13 induce expression of periostin that plays important roles in pathogenesis of these diseases. The elevated levels of periostin in human patients could be detected not only in tissue biopsy samples but also in peripheral bloods using specific antibodies against periostin, because periostin secreted from the disordered tissues is transported into blood vessels and circulates in the cardiovascular system. In this chapter, I introduce the elevated expression of periostin in pathological conditions, and discuss how periostin could be utilized as a biomarker in disease diagnosis.

Keywords

Periostin · Tumor · Fibrosis · Inflammation · Allergy · Antibody · Diagnosis · ELISA · PET · SPECT

18.1 Detection of Periostin with Specific Antibodies

Periostin has been proposed as a biomarker for several diseases including tumor, fibrosis, inflammation and allergy [14, 16, 17, 23, 60, 70, 81, 85]. Elevated expressions of periostin were initially observed in microarray gene expression analyses [5, 22, 29, 33, 51, 82, 90]. Although gene expression of periostin could be evaluated with microarray or quantitative PCR with cDNA samples, these methods require total mRNA of the disordered tissues from biopsy samples of human patients. Biopsy is an invasive procedure that increases the burden on patients. Therefore,

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an alternative method to detect expression of periostin with minimal invasiveness on patients has been needed. As an alternative method, great attention has been paid to periostin in peripheral blood of patients with diseases [13, 24, 47, 48]. Periostin is a secretory protein mainly derived from fibroblastic cells and interacts with several extracellular matrix proteins such as fibronectin, tenascin-C, and collagens [31, 36]. On the other hands, a secreted periostin is transported into circulating blood through blood vessel, which would become permeabilized due to disease progression, and circulates in cardiovascular systems (Fig. 18.1). For example, neovessels formed in tumor tissue are permeable, and are easy to pass relatively large molecules including antibodies [45, 77, 91]. This permeabilization of blood vessels in tumor tissues is recognized as the enhanced permeability and retention (EPR) effect, by which molecules of appropriate sizes, such as liposomes, nanoparticles, and macromolecular drugs, accumulate in tumor tissue more than in normal tissues. Neovessels formed in inflamed tissue are also permeable. Thus, the circulating periostin in peripheral blood indicates existence of disordered tissue such as tumor microenvironment, fibrosis, and inflammation. The highly sensitive detection and quantification method of circulating periostin in peripheral blood of human patients has been evaluated as a minimally invasive procedure for disease diagnosis.

To utilize periostin as a biomarker for diseases, specific detection methods are required. Antibody has usually been used to specifically detect periostin in tissue thin section, blood, and whole body. In the earliest stage of periostin research, Kudo and colleagues developed rabbit polyclonal antibodies against the first FAS 1 domain (anti-RD1) and the carboxyl-terminal end of the CTD (anti-CT) [20, 80], and have revealed the spatiotemporal distribution of periostin in mouse and human tissues. Thereafter, a lot of polyclonal or monoclonal antibodies against periostin have been developed, and some of which are now commercially available. These antibodies against periostin were used to investigate periostin localization. Immunohistochemical analyses using these antibodies demonstrated that periostin physiologically localizes at collagen-dense areas in connective tissue, including the periodontal ligament [20, 30, 71, 84], periosteum [20, 71], cardiac valve [18, 57, 58], and alveolar wall in the lung [7, 35, 62]. Periostin has also been found to pathologically localize in infarcted myocardium [61, 80], fibrosis [22, 49,

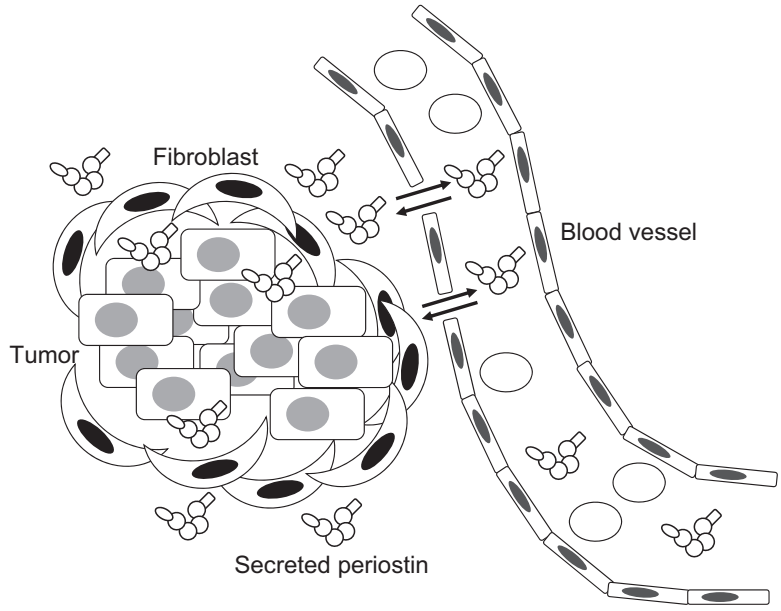

Fig. 18.1 Expression and secretion of periostin into peripheral blood

62, 86, 92], the wound healing process [54, 56, 63, 98], and cancer-associated stroma [15, 28, 32, 33, 43, 55, 65, 68, 73, 83, 89, 94, 96]. Thus, expression of periostin is also closely associated with tissue regeneration post-injury [10]. In addition, function-blocking antibodies against periostin have been developed as rabbit polyclonal antibodies, PN1-Ab, PN21-Ab, PnAb, and mouse monoclonal antibody, MZ-1, OC-20, which were utilized in mouse disease models such as tumor growth and metastasis [39, 50, 52, 64, 88, 99]. These specific antibodies against periostin contribute to highly sensitive detection and quantification of periostin as a biomarker for disease progression.

Periostin-binding DNA aptamer has also been developed [40, 53, 93]. Nucleic acid-based aptamers comprise an emerging class of targeted therapeutic and diagnostic molecules [21, 69]. Aptamers are single-stranded DNAs or RNAs that are designed to bind to proteins with similar or better affinity and specificity, compared with antibodies or small molecules. Periostin-binding aptamers would also be useful in detection of periostin.

18.2 Detection of Periostin in Peripheral Blood

To detect and quantify the circulating periostin in peripheral blood samples, the sandwich enzyme-linked immunosorbent assay (ELISA) using antibodies against periostin has been developed [9]. An antibody against periostin is immobilized on wells of microtiter plate, which captures periostin protein in blood samples. The captured periostin is further labeled with the other antibody against periostin, which binds to the antigen distinct from the other one recognized by the antibody coated on the wells. The antibody on periostin was then recognized with an enzyme-conjugated secondary antibody. The captured periostin protein could be detected with the enzymatic activity as an output. As the enzymes conjugated on secondary antibody, alkaline phosphatase, horseradish peroxidase, and luciferase are utilized. Thus, ELISA enables highly sensitive and specific detection of periostin in peripheral blood samples.

At the beginning of researches on evaluation of periostin in peripheral blood samples, Ben et al. [6] developed ELISA using commercially available antibodies against periostin and measured serum periostin levels in peripheral bloods of human patients with cancers. The authors demonstrated that concentration of periostin in blood samples of patients with colorectal cancer was significantly higher than those of healthy volunteers and of patients with benign colorectal polyps or adenomas. The authors also showed that cancer cells were negative for periostin and their surrounding stromal tissues were positive, indicating that the circulating periostin in peripheral bloods is derived from the surrounding cancer stromal tissues. These results suggest that serum levels of periostin detected by ELISA are of clinical value in identifying patients who may be at a high risk for malignancy of colorectal cancer.

Yamaguchi et al. [97] also evaluated serum periostin levels in peripheral blood samples from patients with progressive skin sclerosis. Skin sclerosis is one of the symptoms of systemic sclerosis that is an autoimmune disorder. Autoimmune reaction causes tissue destruction, resulting in proliferation of activated fibroblasts and accumulation of collagenous extracellular matrix proteins under the skin. Periostin was strongly expressed in the affected dermis biopsy samples from patients with systemic sclerosis. Serum levels of periostin in patients with systemic sclerosis were significantly elevated compared with healthy subjects, indicating that periostin secreted in the affected dermis is transported to blood vessels and circulated in peripheral bloods. This report suggests that an elevated periostin level in patients with systemic sclerosis is associated with severity of skin sclerosis, and that periostin is a potential biomarker for progressive skin fibrosis.

Jia et al. [27] also demonstrated that periostin concentrations in peripheral blood samples from patients with asthma were significantly higher than those from healthy subjects. Asthma is a condition in which airway narrow and swell

produce extra mucus, which makes breathing difficulty and trigger coughing, wheezing and shortness of breath. In a histological view of asthmatic airway, as a result from inflammation and remodeling processes, activated fibroblasts proliferate and deposit collagenous extracellular matrix, causing thickening and increasing density of the basement membrane. In this study, the authors examined several biomarker candidates in peripheral blood samples from patients with severe asthma, and concluded that the serum periostin level was the single best predictor of airway eosinophilia. Other reports also demonstrated periostin as a biomarker for asthma [47]. Thus, periostin is a systemic biomarker of airway eosinophilia in asthmatic patients and has potential utility in patient selection for asthma therapeutics.

In addition to the reports described above, elevated expression of periostin mRNA has been demonstrated in mice with the transient middle cerebral artery occlusion model that is similar to human cerebral ischemia [78, 79]. The elevated expression of periostin promotes neural stem cell proliferation and differentiation, which would contribute to regeneration of brain injury [44]. Furthermore, serum periostin concentrations were significantly increased in peripheral blood samples from patients with traumatic brain injury, compared with those from healthy controls [12]. These reports suggest that brain injury induces expression of periostin in the traumatic region, and that secreted periostin is transported into blood vessels and then circulates in the cardiovascular system [25, 85].

In analogy to these initially reported ELISA experiments, elevated periostin levels in peripheral blood samples from patients with diseases such as cancer, fibrosis, inflammation, allergy, and ischemia have been reported. Diseases with increased periostin expression in disordered tissues should be intended for periostin liquid biopsy as peripheral blood samples with minimal invasiveness, which is useful for patient selection in therapeutics.

Although periostin liquid biopsy is an alternative way having minimal invasiveness and informs us existence of the disordered tissues such as cancer, fibrosis, inflammation and allergy, it is impossible to visualize the disordered tissues. To overcome this problem, a molecular imaging strategy targeting periostin-positive disordered tissues has been examined.

18.3 Molecular Imaging Targeting Periostin in Living Animals

Antibody can be utilized in diagnosis with molecular imaging techniques such as positron emission tomography (PET) and single photon emission computed tomography (SPECT) (Fig. 18.2). PET is a nuclear medicine functional imaging method used to visualize localization of diagnosis probe molecules with radionuclide that emits positron. Positron-emitting radionuclides such as ^{11}C (carbon-11), ^{18}F (fluoride-18), and ^{64}Cu (copper-64), are produced by nuclear reactions in cyclotron, in which hydrogen ions (proton) are accelerated under a high voltage in the magnetic field. These radionuclides are conjugated with probe molecules by chemical reactions. PET system detects pairs of gamma rays emitted indirectly by a positron-emitting radionuclide, which is introduced into a biologically active molecule called a radioactive tracer. For example, small molecule tracers such as ^{18}F-fluorodeoxyglucose (FDG) that is a glucose analogue, have been frequently utilized as diagnosis in clinical oncology for staging, restaging and evaluation of tumor response to treatment, because cancer cells rather than non-malignant cells uptake a lot of glucose as an energy source to proliferate (O'Connor et al. [59]). On the other hand, SPECT is able to visualize localization of radiolabeled diagnosis probe molecules in human patients, which detects gamma ray from radionuclide conjugated on the probe molecule. As gamma-emitting radionuclides, ^{99m}Tc (technetium-99m), ^{123}I (iodine-123), ^{131}I (iodine-131) are utilized. These radionuclides themselves could also be used to scan bone, myocardial perfusion, brain, and tumor.

In case of antibody, the small molecule metal chelator, such as DOTA, NOTA, and NODAGA, is covalently conjugated to antibody, with amine

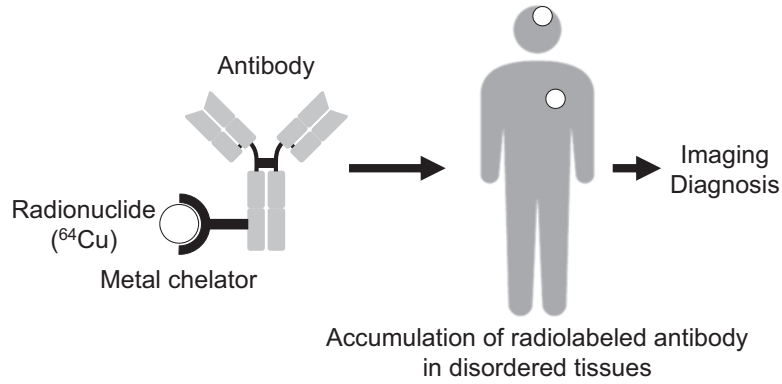

Fig. 18.2 Immuno-PET imaging

coupling reaction for example, and radionuclide metal ion such as ^{64}Cu produced in cyclotron is then introduced to the chelators conjugated on antibody [72]. Radionuclide-labeled antibody is administrated into patients with disease such as cancer, who is thereafter scanned with PET camera and CT (computed tomography). For example, PET scan of patients with aggressive breast cancer were performed with ^{64}Cu-labeled antibody against HER2 (epidermal growth factor receptor 2) (^{64}Cu-trastuzumab) [37, 38, 74, 87]. ^{64}Cu-trastuzumab was administrated to patients with metastatic HER2-positive breast cancer, and scanned with medical PET/CT camera, clearly visualizing its accumulation not only in the primary lesion of tumors but also in brain metastasis. Thus, PET imaging technique with radiolabeled antibody (Immuno-PET) would be useful for diagnosis of diseases [46].

Heidari et al. [19] have utilized an antibody against periostin in PET imaging with mice bearing the esophageal squamous cell carcinoma cell line expressing periostin. The authors used a commercially available anti-periostin monoclonal antibody, and prepared a F(ab')^2 fragment by enzymatic digestion. The F(ab')^2 fragment was then labeled with DOTA chelator and subsequently with ^{64}Cu. PET imaging clearly showed specific accumulation of the radiolabeled antibody to tumor tissue derived from the inoculated cell line. Although this study demonstrated that periostin immuno-PET imaging is a powerful method to visualize periostin-positive tissues, it remains elusive whether periostin immuno-PET imaging could visualize the fibrotic region of dense connective tissues in tumor microenvironment, fibrosis, inflammation and allergy. Further studies on periostin immuno-PET imaging are required in order to visualize periostin-related diseases.

18.4 Clinical Imaging of Tenascin-C and Extracellular Matrix

Tenascin-C, which is one of the interacting proteins with periostin, has been targeted in PET or SPECT imaging studies [1, 3, 4, 34, 66, 67, 75, 76]. Akabani et al. [2] demonstrated that ^{131}I-labeled anti-tenascin 81C6 murine monoclonal antibody accumulated lesion of patients with malignant gliomas in MRI/SPECT imaging. Jacobson et al. [26] developed a radiolabeled single-stranded DNA aptamer that targets tenascin-C and utilized it in PET imaging of mice bearing tenascin-C-positive tumor. Thus, molecular imaging techniques could target extracellular matrix proteins accumulated in the disordered tissues. It should be possible to visualize periostin in the extracellular matrix and matricellular space of the disordered tissues such as tumor microenvironment.

In addition to periostin and tenascin-C, the other extracellular matrix proteins and related transmembrane proteins have also been targeted in molecular imaging. Radiolabeled probes that recognize fibrin, fibronectin, collagens, MMPs, and integrins have been developed to visualize fibrosis and fibrogenesis [11]. These molecular

probes including antibodies against periostin could be useful not only for diagnosis but also for the drug delivery system, which enables targeted delivery of drug only to the disordered tissues and reduces the adverse side effect of drug. For example, tumor microenvironment, which is rich in stromal fibroblasts and extracellular matrix proteins, is one of the crucial factors that cause tumor drug-resistance [8, 41, 42, 95]. Remodeling of tumor microenvironment by the drug delivery system targeting the extracellular matrix or transmembrane proteins described above would improve anti-tumor efficacy in drug treatment. Thus, targeting the fibrotic lesion in the disordered tissues is of importance in therapeutics as well as diagnosis.

18.5 Conclusion

Periostin should be a promising biomarker for diseases such as tumor, fibrosis, inflammation, and allergy. The advantage of periostin is to detect and quantify it in peripheral blood samples of human patients. This liquid biopsy could be performed with minimal invasiveness, which does not burden patients. Periostin in peripheral blood indicates existence of the disordered tissues, in which abnormally activated fibroblastic cells highly express and secret periostin that is transported into circulating blood through blood vessels. Basic researches on periostin in peripheral bloods have utilized ELISA in detection, whereas rapid detection methods, such as the quantitative immuno-chromatography, are required to expand its utility in routine clinical practice.

Antibodies against periostin could be also utilized in clinical immuno-PET diagnosis. Specific and high affinity antibodies against periostin have been developed and utilized for immunological detection, functional blocking, and molecular imaging. Immuno-PET diagnosis could visualize distribution of the disordered tissues in living patients with minimally invasiveness, which contributes to accurate operative treatment as well as evaluation of outcome from therapy. Because clinical molecular imaging such as immuno-PET is an emerging method, periostin in the disordered tissues would be targeted as a useful biomarker for disease diagnosis.

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Part IX

Clinical Applications

19 Clinical Applications Targeting Periostin

Akira Kudo

Abstract

Since periostin is expressed and functioned in incredible diseases, clinical applications have been initiated to directly target periostin for inhibition or activation, or periostin expression is utilized to indicate the disease state or a marker for curing diseases, which will provide novel methods in clinical applications.

Keywords

Clinical application · Incredible disease · RNAi · DNA aptamer · Periostin antibody · Lebrikizumab · Osteoporosis · Biomaterials · Capsular contraction

19.1 Clinical Trials by Inhibition of Periostin Function

Due to the increase in the number for periostin-related diseases (Fig. 19.1), some clinical trials are already underway. The first trial for clinical application of periostin demonstrated that gel-form disks loaded with a recombinant periostin introduced into the pericardial cavity of pigs and lead to increased cardiomyocyte cell cycle activity and angiogenesis [17]. Thereafter, inhibition of periostin action has been getting popular as clinical trials, because excessive activity of periostin induces severe fibrosis and causes a deficiency in organ function. From this concept, a new RNAi agent had a pronounced inhibitory effect on lung fibrosis [20], choroidal neovascularization, and choroidal fibrosis [13] as well as retinal neovascularization [14] and adhesion formation of abraded cecum after surgery [18]. Furthermore, in the clinical trial using periostin expression in lung fibrosis, C-type natriuretic peptide (CNP) expressed by the periostin promoter ameliorates bleomycin-induced pulmonary fibrosis by suppressing TGF-β signaling [8]. Similarly, intraperitoneal administration of the periostin-binding DNA aptamer significantly abrogated peritoneal fibrosis [15]. In addition, benzyl-d(U) TP-modified DNA aptamers targeting human periostin inhibit breast cancer growth and metastasis by selectively binding to the FAS-1 domain of periostin and disrupting the interaction between periostin and αvβ3 and αvβ5 integrins [12]. In a new therapeutic method, overexpression of miR-599, which down-regulates periostin, inhibited glioma cell migration and invasion [22].

Material in this chapter has been adapted from Kudo [10] and Kudo and Kii [11] with permission.

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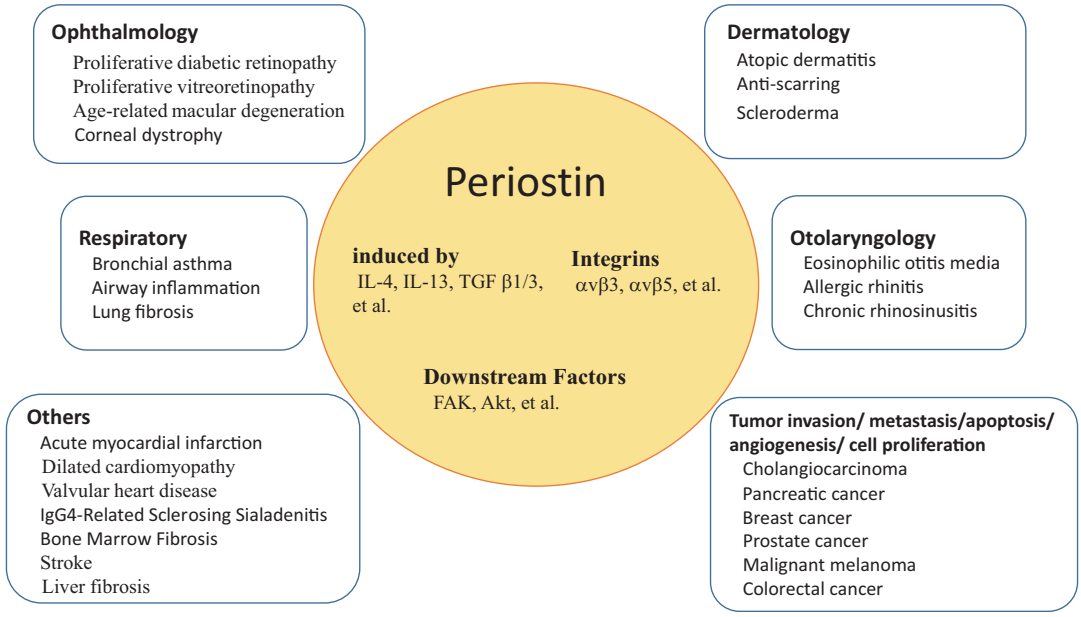

Fig. 19.1 Periostin and related diseases

Inhibition of periostin action by antibodies is a key regulator, and one of many monoclonal antibodies against human periostin has revealed the importance of the first fasciclin I domain, in which 16-amino acid sequence (APSNEAWDNLDSDIRR) is a binding site for the antibody and is a possible binding site for the αvβ3 integrin [4]. Although this antibody was useful for developing a clinical reagent, these results have not been reproduced so far *in vivo*. However, this specific 16-amino acid sequence is a candidate of a novel therapeutic target. The next unique antibody recognizing periostin exon 17 can selectively block the full-length periostin protein but not the alternatively spliced periostin truncated forms that lack exon 17 or exons 17 and 21. Since full-length periostin mainly regulates fibrogenesis in tissue repair after myocardial infarction (MI), this antibody treatment after MI can improve heart function [19].

19.2 New Clinical Applications Due to Periostin Expression

In diagnostic applications, periostin is a useful marker of severe bronchial asthma. In patients with asthma, the high-Th2 phenotype has been associated with a high level of circulating periostin, which is induced by interleukin-13 (IL-13), expressed by airway structural cells, and was detected by anti-periostin antibodies [9]. The effect of an anti-IL-13 antibody-based drug, called lebrikizumab, on asthma was reported [3]. Although there was a beneficial effect on airflow obstruction in all the patients treated with lebrikizumab, the effect was greater in patients who had circulating levels of periostin, indicating that periostin is a good biomarker for determining candidates for lebrikizumab treatment [23]. Currently, the lebrikizumab program is on hold since one of two identical phase 3 studies failed due to a lack of consistent reduction in asthma

exacerbations [6]. In contrast, the simultaneous targeting of both IL-4 and IL-13 by blocking IL-4 receptor with an antibody reduces asthma exacerbations and improves lung function [16], which is consistent with periostin activation by both IL-4 and IL-13. In regard to mechanical stress, under microgravity, periostin can be utilized as a marker of periosteal metabolism, since periostin concentrations in serum increase transiently along with a 15% increase in cortical porosity, which indicates that periostin is a good marker of cortical porosity [21]. As the widely recognized therapy for asthma, corticosteroids are treated, which results in reduction of serum periostin levels and improved airflow limitation, indicating that periostin levels correlate with airway wall thickness and sputum eosinophilia [4]. In the field of calcified tissues for postmenopausal women in osteoporotic bone fracture, plasma protein is highly associated with non-vertebral (limbs, mainly composed of cortical bone) but not vertebral (spine, mainly composed of cancellous bone) fractures [7]. Since a working group of the World Health Organization is searching for clinical risk factors in osteoporotic fracture without the use of bone mineral density (BMD) measurements, periostin is a potential biomarker for predicting osteoporosis-related phenotypes.

In silicone implantation, the most serious complication is capsular contraction. This occurs when excessive fibrotic tissue formation caused by an exaggerated foreign body reaction and a contractile force originating from collagenous capsulation, leads capsular contracture around the implant [1]. This acute inflammatory response against biomaterials induces periostin expression followed by formation of capsular contraction. Similarly, in traditional tissue-engineered cartilage, a biodegradable polymer scaffold can be used for shape retention; periostin contributes to this shape retention by enhancing the mechanical strength of the surrounding fibrous tissues consisted of periostin-mediated collagen structure [5].

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