HHS Public Access

Author manuscript

Cell Tissue Res. Author manuscript; available in PMC 2017 September 01.

Published in final edited form as:

Cell Tissue Res. 2016 September ; 365(3): 453–465. doi:10.1007/s00441-016-2426-6.

Periostin as a Multifunctional Modulator of the Wound Healing Response

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Abstract

During tissue healing, dynamic and temporal alterations occur in the structure and composition of the extracellular matrix (ECM) that are required for effective repair to occur. Matricellular proteins (MPs) are a group of diverse non-structural ECM components, which bind cell surface receptors mediating interactions between the cell and its microenviroment, effectively regulating adhesion, migration, proliferation, signaling and cell phenotype. Periostin (Postn), a pro-fibrogenic secreted glycoprotein, was defined as a MP based on its expression pattern and regulatory roles during development, healing and in disease processes. Postn consists of a typical signal sequence, an EMI domain responsible for binding to fibronectin, four tandem fasciclin-like domains that are responsible for integrin binding and a C-terminal region where multiple splice variants originate. This review will focus specifically on the role of Postn in wound healing and remodeling, an area of intense research in the last 10 years particularly related to skin healing as well as in myocardium post infarction. Postn interacts with cells through various integrin pairs and is an essential downstream effector of TGF-β superfamily signaling. As will be discussed, across different tissues, Postn is associated with pro-fibrogenic process, specifically, the transition of fibroblasts to myofibroblasts, collagen fibrillogenesis and ECM synthesis. Although the complexity of Postn as a modulator of cell behavior in tissue healing is only beginning to be elucidated, its expression is clearly a defining event in moving wound healing through the proliferative and remodeling phases.

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Keywords

Postn; matricellular; myofibroblast; cardiac; skin

1. Introduction

Initially defined by Paul Bornstein in 1995 (Bornstein, 1995), matricellular proteins (MPs) are secreted non-structural extracellular matrix (ECM) molecules which exhibit diverse functions that exert control over development, pathology and tissue healing processes. In general, most MPs show restricted expression to development and re-expressed in post-natal tissues in pathologies or during wound healing (Hamilton, 2008). MPs are non-structural components of the ECM, and modify cell behavior through cell surface receptors such as integrins (Bornstein and Sage, 2002). Since the first definition of MPs based on Bornstein's work on thrombospondin-1 (Reed, et al., 1993, Shingu and Bornstein, 1994), many additional molecules have been given this classification, including (Periostin) Postn in 2008 (Norris, et al., 2008a), the N-Glycoprotein that is the focus of this review.

First cloned in 1993 and initially designated osteoblast specific factor-2 (Takeshita, et al., 1993), Postn was renamed in 1999 by Horiuchi and colleagues based on its restricted expression to the periosteum and the periodontal ligament in mice (Horiuchi, et al., 1999). *Postn* undergoes alternate mRNA splicing (Conway and Molkentin, 2008), which generates variants with respect to the carboxyl tail domain terminus and multiple different isoforms have been identified in both mice and humans. Structurally, full length Postn is a 90 kDa protein that consists of a typical signal sequence, followed by a cysteine-rich region, an EMI domain (protein-protein interactions), four tandem fasciclin-like domains that are responsible for integrin binding and a C-terminal region (Kudo, 2011). There are 10 potential residues on which Postn could also be phosphorylated (Annis, et al., 2015), and although somewhat controversial, there is also evidence that Postn could be a GLA-protein, containing canonical carboxylase recognition sites embedded between or within the fasciclin domains (Coutu, et al., 2008). Therefore, as well as exhibiting alternate splicing, Postn also has the potential to undergo post-translational modification, specifically the GLA-region via γ-carboxylation, maybe in a tissue specific manner. Recent evidence for this hypothesis arises from a study that shows Postn secreted from normal or fibrotic lung is not γ-carboxylated (Annis, et al., 2015), which is in contrast to the results using bone marrow-derived mesenchymal stromal cells where Postn is (Coutu, et al., 2008). The number of different Postn isoforms varies based on tissue of isolation. For example, in the human thyroid, 8 isoforms have been identified (Bai, et al., 2010), but in cardiac tissue, only 4 isoforms are present (Morita and Komuro, 2016). The isoforms described thus far vary between 751 and 836 amino acids in length (Morra, et al., 2011). *Based on the documented information, the possibility exists that although Postn isoforms arise from a single gene, they may more accurately represent a diverse family of proteins with very tissue specific expression profiles*.

In postnatal animals, Postn is primarily associated with collagen-dense connective tissues (Hamilton, 2008). Unlike several of the other described MPs, Postn expression is maintained

in certain adult tissues including periosteum and periodontal ligament, which manifest in the phenotype of the knockout mouse (Rios, et al., 2005). Postn deletion results in significant damage to the tooth-supporting structures (Rios, et al., 2005), with removal of masticatory forces reducing this damage, showing the loss of periostin impairs the ability of the tissue to withstand mechanical loading (Rios, et al., 2008). Postn is heavily expressed in the periodontal ligament and periosteum in adult animals, and we have further described expression in the basal lamina in skin (Elliott, et al., 2012b, Jackson-Boeters, et al., 2009, Zhou, et al., 2010) and gingiva (Wen, et al., 2010) in postnatal tissues, although the significance of these findings has yet to be determined.

Much of our understanding of the role of MPs initially came from analysis of skin healing in knockout animals (for a comprehensive review, see (Walker, et al., 2015), but in the case of Postn, initial descriptors of its role in tissue healing came from the cardiac system (Conway and Molkentin, 2008, Dorn, 2007, Ladage, et al., 2013, Minicucci, et al., 2013, Norris, et al., 2008a, Oka, et al., 2007, Shimazaki, et al., 2008). Postn, like most MPs, is most noticeably re-expressed as a result of tissue injury, temporally and spatially regulating different aspects of the repair process by modifying cell behavior (Walker, et al., 2015). In this review, we will focus on the role of Postn in the healing of skin and cardiac muscle, the two tissue systems where it has been most intensely investigated and its actions defined (Dorn, 2007, Elliott, et al., 2012a). Additionally, we will discuss Postn in tissues where it is known to play prominent roles in development and disease, and although described, its role in repair is not well defined.

2. Skin healing

Postn is prominently expressed during skin development in the dermis, basement membrane and hair follicles from embryonic through neonatal stages and in the basement membrane and hair follicle only in adult (Zhou, et al., 2010). The role of Postn in skin pathologies (Arima, et al., 2015, Crawford, et al., 2015, Mineshige, et al., 2015, Shiraishi, et al., 2012, Song and Qin, 2008, Supp, et al., 2012, Yamaguchi, et al., 2013, Zhang, et al., 2015, Zhou, et al., 2010) and wound healing (Elliott, et al., 2012a, Elliott, et al., 2012b, Jackson-Boeters, et al., 2009, Nishiyama, et al., 2011, Ontsuka, et al., 2012, Walker, et al., 2015, Zhou, et al., 2010) has been the subject of intense research, which has pointed to very specific roles for Postn.

Skin wounds heal in a relatively rapid manner depending on their initial size. In mice, termination of bleeding (hemostasis) is followed by the well described temporal phases of inflammation, proliferation and remodeling (Walker, et al., 2015). As a result of hemostasis, a provisional matrix of fibrin and fibronectin forms, which temporally matures into granulation tissue (Greiling and Clark, 1997). Transforming growth factor-β1 (TGF-β1) is secreted by macrophages (Assoian, et al., 1987) and chemotactically initiates fibroblast migration into the loose granulation tissue matrix. We first published the expression pattern of Postn in full thickness excisional wound healing in 2009 (Jackson-Boeters, et al., 2009), demonstrating that Postn is first detected in maturing granulation tissue at day 3 post-wounding, peaking at day 7, that latter correlating with the peak of myofibroblast presence during healing. Although Postn has been implicated in the regulation of inflammation in

certain pathological situations (Bentley, et al., 2014, Huang, et al., 2015, Izuhara, et al., 2014, Koh, et al., 2016, Mael-Ainin, et al., 2014, Nair and Kraft, 2012, Schwanekamp, et al., 2016), its upregulation at day 3 post wounding suggested that it plays no significant role in regulating inflammatory processes during acute skin healing. Moreover, we found an inverse correlation between inflammatory cell infiltration ($CD68^+$ macrophages) and Postn expression, suggesting that Postn is not associated with the inflammatory phase of healing in acute wounds (Jackson-Boeters, et al., 2009).

In subsequent years, our group and that led by Professor Akira Kudo at the Tokyo Institute of Technology, independently performed analysis of excisional skin healing processes in *Postn* knockout ($Postn^{-/-}$) mice in comparison to wild-types ($Postn^{+/+}$). Representing two different $Postn^{-/-}$ mouse alleles, the studies both reported very similar delays in closure kinetics in the $Postn^{-/-}$ mice. Reduced closure was evident in $Postn^{-/-}$ mice from day 3 to day 9 post wounding, which corresponds temporally with transition from the inflammatory phase (day 3) into the proliferative phase of healing (days 4–9) (Elliott, et al., 2012b, Nishiyama, et al., 2011). Both studies similarly demonstrated that the peak of *Postn* mRNA was at 7 days post wounding (Figure 1), before declining back to baseline levels. As will be described below, despite these similarities, the papers identified two different mechanisms underlying the closure defect in $Postn^{-/-}$ mice.

In mice, wound closure occurs as a result of re-epithelialization and contraction. Nishimyura and colleagues focused on re-epithelialization of the wounds, identifying that the percent closure of the wounds was significantly reduced in the $Postn^{-/-}$ mice in comparison with their $Postn^{+/+}$ counterparts at day 3 and 5 post-wounding (Nishiyama, et al., 2011). This defect in re-epithelialization was attributed to a reduction in proliferation of keratinocytes surrounding the hair follicles in $Postn^{-/-}$ mice. *In vitro* analysis of the role of Postn in proliferation using the human keratinocyte cell line HaCaT transfected either with or without a mouse Postn-HA overexpression vector demonstrated that Postn induced cell proliferation, but not migration in scratch wound assays (Nishiyama, et al., 2011). Thus the presence of Postn in the extracellular matrix appears to be important for stimulating keratinocyte proliferation, possibly through the association of Postn with laminin γ2, fibronectin, and bone morphogenetic protein-1.

In contrast to Nishiyama and colleagues, in our analysis of the $Postn^{-/-}$ mouse, we initially observed a reduction in wound contraction based on wound shape (also noted in (Nishiyama, et al., 2011) in the $Postn^{-/-}$ mice versus $Postn^{+/+}$ mice). We therefore focused on cellular processes related to mesenchymal cell recruitment into the granulation tissue and transition of these cells to α-smooth muscle actin expressing myofibroblasts which facilitate ECM contraction. As fibroblasts migrate into the wound bed on the developing ECM in the granulation tissue, they undergo a phenotypic change triggered by TGF-β1, becoming α-smooth muscle actin-expressing myofibroblasts (Hinz and Gabbiani, 2003). In the *Postn*−/− mouse at day 7 post-wounding, a significant reduction in myofibroblasts in the granulation tissue was observed, which we subsequently showed could not be attributed to a lack of mesenchymal cell infiltration of the wound bed, nor any changes in canonical TGF-β signaling. TGF-β1 signaling is classically linked to Smad2 and Smad3, which are activated in response to ligand binding to the TGF-β type I and II receptors (Attisano and Wrana,

2002), but in recent years it has been shown that FAK/TAK/JNK pathways are required for myofibroblast induction by TGF-β 1 (Zhang, 2009). Using *Postn*$^{-/-}$ and *Postn*$^{+/+}$ dermal fibroblasts *in vitro*, we noted that when cultured on tissue culture plastic, cells from both WT and KO animals assumed a myofibroblast phenotype. Only when cells were cultured in collagen gels did the non-contractile phenotype of the *Postn*$^{-/-}$ fibroblasts manifest. Addition of recombinant Postn to the collagen gels was sufficient to recover myofibroblast differentiation and gel contraction. Addition of antibodies to β1 integrins or PP2 kinase inhibitor to inhibit Src/FAK signaling both inhibited Postn mediated contraction, demonstrating that Postn induces myofibroblast differentiation through non-canonical TGF-β signaling.

β1 integrin expression in fibroblasts is required for skin healing (Liu, et al., 2010), and mice containing a fibroblast-specific deletion of integrin β1 show impaired healing and a reduction in both myofibroblasts and granulation tissue formation. Furthermore, these mice are also resistant to bleomycin induced skin fibrosis (Liu, et al., 2009). Interestingly, in atrioventricular mesenchyme, Postn localizes with β1 integrins in regions of matrix compaction (Butcher, et al., 2007), suggesting further that the interaction of mesenchymal cells with Postn through β1 integrins is a pivotal step in contraction. Postn is known to be a ligand for αVβ3 and αVβ5 (Gillan, et al., 2002), but β1 integrins are more associated with fibrillar or supermature adhesion sites (Tomasek, et al., 2002), suggesting that the role of Postn in matrix contraction is localized through tensin-containing fibrillar adhesions, not focal adhesions.

The role of ECM stiffness in myofibroblast differentiation is well defined (for comprehensive review see Tomasek et al (Tomasek, et al., 2002). To further investigate the role of ECM compliance on the relationship between Postn and myofibroblast differentiation, we utilized a collagen-coated flexible polyacrylamide gels of varying stiffness (Young's moduli of 4800, 19200 and 50,000 Pa). As the stiffness increased *Postn*$^{-/-}$ fibroblasts increasingly differentiated into myofibroblasts and at 50,000 Pa, levels were similar between *Postn*$^{-/-}$ and *Postn*$^{+/+}$ dermal fibroblasts. Coating of Postn with collagen on 4800 Pa surfaces was sufficient to induce FAK phosphorylation and induce activation of α-SMA in *Postn*$^{-/-}$ dermal fibroblasts. Therefore, Postn facilitates myofibroblast differentiation and matrix contraction through integrin engagement and FAK phosphorylation in ECM matrices that are more compliant, which would include granulation tissue (Grinnell, 2003). In rigid environments such as cell culture plastic, the stiffness of the material is sufficient to compensate for the absence of Postn to induce myofibroblast differentiation. *In vivo* evidence from *Postn*$^{-/-}$ *mice* support this hypothesis (Figure 2), myofibroblasts were present at the wound border, which has been modeled to be an area of peak matrix stiffness (Murray, 2003), but not in the granulation tissue itself (Grinnell, 2003). This data suggests that within a single wound, it is possible that multiple mechanisms may give rise to the myofibroblast populations evident.

From our study and that of Nishiyama et al, (Nishiyama, et al., 2011) two mechanisms for Postn in skin healing were identified, wound contraction and re-epithelialization. Interestingly, we did not quantify any change in total epithelial migration distance between *Postn*$^{-/-}$ mice versus *Postn*$^{+/+}$ mice at day 7 post-wounding (Elliott, et al., 2012b), which

appears to contradict the work of Nishiyama (Nishiyama, et al., 2011). However, they observed a significant reduction in % re-epithelialization at days 3 and 5 post-wounding in *Postn*$^{-/-}$ mice. Therefore, the possibility exists that the initial reduction in re-epithelialization rates is recovered by day 7. Alternatively, as healing progresses, it is possible that other molecules compensate for *Postn* deletion. For example, Postn shares high homology with another member of the fasciclin I family, βig-h3, and using *in situ* hybridization, we have shown that *βig-h3* is highly expressed in the migrating keratinocyte layer during excisional healing at comparative levels in *Postn*$^{-/-}$ and *Postn*$^{+/+}$ mice at day 7 (Figure 3). Whether βig-h3 is involved in re-epithelialization in skin has, however, yet to be determined, although *βig-h3* null mice are viable (Ahlfeld, et al., 2016). It must also be considered that assessment of re-epithelialization in murine models is problematic; their skin is loose, ie, is not attached to the underlying fascia and muscle beds, and skin healing in mice predominantly occurs as a result of wound contraction, not re-epithelialization. Future studies using splinted wounds to inhibit contractions may be beneficial in clearly quantifying whether re-epithelialization is in fact inhibited.

Based on our *in vitro* analysis, we next investigated whether addition of exogenous Postn was sufficient to rescue the wounding phenotype in the *Postn*$^{-/-}$ mice. Using electrospinning techniques, collagen and Postn fibrous scaffolds were produced and placed into the animals at the time of wounding. Analysis of α-SMA at day 7 post-wounding demonstrated that the addition of the Postn containing scaffolds was both necessary and sufficient to recover wound contraction (Elliott, et al., 2012b). These results which have been replicated by Ontsuka et al, who demonstrated that by “painting” recombinant Postn protein onto the wounded area of *Postn*$^{-/-}$ mice every 2 days, the wound closure phenotype could be rescued (Ontsuka, et al., 2012).

Further evidence for the importance of Postn in skin comes from analysis of its expression patterns in scarring (Crawford, et al., 2015, Li, et al., 2013, Song and Qin, 2008, Zhang, et al., 2014, Zhou, et al., 2010) and in direct contrast, non-healing skin lesions (Elliott, et al., 2015). Postn is overexpressed in both hypertrophic and keloid scarring (Crawford, et al., 2015, Zhou, et al., 2010). Interestingly, we have recently shown that Postn expression is almost completely suppressed in the wound bed of human non-healing skin lesions, which correlates with a complete absence of myofibroblasts in the tissue (Elliott, et al., 2015). Based on the findings that exogenous Postn can rescue the wound healing process in *Postn*$^{-/-}$ mice, it opens the intriguing possibility of using wound dressings containing exogenous Postn as method for speeding and/or inducing wound closure in situations of impaired healing.

3. Cardiac Tissue

Early studies described Postn expression during heart development, specifically in fibrous regions, such as the chordae tendinae, and in areas that experience high sheer stress such as the developing endocardial cushions and later in the mature valves (Kern, et al., 2005, Kruzynska-Frejtag, et al., 2001, Norris, et al., 2007, Norris, et al., 2008b). Following development, Postn expression remains mostly absent in healthy heart tissue, however upon injury is re-expressed by cardiac fibroblasts (Oka, et al., 2007, Snider, et al., 2008, Zhao, et

al., 2014). The effect of this upregulation has been investigated using both gain-of-, and loss-of-function models.

Heart function has been investigated in three independently developed *Postn* knockout mouse lines. Knockout results in ~14% perinatal lethality caused by impaired valvulogenesis within the heart (Rios, et al., 2005, Snider, et al., 2008). However, surviving mice are grossly normal although display stunted growth (Oka, et al., 2007, Rios, et al., 2005, Shimazaki, et al., 2008). In contrast, following cardiac insult these knockout mice display more divergent phenotypes compared to the wild type counterparts. After experimental induction of myocardial infarction, $Postn^{-/-}$ mice exhibited a significantly decreased survival rate attributed to these mice having roughly twice the incidence of cardiac rupture within the first 8 days following the ischemic insult (Oka, et al., 2007, Shimazaki, et al., 2008). Biomechanical investigation demonstrated that infarcted knockout hearts required a significantly lower applied pressure in order to rupture compared to the wild-type counterparts, however this was not seen in non-infarct hearts (Shimazaki, et al., 2008). These results were further attributed to deficits in cardiac fibroblast infiltration into the infarcted area and impaired ECM deposition. Importantly, in mice that did survive beyond this initial 8-day period, $Postn^{-/-}$ mice displayed reduced fibrosis and improved heart function in comparison with wild types (Oka, et al., 2007). Similarly, following insult by pressure overload, $Postn^{-/-}$ mice did not progress to hypertrophy and maintained function, in contrast to wild type mice (Oka, et al., 2007). In a comparable study investigating Dahl salt-sensitive rats fed a high salt diet as a model for hypertension, anti-sense oligonucleotides for *Postn* mRNA led to improved survival and heart function (Katsuragi, et al., 2004). These studies emphasize that while increasing Postn expression may be an adaptive response following acute injury to quickly regain ECM and tissue homeostasis, prolonged expression as seen following acute injury, or in chronic stress conditions leads to an excessive fibrotic response and ultimately impairs function. While there is a strong link between Postn and the fibrotic response in several tissues, its effect on hypertrophy appears to be context dependent. Whereas above, pressure overload induced hypertrophy was reduced in $Postn^{-/-}$ mice (Oka, et al., 2007), in a genetic model of hypertrophic cardiomyopathy established by mutation of the α-myosin heavy chain (MHC) gene, $Postn^{-/-}$ mice did not exhibit reduced hypertrophy, but lesser proliferation of non-cardiomyocyte cells and a reduction in the extent of fibrosis was quantified (Teekakirikul, et al., 2010). Conversely, liposomal delivery of *Postn* cDNA impaired heart function (Katsuragi, et al., 2004), in uninjured hearts.

4. Periodontium

Periodontium refers to the specialized connective tissues that anchor teeth in the jaw, facilitating transmission of the stresses of mastication. Structurally, it consists of the gingiva, periodontal ligament (PDL), cementum, and alveolar bone. Postn is expressed during tooth development (Ma, et al., 2011, Suzuki, et al., 2004) and expression is maintained throughout adulthood in the PDL. In the adult periodontium, Postn is highly expressed in the PDL and in periosteal and endosteal progenitor cells associated with the alveolar bone demonstrating that Postn plays a specific role in the periodontium (Rios, et al., 2008, Wen, et al., 2010). A more recent study demonstrated that Postn expression in the PDL temporarily decreases following the removal of the occlusal force in mice and human (Afanador, et al., 2005).

Analysis of the *Postn*$^{-/-}$ mouse phenotype revealed significant periodontal disease-like characteristics, including resorption of the alveolar bone and a rapid degeneration of the PDL (Rios, et al., 2005, Rios, et al., 2008). In adult humans, Postn is highly expressed in the PDL and periosteum of alveolar bone (Wen, et al., 2010). Postn is known to organize the extracellular matrix at the tooth interface of the alveolar bone and PDL throughout adulthood (Kruzynska-Frejtag, et al., 2004), likely through its regulation of collagen fibrillogenesis (Choi, et al., 2011, Norris, et al., 2007) and possibly modulation of progenitor-cell phenotype (Coutu, et al., 2008, Kawanami, et al., 2009, Tkatchenko, et al., 2009). Despite extensive knowledge on Postn expression in the periodontium, comparatively little is known about its role in healing of periodontal structures.

There are two areas that Postn has been examined in 1) periodontal surgery (Padial-Molina, et al., 2015) and 2) experimental tooth movement (Rangiani, et al., 2015, Wilde, et al., 2003). Using an observational prospective case-control study Padial-Molina and colleagues assessed Postn expression in patients with and without periodontitis. Histologically, they identified that Postn protein levels were reduced in the sub-epithelial tissues in patients with periodontitis, which has been shown previously in a rat model to result from inflammatory processes (Padial-Molina, et al., 2012). Following surgery in humans however, Postn levels increased in gingival crevicular fluid (GCF) in both healthy and disease patients, although was higher in the latter group. With increasing time post-surgery, levels of Postn decline in the GCF in both patient groups, possibly due to its incorporation into the maturing ECM. This study clearly shows however, that Postn protein levels show temporal changes during the healing phase post surgery in human subjects.

Postn has also been investigated in experimental tooth movement, an orthodontic procedure that can be considered to induce damage, healing and remodeling as a result of the application of force to teeth, particularly in the periodontal ligament and bone where Postn is prominently expressed (Hamilton, 2008, Rios, et al., 2005, Rios, et al., 2008, Wen, et al., 2010, Yamada, et al., 2014). Three phases of orthodontic tooth movement have been proposed. The first phase (1 to 2 days) is a tooth movement immediately after the force application. In the second phase (20 to 30 days), tooth movement ceases because of the hyalinization in the compressed zone of the PDL. The hyalinized zone of the PDL is necrotic, cell-free, and lacks normal tissue architecture. The last phase involves the removal of the necrotic tissue and at this stage, the tooth movement resumes. During experimental tooth movement, Postn expression has been shown to be upregulated in the compression sites compared to tension sites of the PDL following experimental tooth movement in mice (Wilde, et al., 2003). In a subsequent study by Rangiani and colleagues, they assessed experimental tooth movement, comparing the process directly in *Postn*$^{-/-}$ and *Postn*$^{+/+}$ mice (Rangiani, et al., 2015). Interestingly, the process of tooth movement was significantly slower in *Postn*$^{-/-}$ mice compared to wild types, and this was concomitant with no change in sclerostin expression, but reduced numbers of osteoclasts and the integrity of collagen fibers was also significantly compromised. However, no mechanistic studies were performed, so whether the role of Postn is direct or indirect in these processes is yet to be determined.

5. Tendon and Ligaments

Upon tendon injury, the inflammatory phase of tissue repair is activated during which inflammatory cells rid the tissue of necrotic material. Tenocytes then migrate to the wound where they secrete type III collagen during the first few weeks of repair, followed by type I collagen as the repair becomes fibrotic. This fibrous tissue eventually remodels giving a tendon with a scar-like composition which (Sharma and Maffulli, 2006), although repaired does not match the biochemical and biomechanical properties of the original undamaged tissue.

The first observation of Postn in the tendon came from Norris et al who reported Postn co-localized with collagen type I in the tendons of adult mice (Norris, et al., 2007). While investigating the role of Postn in collagen I fibrillogenesis, they found that collagen I obtained from the tendons of *Postn*$^{-/-}$ mice had both reduced fibril diameters and denaturation temperatures, which was indicative of impaired fibril organization and crosslinking (Norris, et al., 2007). The finding that Postn regulates collagen I fibrillogenesis has important implications for the tendon, as the healthy tendon extracellular matrix consists largely of type I collagen (Sharma and Maffulli, 2006). Postn localization in the tendon has also been reported during mouse mandible development, where it was shown to have distribution patterns similar to TIMP-2 (Yoshiba, et al., 2007), in studies involving murine achilles tendons (Chamberlain, et al., 2013, Noack, et al., 2014) where it is expressed by both tenocytes and chrondrocytes (Noack, et al., 2014) and in the patellar tendon (Little, et al., 2014).

Although its role in tendon repair has not been fully elucidated, a recent surgical model of tendon repair demonstrated that Postn expression was upregulated at all time points following tendon injury, corresponding with an increase in type III collagen expression (Chamberlain, et al., 2011). Postn has also been found to be upregulated in human tendinopathy (Jelinsky, et al., 2011). Another study showed Postn upregulation in BMP2/Smad8ca-expressing C3H10½ cells, a tenogenic model cell line that forms tendon-like structures both *in vitro* and *in vivo*, suggesting that its secretion contributes to tendon formation (Noack, et al., 2014). To confirm Postn's role in tenogenesis, the group compared Postn expression between the achilles tendon and muscle of adult mice, finding not only higher expression in the achilles tendon relative to muscle, but also higher expression of Postn in the tendon relative to tendon-specific genes such as scleraxis and tenomodulin. Finally, when Postn was overexpressed in human MSCs *in vivo*, it led to the formation of wavy fibroblastic tendon-like tissue (Noack, et al., 2014). Taken together these studies suggest that Postn plays a role in both tendon formation and repair, by interacting with and organizing the extracellular matrix of the tendon (Chamberlain, et al., 2013, Noack, et al., 2014).

Fewer investigations have been performed with respect to potential roles for Postn role in ligament repair. Postn expression has been documented in rat medial collateral ligament (MCL) (Chamberlain, et al., 2013), in the human anterior cruciate ligament (ACL) (Little, et al., 2014) and in human transverse carpel ligaments (Shih, et al., 2009). Microarray analysis to identify genes that contribute to the early stages of tendon repair found that *Postn* mRNA

was significantly upregulated at 7 days following bilateral transection of rat MCLs (Chamberlain, et al., 2013). Using immunohistochemistry, the group also detected an increase in Postn protein at day 3 post injury, with a further increase at day 7. Therefore, as is evident in skin, Postn increases in the first week of healing, where it influences fibroblast phenotype and regulates collagen synthesis (Chamberlain, et al., 2013).

6. Skeletal Muscle

During skeletal muscle regeneration, Postn has been found to be highly upregulated following cardiotoxin induced injury in both the gastrocnemius (Goetsch, et al., 2003) and tibialis anterior (Ozdemir, et al., 2014) muscles in mice. Although absent from healthy adult muscle, following injury, Postn is expressed within fibroblasts and macrophages that infiltrate the damaged tissue as well as within the regenerating myofibers (Goetsch, et al., 2003, Ozdemir, et al., 2014). This expression profile has prompted others to investigate its expression in highly relevant muscular dystrophy models. Postn displayed upregulation in dystrophic gastrocnemius muscle from *mdx* mice (Marotta, et al., 2009), as well as in the diaphragm of *mdx-4cv* mice (Holland, et al., 2015). These data were further supported using the *Sgcd*−/− mouse model for muscular dystrophy and this expression profile correlated well with biopsies from patients with Duchenne muscular dystrophy (Lorts, et al., 2012). Interestingly, when crossed with *Postn*$^{-/-}$ mice to produce *Sgcd*−/− *postn*−/− double knockouts, the pathology was less severe in both the gastrocnemius and quadriceps muscles investigated. Importantly, these mice displayed enhanced ECM turnover, decreased fibrosis and enhanced myofiber regeneration. Moreover, the double knockout mice exhibited improved muscle function.

6. When Healing Fails to Stop: Periostin in Fibrosis

Postn has been associated with development and the severity of fibrotic conditions in numerous tissues (Bentley, et al., 2014, Huang, et al., 2015, Izuhara, et al., 2014, Kim, et al., 2013, Lorts, et al., 2012, Mael-Ainin, et al., 2014, Naik, et al., 2012, Norris, et al., 2008a, Oka, et al., 2007, Zhou, et al., 2010) and a search for Postn and fibrosis in pubmed returns 136 papers (out of 836 total on Postn). In many instances, fibrosis is idiopathic (Nanthakumar, et al., 2015), but the same cellular processes underlying fibrotic conditions are also required in the healing response in tissues such as skin (Elliott and Hamilton, 2011). In normal tissue following the proliferative/fibrotic phase of healing, myofibroblast populations undergo apoptosis, leaving a relatively acellular collagen-dense scar tissue, and it is the persistence of these myofibroblast populations that results in excessive ECM production/remodeling and increased tissue contraction (Hinz and Gabbiani, 2003, Tomasek, et al., 2002).

In healthy skin, Postn is normally expressed in the basal lamina (Zhou, et al., 2010), but it is overexpressed in both keloid and hypertrophic scarring post-injury (Crawford, et al., 2015, Song and Qin, 2008, Zhou, et al., 2010), where it increases fibroblast proliferation and contractility (Crawford, et al., 2015). In addition, Postn has been shown to promote angiogenesis in keloid scars (Zhang, et al., 2015), which is interesting as keloid scars are often hypoxic in nature (Ueda, et al., 2004). Consistent with these findings, Postn is

upregulated in keloid fibroblasts in response to hypoxia, where the presence of the protein mediates migration, proliferation and collagen synthesis of fibroblasts (Zhang, et al., 2014). Based on the studies performed to date, continued expression of Postn is associated with scar formation, and its inhibition may represent a legitimate target to reduce fibrosis after skin injury (Figure 4).

Similar to skin, in healthy human cardiac tissue, *Postn* mRNA is relatively low and the protein is not detectable by Western blotting (Zhao, et al., 2014), but in myocardial fibrosis (Zhao, et al., 2014) or post infarction (Minicucci, et al., 2013), Postn increases significantly, where it correlates with remodeling events. Interestingly, genetic deletion of Postn results in an increased risk of ventricular rupture, but in mice that survive, less fibrosis is evident in the remodeled myocardium in comparison with wild-type mice (Oka, et al., 2007). This is further demonstrated in a swine model, where controlled release of Postn post infarction improved heart function, but stimulated fibrosis (Ladage, et al., 2013). Whether Postn represents a target to inhibit fibrosis in the cardiac system is far from clear, as its complete inhibition may also prevent necessary remodeling to prevent wall thinning and rupture.

7. Concluding Remarks

Analysis of Postn in multiple wound healing models place this matricellular protein as a key modulator of cell phenotype and processes essential for proper wound resolution, but as discussed, it also is associated with fibrosis. The patterns of Postn expression are very similar between divergent tissues, suggesting a similar requirement temporally for the presence of the protein in healing. As the molecule itself becomes better defined, it will be of great interest to see how just how tissue specific certain isoforms are and whether post-translational modifications further enhance the activity of the molecule. However, the research performed thus far place Postn as a critical regulator of cell processes underlying the proliferative and remodeling phases of healing.

Acknowledgments

This work was funded in part by: Canadian Institutes of Health Research (Operating grant RN247506) and the Canadian Foundation for Innovation Leaders Opportunity Fund (no. 18742) to D. W. Hamilton, and National Institutes of Health R01 HL115619 to S. J. Conway.

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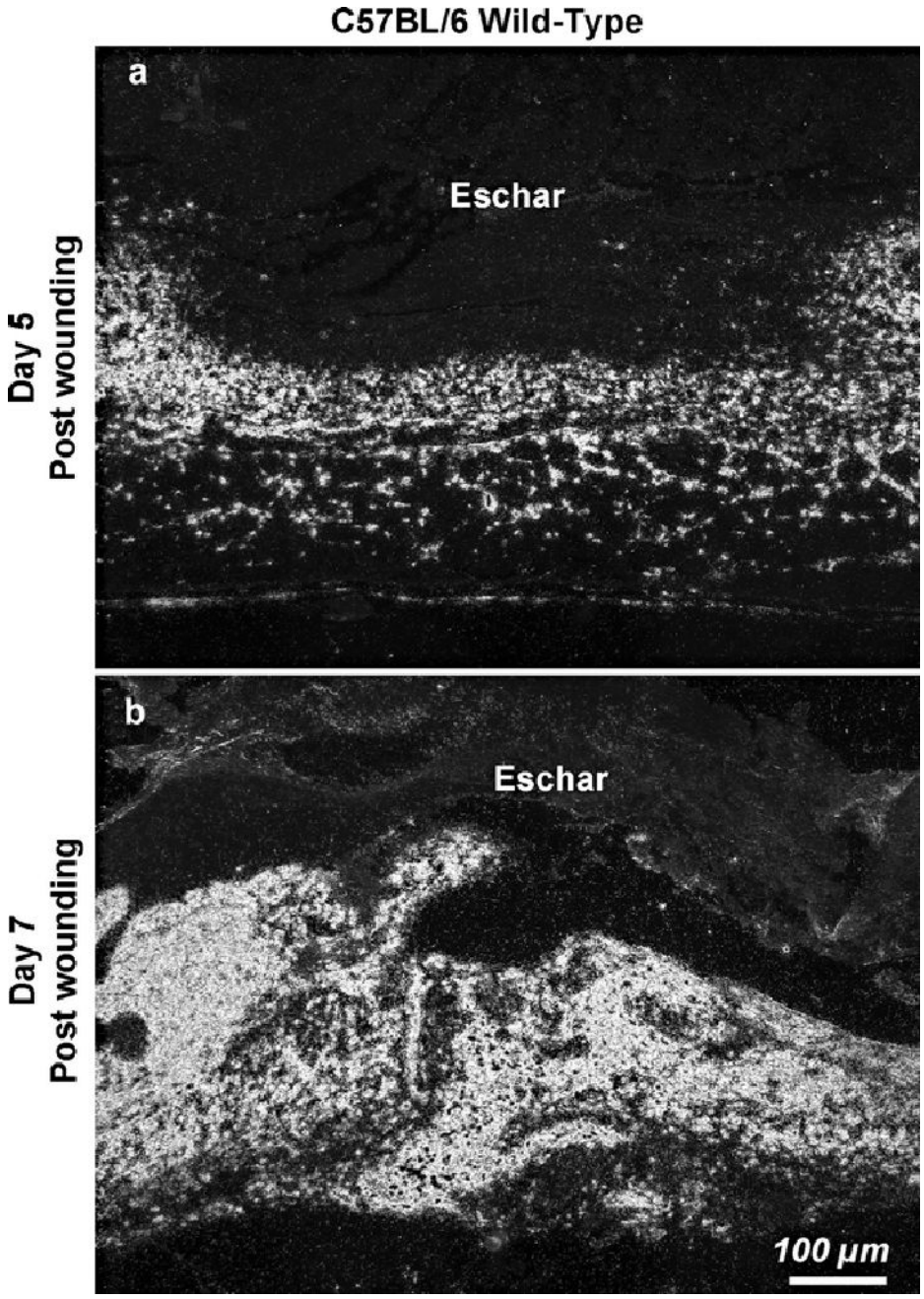

Figure 1.

Postn message detection in (a) day 5 and (b) day 7 *Postn*$^{+/+}$ wounds by in situ hybridization, showing Postn expression localizing to the granulation tissue in the wound. The presence of *Postn* message increases significantly from day 5 to day 7. Arrowheads indicate the wound borders.

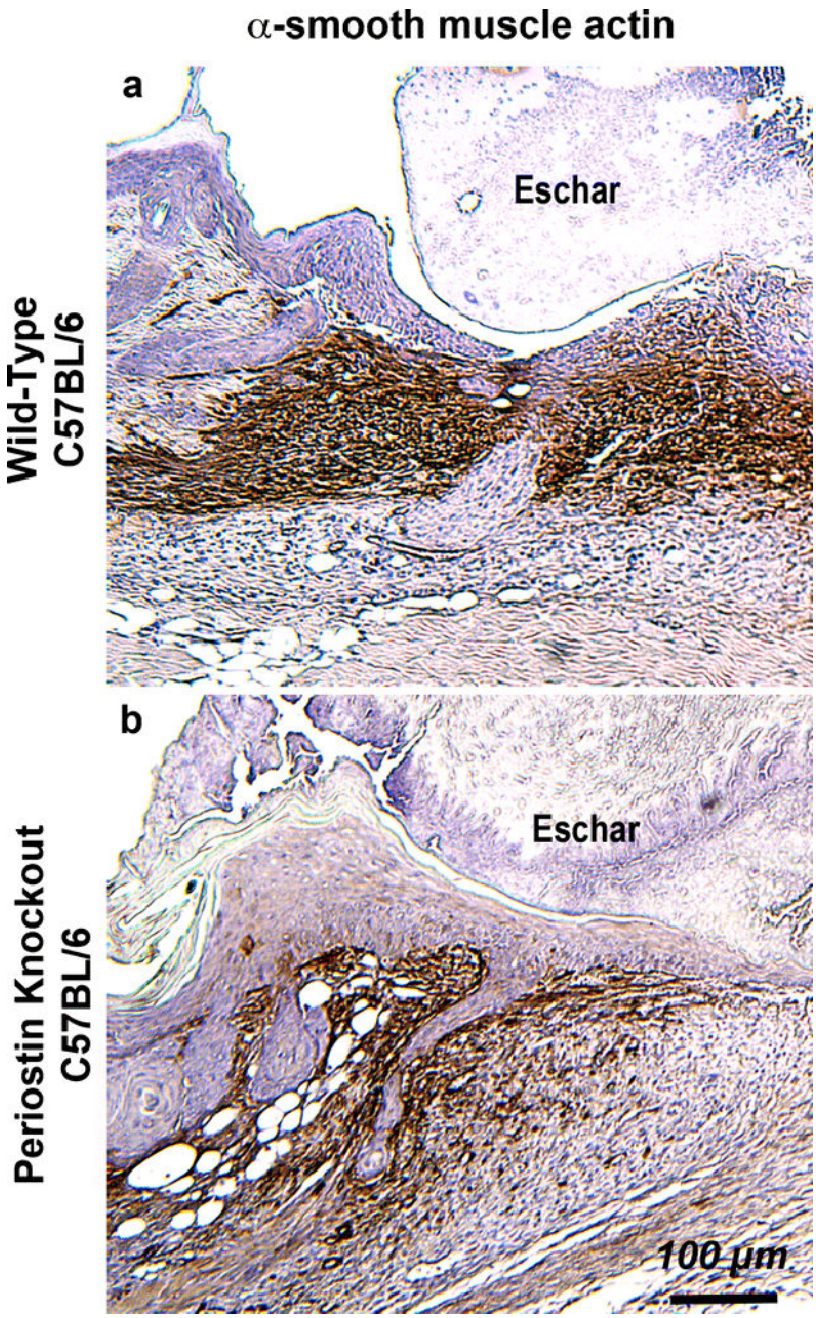

Figure 2.

α-smooth muscle actin localization using immunohistochemistry in day 7 (a) *Postn*$^{+/+}$ and (b) *Postn*$^{-/-}$ wounds. In *Postn*$^{+/+}$, α-SMA is present at the wound border and throughout the granulation tissue, but in *Postn*$^{-/-}$, it is only present at the wound border, which corresponds to the highest area of stress in the wound. Red arrowheads indicate the wound borders.

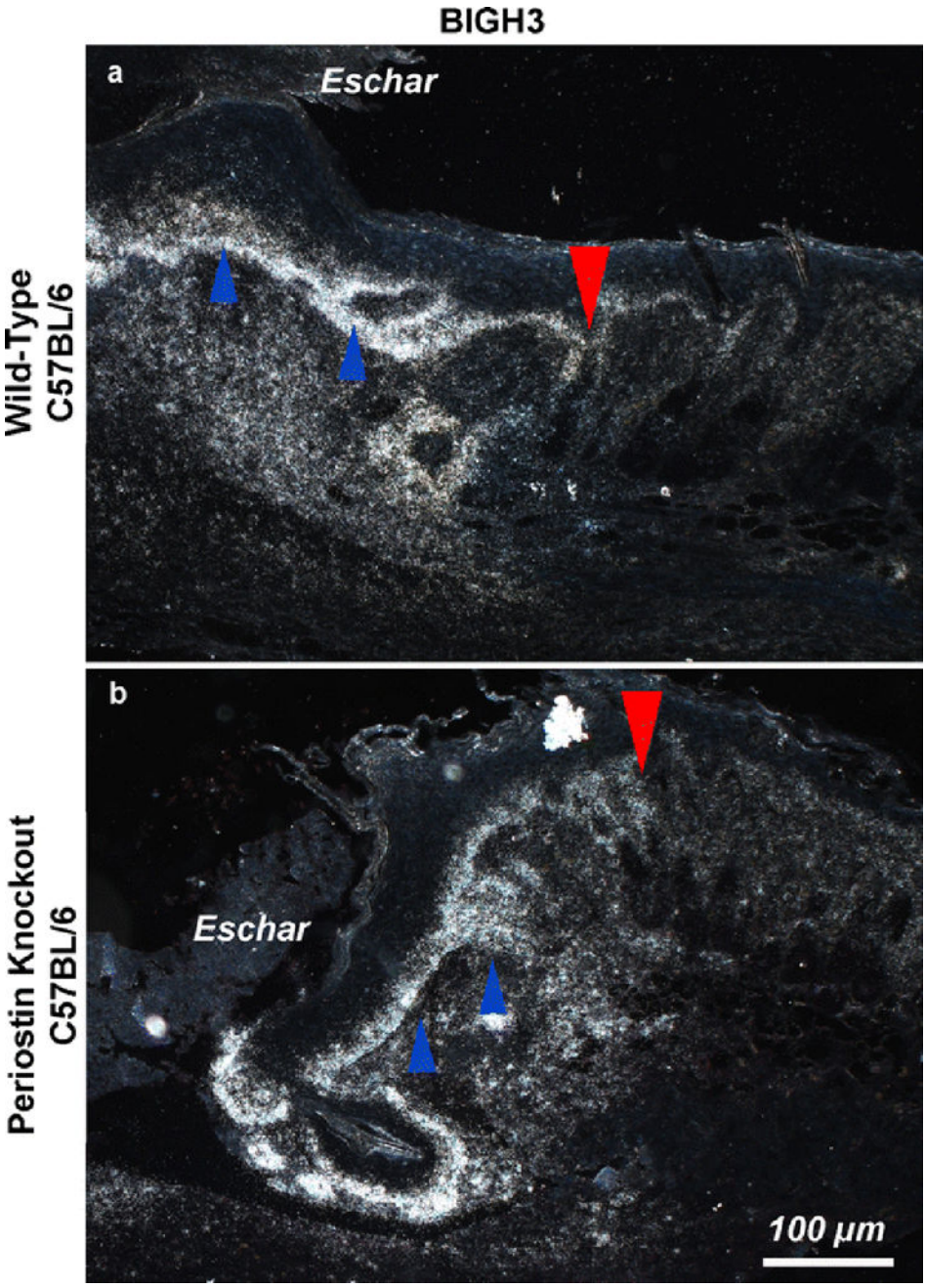

Figure 3.

βig-h3 message detection in day 7 (a) *Postn*$^{+/+}$ and (b) *Postn*$^{-/-}$ wounds by in situ hybridization, showing βig-h3 expression associated with the migrating epithelial tongue. Red arrowheads indicate the wound borders. Blue arrowheads indicate migrating epithelial tongue. White arrows indicate direction of the wound centre.

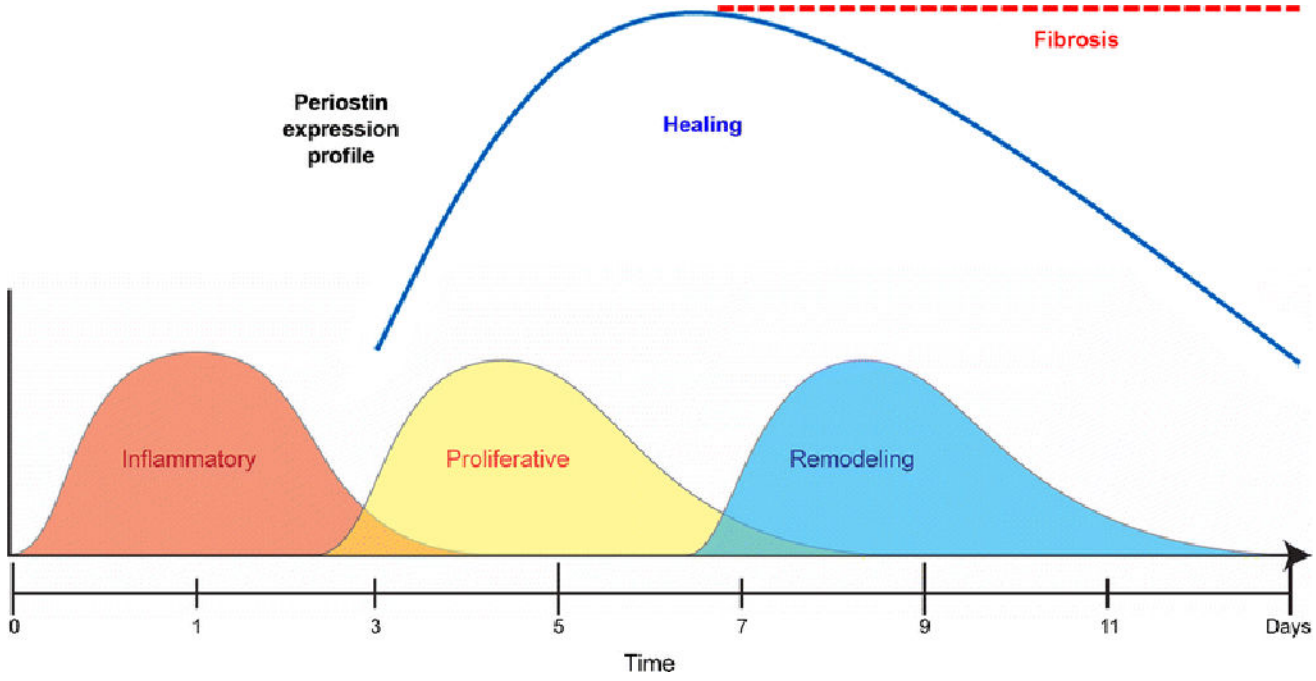

Figure 4.

Schematic representation of periostin expression profile in wound healing and fibrosis. Periostin is expressed during the proliferative phase of healing, peaking at day 7, with expression continuing, but declining during the remodeling phase. However, in fibrosis, periostin expression is maintained.