CRITICAL REVIEWS

Fibrinogen-Related Proteins in Tissue Repair: How a Unique Domain with a Common Structure Controls Diverse Aspects of Wound Healing

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Significance: Fibrinogen-related proteins (FRePs) comprise an intriguing collection of extracellular molecules, each containing a conserved fibrinogen-like globe (FBG). This group includes the eponymous fibrinogen as well as the tenascin, angiopoietin, and ficolin families. Many of these proteins are upregulated during tissue repair and exhibit diverse roles during wound healing.
Recent Advances: An increasing body of evidence highlights the specific expression of a number of FRePs following tissue injury and infection. Upon induction, each FReP uses its FBG domain to mediate quite distinct effects that contribute to different stages of tissue repair, such as driving coagulation, pathogen detection, inflammation, angiogenesis, and tissue remodeling.
Critical Issues: Despite a high degree of homology among FRePs, each contains unique sequences that enable their diversification of function. Comparative analysis of the structure and function of FRePs and precise mapping of regions that interact with a variety of ligands has started to reveal the underlying molecular mechanisms by which these proteins play very different roles using their common domain.
Future Directions: Fibrinogen has long been used in the clinic as a synthetic matrix serving as a scaffold or a delivery system to aid tissue repair. Novel therapeutic strategies are now emerging that harness the use of other FRePs to improve wound healing outcomes. As we learn more about the underlying mechanisms by which each FReP contributes to the repair response, specific blockade, or indeed potentiation, of their function offers real potential to enable regulation of distinct processes during pathological wound healing.

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SCOPE AND SIGNIFICANCE

MANY EXTRACELLULAR MATRIX (ECM) proteins are expressed during wound healing and are essential for the correct progression of tissue repair. In this study, we discuss the contribution of one particular subset of these molecules to a successful healing response. Fibrinogen-related proteins (FRePs) contain a common and ancient domain that has evolved to play key roles in tissue repair. We highlight the different and overlapping roles of these proteins, focus on how these domains interact with and signal to cells, and summarize the potential of targeting these molecules in pathological wound healing.

TRANSLATIONAL RELEVANCE

FRePs provide a temporary scaffold to support cell adhesion and infiltration, upon which to lay new tissue during rebuilding, but they also actively modulate cell behavior, including adhesion, migration, differentiation, proliferation, and survival. Expression of these molecules

during tissue injury is extraordinarily orchestrated and their induction, downregulation, and clearance from the tissue once repair is completed are very tightly regulated. This creates a dynamic microenvironment at the wound site where fluctuating levels of extracellular proteins direct the cell phenotype to mediate tissue repair.

CLINICAL RELEVANCE

Defects in wound healing, such as excessive scarring or fibrosis and delayed wound repair, pose significant clinical challenges. The pivotal role of FRePs in controlling wound healing and their restricted expression in healthy tissues implicates them as eminently tractable targets for the improvement of repair-related pathological conditions. Some are already well established in clinical use, while the potential of others is only just becoming apparent.

DISCUSSION

Definition of FRePs

Fibrinogen is a large glycoprotein comprising pairs of three nonidentical polypeptide chains $(\alpha \beta \gamma)_2$, which radiate out from a central nodule containing the N-terminus of each peptide. The C-terminal regions of the β and γ chains, each forms a 30 kDa globular domain that is absent in the α chain and exhibits a unique fold and structure containing three distinct subdomains, A, B, and P (Fig. 1).[1] Domains with high homology to this globular region are also found in other proteins; this structure has become defined as a fibrinogen-like globe (FBG) and FRePs as proteins that contain an FBG domain.[2–4]

Although the FBG domain was first described in mammalian fibrinogen, it is an ancient domain. There are 2,515 FRePs in eukaryotes, all in multicellular organisms. Some molecules exist that are homologous to the A subdomain in choanoflagellates[5]; however, the sea sponge (Phylum Porifera) contains the first complete FBG domain with all three subdomains present. In invertebrates, FRePs are essential for protection against infection with additional functions in development and allorecognition (reviewed in Hanington and Zhang[4]). In mammals, there are 541 FRePs with 21 different human FRePs, including fibrinogen, tenascins, ficolins, angiopoietins, angiopoietin-related proteins, fibroleukin, and fibrinogen C domain-containing protein-1 (FIBCD-1; Table 1). Typically, FRePs are multidomain proteins where the FBG domain is at the C-terminus of the molecule and this is true for all human FRePs (Fig. 2). There is ~30–40% sequence homology among the FBG domains of these proteins, indicating a high level of conservation (Fig. 3), and crystal structures of the FBG domains of human ficolin-H, -L, and -M, angiopoietin-2, FIBCD-1, and crab thachylectin-5A exhibit the same subdomain organization and structural folds.[6–10]

Human FRePs and wound healing

A large body of literature reveals that FRePs play key roles during tissue repair. Each domain within these large multimodular molecules can interact with a wide variety of binding partners to mediate diverse effects on cell behavior. For example, the epidermal growth factor-like (EGF-L) repeats of tenascin-C promote mesenchymal stem cell (MSC) survival in wound healing by activating
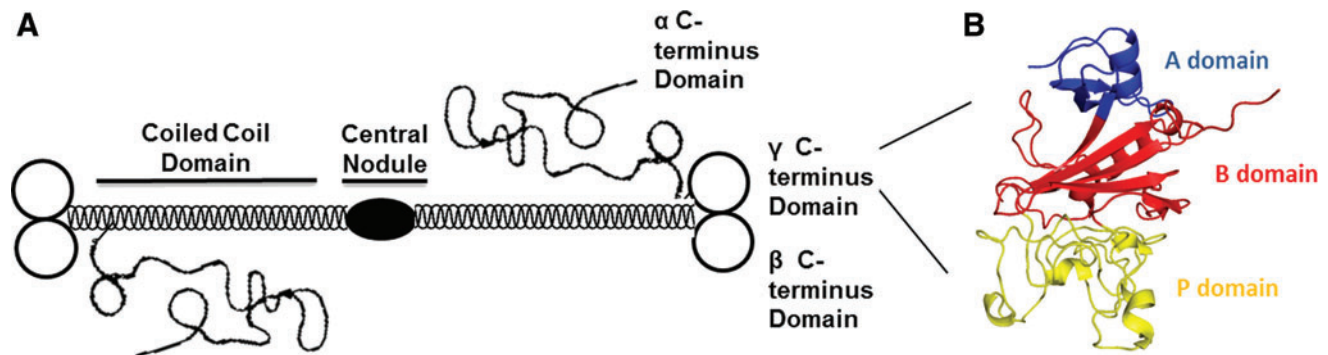
Figure 1. Fibrinogen and its C-terminal globular domain. **(A)** Schematic structure of fibrinogen showing the central nodule, coiled-coil domain, and α, β, and γ C-terminus domains. **(B)** Three-dimensional structure of the γ C-terminus globule showing its constituent three subdomains: A, B, and P. The A subdomain is at the N-terminus of the globule and consists of ~50 amino acids. The B subdomain is in the *middle* of the structure and comprises ~100 residues. Finally, at the C-terminus is the P subdomain, which contains ~100 amino acids. This image was modified from the Yee *et al.*[1] protein data bank number 1FID and colored using the PyMOL Molecular Graphics System, Version 1.5.0.4 Schrödinger, LLC. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

Table 1. *The human fibrinogen-related proteins*

FBG domain	Accession number	Amino acids position	Molecular weight (kDa)	Number of amino acids	pI
Angiopoietin 1	Q15389	277–497	27.1	237	8.40
Angiopoietin 2	O15123	275–495	28.2	248	5.78
Angiopoietin 4	Q9Y264	282–502	30.1	221	8.89
Angiopoietin-related protein 1	O95841	271–491	25.7	221	6.18
Angiopoietin-related protein 2	Q9UKU9	269–493	25.9	223	7.83
Angiopoietin-related protein 3	Q9Y5C1	237–460	26.1	224	6.89
Angiopoietin-related protein 4	Q9BY76	179–406	25.6	228	8.71
Angiopoietin-related protein 5	Q86XS5	141–390	28.2	248	7.17
Angiopoietin-related protein 6	Q8NI99	251–270	25.2	220	6.64
Angiopoietin-related protein 7	O43827	122–343	26.2	225	7.18
Fibrinogen β	P02675	232–491	29.8	260	7.06
Fibrinogen γ	P02679	170–453	32.1	284	5.35
Fibroleukin	Q14314	204–437	27.2	234	8.58
FIBCD-1	Q8N539	235–461	25.7	227	5.44
Ficolin-H	O75636	84–299	24.8	219	6.31
Ficolin-L	Q15485	96–313	24.0	221	6.07
Ficolin-M	O00602	109–326	25.6	231	5.87
Tenascin-C	P24821	1,974–2,202	26.1	229	8.78
Tenascin-R	Q92752	1,128–1,359	27.0	232	8.63
Tenascin-W	Q9UQP3	1,060–1,300	27.6	240	9.18
Tenascin-X	P22105	4,013–4,243	26.1	231	5.54

FBG, fibrinogen-like globe; FIBCD, fibrinogen C domain-containing protein-1.

the EGF receptor,[11] protecting them from Fas ligand-induced nuclear DNA damage.[12] Likewise, serine 89, located outside the FBG domain of fibroleukin, has prothrombinase activity that drives blood coagulation.[13] In this study, however, we focus solely on the functions of the FBG domains of FRePs. Known ligands for the FBG domains of human FRePs are listed in Table 2, and the roles of these interactions during the different stages of wound healing are discussed below.

Blood clotting. Upon injury, blood pervades the wounded space and thrombin cleaves soluble fibrinogen and platelet-associated protease-activated receptors, leading to thrombus formation and deposition of a fibrin matrix creating the hemostatic blood clot that also serves as a scaffold for tissue repair.

Fibrinogen is essential to coagulation, and the FBG domains of the β and γ chains play vital roles in these initial stages of wound healing. Fibrinogen produced by hepatocytes circulates in plasma until tissue injury, whereupon thrombin catalyzes it into fibrin. In this reaction, N-fibrinopeptides from the α chain of fibrinogen are released exposing an N-terminus motif (GPR) that binds to the FBG domain of the γ chain of an adjacent molecule. Using short peptide mimics of the α chain N-terminus sequences, a complementary "a" binding pocket was mapped in the P domain of the γ chain (Table 2).[14,15] Blocking this pocket with the GPRP peptide or point mutations of residues in this

region of the FBG domain results in impaired fibrinogen polymerization.[16,17] The β chain N-terminus is also cleaved, but at a slower rate, and interaction of this region with the FBG domain at the C-terminus of the β chain has been speculated to contribute to lateral fibril association; for a detailed explanation of fibrinogen polymerization see review.[18] Fibrin molecules are further cross-linked by factor XXIIa, a transglutaminase, which creates covalent bonds between 405Lys of one γ chain and 398/399Gln of another.[19] Calcium is pivotal in accelerating fibrin formation, protecting the molecule against plasmin degradation, and stabilization against heat denaturation, and the calcium-binding site in fibrinogen is located in the FBG domain of the γ chain proximal to the "a" pocket.[14] Fibrinogen also plays a key role in aggregating activated platelets; residues H271-V279 in the P subdomain of the γ chain FBG domain bind to platelet $\alpha_{IIb}\beta_3$ integrins[20] and N236-T254 in this domain bind to $\alpha_5\beta_1$ integrin to mediate platelet adhesion and clot retraction.[21]

Fibrinogen is subject to alternative splicing. One common variant of FBG domain of the γ chain (γ') is found in 8–15% of plasma. This variant lacks four amino acids at the end of the γA chain, which are replaced by an additional 20 amino acids.[22] This modification creates new binding sites for thrombin and factor XIII (Table 2)[23,24]; however, this variant cannot bind to platelets due to loss of $\alpha_{IIb}\beta_3$ integrin-binding sites.[25] γ' exhibits slower polymerization, thinner fibers, smaller pores, and a stiffer matrix that is more resistant to lysis
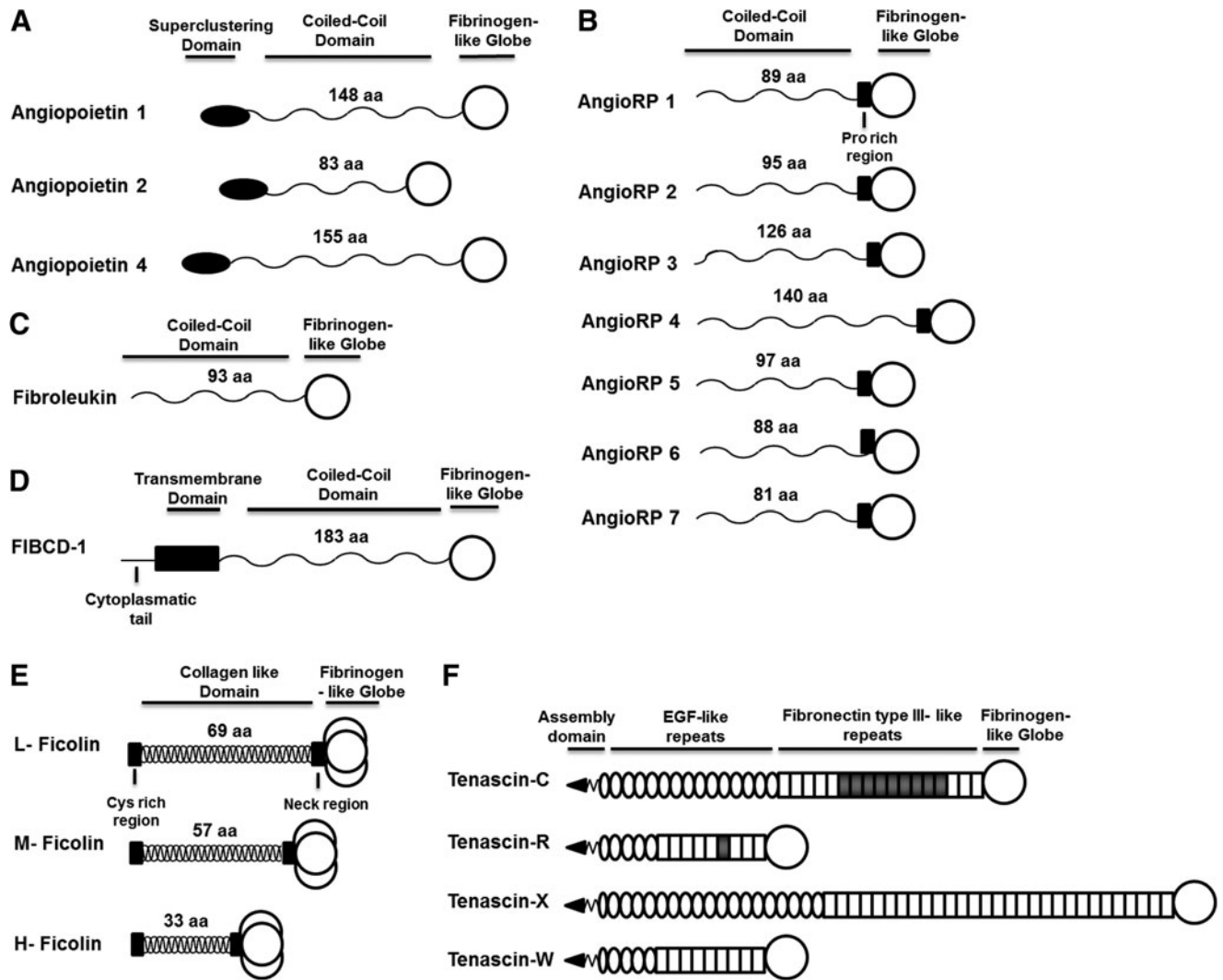
Figure 2. Domain organization of human FRePs. **(A)** Angiopoietins 1, 2, and 4 are found in humans; angiopoietin 3 exists only in mice. Angiopoietins contain an N-terminal superclustering domain, followed by a coiled-coil domain, in which the number of amino acids varies between family members, and the C-terminus is capped by the FBG domain. **(B)** Angiopoietin-related proteins are structurally similar to angiopoietins. Seven have been reported in humans. They lack a superclustering domain, instead containing a proline-rich region before the FBG domain. **(C)** Fibroleukin contains a coiled-coil domain and a C-terminal FBG domain. **(D)** FIBCD-1 is the only FReP that is not secreted; it contains a transmembrane domain, followed by coiled-coil domain and FBG domain. **(E)** Three ficolins are found in humans: L-, M-, and H-ficolin. They contain an N-terminal collagen-like domain, which includes a cysteine-rich region and a neck region that connects with the FBG domain. These proteins have a unique mode of oligomerization; three coil-coiled chains interlace forming a collagen-like helix that brings three FBG domains together to create a trimer at the C-terminus, and **(F)** Tenascins are a family of four extracellular matrix glycoproteins, tenascin-C, -R, -W, and -X. Tenascins have an N-terminal region that contains a coiled-coil structure and disulfide bonds necessary for oligomer formation. Next are the EGF-like repeats, followed by FNIII, whose number varies among the tenascins, and then the FBG domain. EGF, epidermal growth factor; FBG, fibrinogen-like globe; FIBCD-1, fibrinogen C domain-containing protein-1; FNIII, fibronectin type III-like domain; FRePs, fibrinogen-related proteins.

compared with nonspliced γ chains.[26,27] Plasma levels of γ' are a risk factor for the development of cardiovascular diseases.[28] During the healing of full-thickness wounds in rats, those covered with γ fibrin showed higher dermal vasodilator responses and increased breaking strength, while those treated with γ' fibrin exhibited modest increases in macrophage infiltration. *In vitro*, the higher fibrinolysis of γ fibrin aided endothelial cell sprouting and vascularization,[29] although further analysis is

required to understand the effects of these variants on angiogenesis *in vivo*.

Inflammation. Infection after tissue injury is sensed by pattern recognition receptors (PRRs) of the immune system, which detect pathogen-associated molecular patterns and rapidly activate inflammation. PRRs also mount an inflammatory response upon recognition of endogenous signals of tissue damage (damage-associated molecular
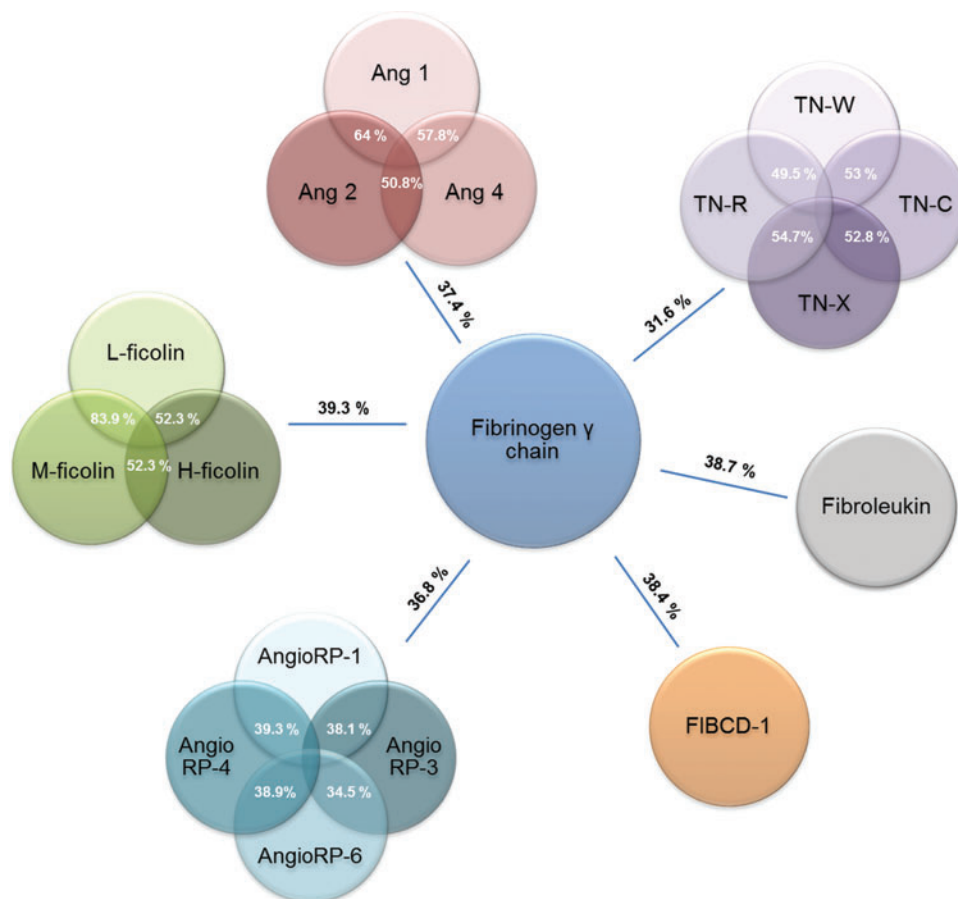
Figure 3. Degree of identity among human FRePs. In *white* is the % of identity among FReP family members, and in *black* is the average of identity of this family with the fibrinogen γ chain. The % of identity was calculated using ClustalW pairwise alignment. There is 40% identity between the C-terminus globular domains of fibrinogen γ and β chains. Ang, angiopoietins; AngioRP, angiopoietin-related proteins; TN, tenascin. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

patterns [DAMPS]). Invasion of the blood clot by immune cells, including neutrophils and macrophages, enables pathogen and tissue debris removal.

Ficolins are secreted soluble oligomeric proteins that act as PRRs and activate complement-mediated inflammatory pathways (reviewed in Ren *et al.*[30]). L-ficolin is made in the liver and secreted into serum; M-ficolin is produced by monocytes, neutrophils, and type II alveolar epithelial cells and is found in serum and lungs. The FBG domain of both these proteins binds to different types of sugar epitopes, such as *N*-acetylglucosamine (GlcNAc), galactose, 1,3-β-D-glucan, *N*-acetylgalactosamine, *N*-acetyl-D-mannosamine (ManNAc), *N*-acetyl-D-glucosamine, *N*-acetylcysteine, and lipoteichoic acid,[6,31,32] from gram-negative and gram-positive bacteria, such as *Salmonella typhimurium*, *Pseudomonas aeruginosa*,[33] and type III group B *Streptococci* and *Staphylococcus aureus*.[34–36] Sugar-binding sites are predominantly located in a series of residues conserved in both ficolins in the

P-subdomain (Table 2). L-ficolin can activate Jun kinase phosphorylation and induce interferon-γ, interleukin (IL)-17, IL-6, tumor necrosis factor alpha, and nitric oxide expression in mouse peritoneal macrophages upon acute *Mycobacterium tuberculosis* infection,[37] but little is known about how L-ficolin does this. High levels of L-ficolin are also found in the serum of patients with influenza A and hepatitis C, where it is associated with favorable antiviral responses and clearance.[38,39] Interestingly, in addition to binding pathogenic moieties, the FBG domain of L-ficolin can also bind to DNA derived from late apoptotic cells and necrotic cells in a calcium-dependent manner, aiding clearance of dead cells by increasing opsonization and macrophage uptake. This binding was not inhibited by addition of GlcNAc, indicating a distinct binding site for nucleic acids in the FBG domain.[40]

Polymorphisms in the FBG domain of M-ficolin dictate expression levels and binding ability.

Table 2. *Binding sites of the ligands for the fibrinogen-like globe domains of human fibrinogen-related proteins*

FBG domain	Ligand	Binding amino acids	Subdomain
Angiopoietin 1	Tie2	D218, A219, P222, K238, K243, Y246	P [103]
Angiopoietin 2	Tie 2	D218, A219, P222, N237-F239, Y245, Y246, S250	P [103]
	Integrin $\alpha_5\beta_1$	E352-Q366	P [66]
Angiopoietin-like 3	Integrin $\alpha_v\beta_3$	Not mapped	
Angiopoietin-like 4	Integrin β_1/β_5	Not mapped	
Fibrinogen γC	Integrin $\alpha_{IIb}\beta_3$	H271-V279	P [20]
	Integrin $\alpha_M\beta_2$	G48-V60/Y248-G266	A, P [104]
	Integrin $\alpha_v\beta_3$	G217-S229	P [80]
	Integrin $\alpha_5\beta_1$	N236-T254	P [21]
	"a" peptide GPRP	Q245, D246, K254-H256, Y279-G282, R291	P [14]
Fibrinogen γ'C	Thrombin	Y418, Y422, P413, A414	P [105]
	Factor XIII	V408-L427	P [24]
FIBCD-1	Chitin	Y250, H260, Y283, A284	P [49]
	GlcNAc, ManNAc, acetylated amino acids	Not mapped	
H-ficolin	D-fucose, galactose	C189, Y190, Y208, V218	P [6]
	LPS	Not mapped	
L-ficolin	Acetylated sugars	F180, C189, H190, G204, F206, Y218, N219	P [6]
	1,3-β-D-glucan	E226, D53, D55, S34, S86	A, B [6]
	DNA	Not mapped	
M-ficolin	Acetylated sugars	F180, C189, H190, Y206, A207, Y218	P [7]
Tenascin-C	Integrin $\alpha_v\beta_3$	W251-K289	P [80]
	TLR4	Not mapped	
Tenascin-X	$\alpha_{11}\beta_1$	Not mapped	
	Small latent complex of TGF-β	Not mapped	
	Tropoelastin	Not mapped	

Numbers in superscripts indicate cited references.
FIBCD-1, fibrinogen C domain-containing protein-1; GlcNAc, *N*-acetylglucosamine; LPS, lipopolysaccharide; ManNAc, *N*-acetyl-D-mannosamine; TGF-β, transforming growth factor beta; TLR4, toll-like receptor 4.

Ser268Pro reduces M-ficolin levels in heterozygote individuals and completely abolishes expression in homozygotes. Ser268 is an essential part of the calcium-binding site in the FBG domain of M-ficolin, suggesting that calcium plays an important role in its stability. Other mutations, *Ala218Thr* and *Asn289Ser*, also alter M-ficolin levels, and recombinant proteins with these mutations showed impaired binding to the sialic acid residues in the polysaccharide capsule of group B *Streptococcus*. These residues are proximal to bacterial-binding sites in the P domain and likely give stability to the protein structure and exposure of interaction sites.[41]

The third member of the ficolin family, H-ficolin is expressed in the liver and the lung and is also found in serum. Its FBG domain binds to galactose and D-fucose through the same residues that L-ficolin and M-ficolin use to bind acetylated sugars (Table 2).[6] It also binds to the gram-positive bacterium *Aerococcus viridans*.[42] H-ficolin is specifically elevated in the airways during influenza A virus infection, where it interacts with viral surface glycoproteins inhibiting infection *in vivo*.[43] More recently, H-ficolin has been shown to agglutinate *Hafnia alvei* and induce bacterial phagocytosis, possibly by the activation of the lectin pathway of complement. The FBG domain of H-ficolin recognizes lipopolysaccharide (LPS) O-specific polysaccharide, and this binding is calcium and magnesium dependent.[44] Interaction of H-ficolin with LPS of *H. alvei* inhibits toll-like receptor 4 (TLR4)/MD-2/cluster of differentiation 4 activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) transcription factor.[45] Like L-ficolin, the FBG domain of H-ficolin binds to apoptotic cells and necrotic cells and activates opsonization; however, H-ficolin does not bind to nucleic acids and it is unknown what the ligand is in this context.[46] An H-ficolin variant exists where a frameshift in exon 5 (Leu117fs) leads to complete deletion of the FBG domain of this protein. In heterozygous individuals this reduces serum ficolin levels. Levels are abolished in homozygous patients who suffer from severe recurrence of bacterial and lung infection, lung fibrosis, bronchiectasis, and obstructive lung disease as a result,[47] indicating the essential role of the FBG domain in this protein against infection.

Another FReP that can bind acetyl groups is FIBCD-1. This transmembrane PRR is expressed by epithelial cells in large and small intestinal walls and in salivary glands.[48] The FBG domain of FIBCD-1 forms a tetrameric structure through

noncovalent interactions of adjacent molecules within the cell membrane and this form binds specifically to chitin (an *N*-acetyl homopolymer) through residues in the P-subdomain (Table 2), as well as interacting with GlcNAc, ManNAc, and acetylated amino acids.[48,49] After ligand binding, FIBCD-1 can endocytose acetylated compounds[48] and potential phosphorylated sites in the cytoplasmic region of this protein infer its role as a signaling molecule; however, still little is known about the events downstream of endocytosis.

Fibroleukin (fibrinogen-like protein 2) is a membrane-bound protein expressed by macrophages and endothelial cells, but which is also found circulating in serum.[50] Increased soluble fibroleukin is observed in alveolar macrophages from patients with peripheral lungs of chronic obstructive pulmonary disease,[51] chronic hepatitis C,[52] and myocardial ischemia/reperfusion injury.[53] Inhibition of fibroleukin has shown beneficial effects in a model of hepatic reperfusion, decreasing liver injury by lowering the apoptotic levels of mice hepatocytes and sinusoidal endothelial cells.[54] In addition to the procoagulatory role of regions outside the FBG domain, the FBG domain of fibroleukin has shown some immune-suppressive effects. It can abolish LPS-induced maturation of bone marrow-derived dendritic cells, resulting in inhibition of T cell proliferation, and this phenomenon could be rescued by adding antibodies against the FBG domain of fibroleukin. Soluble fibroleukin favors the Th2 cytokine profile by increasing levels of anti-inflammatory cytokines, IL-4 and IL-10,[55] although it is not known how this change in cytokine profile is mediated.

Indeed, other FRePs can induce cytokine synthesis. The FBG domain of the ECM glycoprotein tenascin-C activates TLR4-mediated inflammation in primary human cells and murine models of inflammation *in vivo*, driving a persistent innate immune response. Tenascin-C is upregulated in acute inflammation and tissue injury, where it is thought to act as a DAMP in cases of sterile inflammation.[56] However, it is not yet known whether this is caused by a direct interaction of the FBG domain with TLR4. At sites of inflammation, increased vascular permeability allows extravasation of intact fibrinogen.[57] Fibrinogen can also activate TLR4 stimulating the synthesis of cytokines and chemokines that attract T-cells, neutrophils, and macrophages; however, it is not known if this function is mediated by the FBG domain of the γ or β chain.[58] Given the activation of TLR4 by the FBG domain of tenascin-C, it is tempting to speculate that either the FBG domains of fibrinogen or fibroleukin can also activate TLR4. Finally, both fibrinogen and fibrin promote leukocyte adhesion during the inflammatory response through binding of residues G48-V60/Y248-G266 of the γ chain FBG domain to $\alpha_M\beta_2$ integrins.[59]

Angiogenesis. Wound neovascularization is mediated by endothelial cells infiltrating into the provisional matrix and proliferating, forming new blood vessels. Angiopoietins are one of the major angiogenic players in wound healing. These secreted proteins expressed by endothelial cells control blood vessel development and stability, promoting or inhibiting angiogenesis, through activation of tyrosine kinase (Tie) receptors by the FBG domain.[60]

Angiopoietin-2 is expressed from day 3 of dermal wound healing[61] and is present during re-epithelialization, granulation tissue formation, and remodeling.[62,63] Binding of the angiopoietin-2 FBG domain to Tie2 receptor mediates the destabilization of existing vessels promoting vascular remodeling in the presence of vascular endothelial growth factor (VEGF).[64] In addition, it can promote endothelial cell survival.[65] A recent study also found that the FBG region of angiopoietin-2 binds directly to $\alpha_5\beta_1$ integrin (Table 2) and Gln362 in the P-subdomain is essential for this interaction. This binding site does not overlap with the binding motif of Tie-2 receptor. Interaction with $\alpha_5\beta_1$ integrins plays a role in the adhesion, migration, and invasion of human umbilical vein endothelial cells.[66] Angiopoietin-2 null mice are born normally, but die in the first 14 days due to vascular defects and impaired inflammatory responses,[67] while overexpression of angiopoietin-2 in endothelial cells or in the dermis results in embryonic death.[64] Diabetic wound healing is characterized by a decrease in angiogenesis and granulation tissue formation.[68,69] In genetically diabetic *db/db* mice, angiopoietin-2 and VEGF expression increased in dermal wounds associated with vessel destabilization and neovessel formation. However, VEGF induction was transient and, without sustained elevated levels of this factor, angiopoietin-2 promoted further vessel destabilization and regression, leading to a marked vascular death. In addition, in *db/db* mice wound healing, there is reduced expression and activation of Tie2 in the skin.[61]

Angiopoietin-1 is expressed later in dermal wound healing during granulation tissue formation and remodeling.[63] In contrast to angiopoietin-2, it enhances the stabilization and maturation of blood vessels through the activation of Tie -2 by its FBG domain[64,70,71] and promotes adhesion and

migration of endothelial cells.[72] Angiopoeitin-1 knockout mice are not viable, but overexpression of angiopoietin-1 in the skin results in an enlargement of vessels that contain increased numbers of endothelial cells and pericytes.[70] New therapeutic approaches have focused on restoring Tie2 activation in diabetic wound healing and enhancing levels of angiopoietin-1. Adeno-associated virus (recombinant adeno-associated virus [rAAV]) mediated transfection of the angiopoietin-1 gene into diabetic mice and improved healing of incisional wounds with complete reepithelialization, well-formed granulation tissue, increased matrix maturation, organization, and breaking strength. Angiopoietin-1 rAAV mice also showed a significant increase in the formation of new blood vessels, but no changes in VEGF mRNA and protein expression,[73] indicating that angiopoietin-1 can improve wound healing independently of VEGF expression. More recently, MSCs modified to continuously release angiopoietin-1 were implanted in excisional full-thickness wounds in rats and improved wound healing, increasing angiogenesis, and epidermal regeneration were observed.[74] Other approaches use Tie receptor agonists, for example, vasculotide, a peptide mimic of angiopoietin-1 can bind Tie2 promoting receptor phosphorylation and induction of mitogen-activated protein kinase, protein kinase B, and endothelial nitric oxide synthase. *In vitro*, vasculotide protects endothelial cells from serum withdrawal death and promotes cell migration and MMP2 expression. *In vivo*, delivery of vasculotide in a matrigel plug to excisional wounds of *db/db* mice enhanced angiogenesis and stabilization of blood, as well as increased granulation tissue and accelerated wound closure.[75] Vasculotide also improved murine acute radiation skin damage, reducing inflammation and increasing endothelial cell density and survival.[76] Thus, studies focused on restoring the balance in the Tie2 signaling axis show beneficial effects for the treatment of diabetic wound healing and beyond.

Angiopoietin-related proteins or angiopoietin-like proteins are a family of secreted glycoproteins that do not bind to Tie receptors. However, some members of this family participate in the regulation of angiogenesis.[77] For example, angiopoietin-like 3, which is found in the liver and serum, induces angiogenesis through its FBG domain, stimulating endothelial cell adhesion and migration through binding to $\alpha_v\beta_3$ integrin.[78] Other FRePs also drive angiogenic processes through ligation of endothelial cell $\alpha_v\beta_3$ integrin; G217-S229 within the FBG domain of the fibrin γ chain inter-

acts with $\alpha_v\beta_3$ to induce clot retraction during wound healing and increase angiogenesis and neovascularization by promoting cell adhesion, proliferation, and migration.[79,80] The FBG domain of tenascin-C, also modulates endothelial cell adhesiveness and migration, enhancing endothelial sprouting through binding of W251-K289 to $\alpha_v\beta_3$ integrin.[80–82]

Tissue remodeling. In the latter stages of wound healing, fibroblasts invade and deposit new ECM initiating granulation tissue formation. Keratinocytes migrate above the provisional matrix reepithelializing dermal wounds. Finally, fibroblasts differentiate into myofibroblasts and initiate wound contraction and further collagen deposition. The provisional matrix is degraded as the wound contracts and collagen fibers are rearranged and aligned as new scar tissue.

Angiopoietin-like 4 is expressed by keratinocytes in dermal wound healing during reepithelialization[83] after activation of peroxisome proliferator-activated receptors β/δ, which regulate epidermal differentiation.[84] This FReP promotes keratinocyte migration[83] and interacts with other ECM proteins such as fibronectin and vitronectin to delay their proteolytic degradation, although the role of the FBG domain in this process is not clear. The FBG domain does, however, interact with β_1/β_5 integrins[85] activating focal adhesion kinase (FAK)-Src, signaling pathways that enhance keratinocyte migration and accelerate wound healing; indeed, angiopoietin-like 4 knockout mice present delayed wound reepithelialization and impaired keratinocyte migration.[85]

Angiopoietin-like 4 is considered a potential therapeutic target in impaired wound healing in diabetic mice where levels of this FReP remain low. Topical application of the recombinant FBG domain of angiopoietin-like 4 to full-thickness excisional wounds in db/db mice improved wound closure and accelerated reepithelialization compared with saline-treated wounds. Further study of the mechanisms underlining enhanced wound healing by angiopoietin-like 4, found that FBG domain binding to β_1 integrin activates janus kinase 1/signal transducer and activator of transcription 3 signaling pathways, which in turn, upregulate the expression of inducible nitric oxide synthase in keratinocytes and enhance angiogenesis. Thus, cross talk between keratinocytes and epithelial cells may prove to be a unique therapeutic target in the improvement of angiogenesis in diabetic wounds.[86]

Tenascin-C is expressed by keratinocytes[87] and fibroblasts in dermal wound healing during the

phases of reepithelialization and granulation tissue formation.[88] However, tenascin-C knockout mice show very minor defects in wound healing exhibiting temporarily reduced fibronectin in the granulation tissue.[89] Impaired tissue repair was observed in these mice after corneal incision injury[90] and articular cartilage damage,[91] implying a possible role for tenascin-C in remodeling during repair. Tamaoki *et al.*[92] showed in a model of myocardial injury that the tenascin-C FBG domain enhanced the migration of myocardial fibroblasts and promoted differentiation into myofibroblasts, in addition to increasing wound contraction.

Another family member, tenascin-X, is predominantly expressed during the late phases of matrix maturation after wound remodeling. Tenascin-X deficiency results in the Ehlers–Danlos Syndrome, characterized by skin fragility and hyperextensible skin. No major differences have been found in the wound healing of these patients, although tenascin-X knockout mice exhibit lower wound strength than wild-type mice.[93] The FBG domain of tenascin-X can bind to tropoelastin, an interaction which is likely to mediate stability and maturation of elastic skin fibers.[94] Tenascin-X also influences epithelial-to-mesenchymal transition, through the interaction of its FBG domain with the small latent complex of transforming growth factor beta (TGF-β), producing a conformational change that exposes active TGF-β. This mechanism is dependent on the binding of the FBG domain to $\alpha_{11}\beta_1$ integrin in the cell surface.[95]
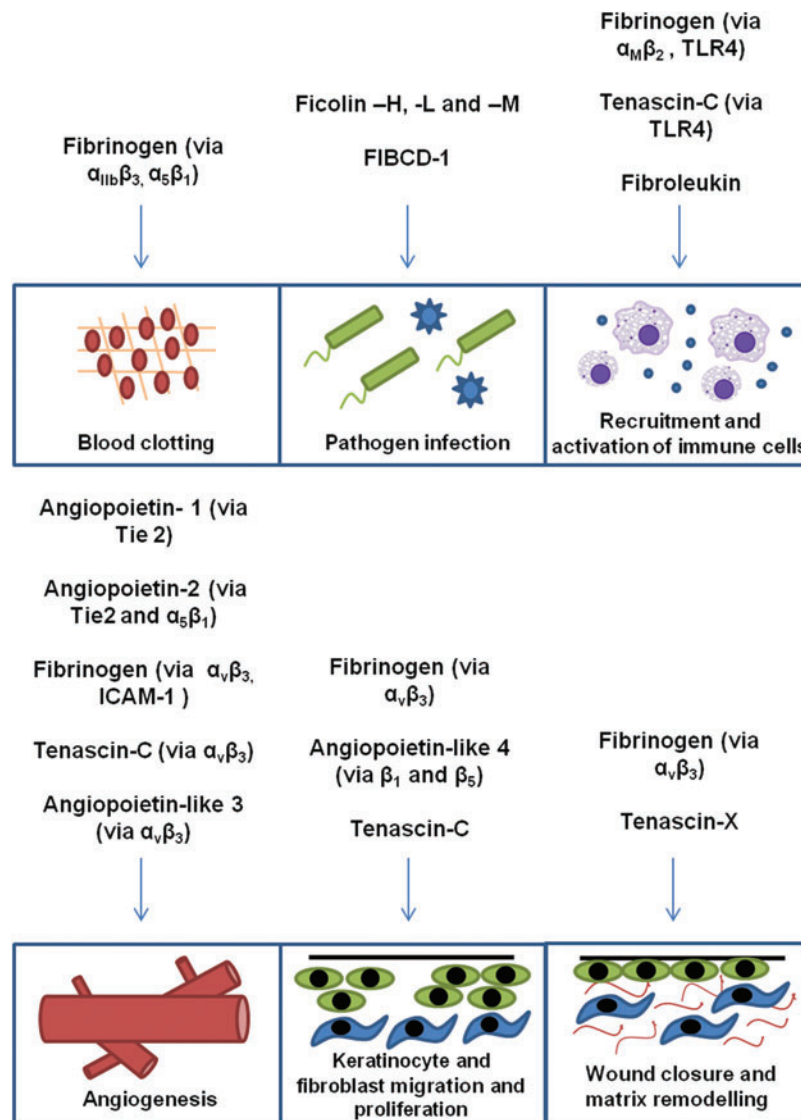
Figure 4. Functions of FREPs during wound healing. Schematic representation of the roles of FRePs in the stages of wound healing. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

SUMMARY

The FBG domains of human FRePs interact with multiple diverse binding partners. A large degree of homology exists among FBG domains of FRePs from the same family and also among members of different families. However, each also contains unique sequences. This is reflected in the overlapping functions of some FRePs and in the unique functions of others during different stages of wound healing (Fig. 4). For example, while the Tie2 receptor-binding site is only present in angiopoietins, the ficolin-P domain sugar-binding sites are conserved not only in all ficolins, but in each human FReP. This raises the question of ficolin redundancy and specificity; it may be that their distinct expression pattern confers tissue-specific functions; moreover, it appears that additional binding sites in the A and B domains confer ligand specificity, for example, L-ficolin binding to elongated carbohydrates, including 1,3-β-D-glucan.[6] These data also raise the intriguing possibility that all human FRePs may act as PRRs; however, it is not clear if other FRePs can bind pathogenic ligands. There are data to suggest that this may not be the case; the GPRP peptide mimic of the fibrin α chain that binds to the "a" pocket of fibrinogen during polymerization also prevents the binding of the FBG domain of M-ficolin to GlcNAc,[96] suggesting that nonconserved residues surrounding this region allow each FReP to use the same binding sites differently. Many more details remain to be uncovered before we fully understand FRePs; for example, mapping the binding sites for TLR4 and refining the precise amino acids that form integrin-binding sites. Together, these data will enable us to discern whether universal binding sites exist in all FRePs and how these are differentially ligated.

Precise mapping of FBG-binding sites will also enable the development of specific inhibitors that might be useful in controlling coagulation, inflammation, angiogenesis, and tissue rebuilding during wound repair. Fibrinogen is already used extensively in dermal wound repair, its abundance, ease of isolation, and natural properties as a coagulant and provisional matrix making it ideal to aid wound healing (reviewed in Ahmed *et al.*[97] and Janmey *et al.*[98]). Preclinical studies described above exemplify how modulation of angiopoietin activity may improve angiogenesis in diabetic and other nonhealing wounds, as well as the potential of angiopoietin-like 4 in controlling the keratinocyte phenotype. Antagonists of other FRePs may also prove useful, for example, blockade of ficolin

activity in infectious diseases and of TLR4 activating FRePs to reduce excessive inflammation. Clues may also be gained from studying scar-free tissue regeneration. For example, high levels of tenascin-C are associated with wounds that heal in a scar-free manner in axolotls,[99] in cells derived from ear wounds of MRL/MpJ mice, a strain that can regenerate scar-free ear wounds,[100] and in fetal wounds where tenascin-C expression increases rapidly before reepithelialization, enhancing cell migration and increasing wound epithelialization rates.[101] In contrast, high levels of tenascin-C are found in fibrotic chronic wounds and keloids[102] indicating that regulation of tenascin-C expression during wound healing may provide future avenues to improve tissue repair and reduce fibrosis and abnormal scarring.

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TAKE-HOME MESSAGES

- FRePs each contain a globular highly homologous FBG domain.
- Human FRePs include fibrinogen, tenascins, ficolins, angiopoietins, angiopoietin-related proteins, fibroleukin, and FIBCD-1.
- FRePs are specifically upregulated upon tissue injury and infection.
- The FBG domain of each FReP has diverse functions playing distinct roles during tissue repair.
- These diverse functions are mediated by nonconserved residues within the FBG domain, unique to each family.
- Targeting these proteins has the potential to aid pathological wound healing.

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Abbreviations and Acronyms

Ang = angiopoietin
AngioRP = angiopoietin-related protein
DAMPS = damage-associated molecular patterns
ECM = extracellular matrix
EGF = epidermal growth factor
FAK = focal adhesion kinase
FBG = fibrinogen-like globe
FIBCD-1 = fibrinogen C domain-containing protein-1
FNIII = fibronectin type III-like domain
FRePs = fibrinogen-related proteins
GlcNAc = *N*-acetylglucosamine
ICAM-1 = intracellular adhesion molecule 1
IL = interleukin
JAK1 = janus kinase 1
LPS = lipopolysaccharide
ManNAc = *N*-acetyl-D-mannosamine
MSCs = mesenchymal stem cells/multipotent stromal cells
PRR = pattern recognition receptor
rAAV = recombinant adeno-associated virus
TGF-β = transforming growth factor beta
Tie = tyrosine kinase receptors
TLR4 = toll-like receptor 4
TN = tenascin
VEGF = vascular endothelial growth factor