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Microfibril-associated glycoproteins MAGP-1 and MAGP-2 in Disease

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Abstract

Microfibril-associated glycoproteins 1 and 2 (MAGP-1, MAGP-2) are protein components of extracellular matrix microfibrils. These proteins interact with fibrillin, the core component of microfibrils, and impart unique biological properties that influence microfibril function in vertebrates. MAGPs bind active forms of TGFβ and BMPs and are capable of modulating Notch signaling. Mutations in MAGP-1 or MAGP-2 have been linked to thoracic aneurysms and metabolic disease in humans. MAGP-2 has also been shown to be an important biomarker in several human cancers. Mice lacking MAGP-1 or MAGP-2 have defects in multiple organ systems, which reflects the widespread distribution of microfibrils in vertebrate tissues. This review summarizes our current understanding of the function of the MAGPs and their relationship to human disease.

Keywords

microfibril-associated glycoprotein; MAGP; fibrillin; microfibril; bone; obesity; cancer; aneurysms

1. INTRODUCTION

The extracellular matrix (ECM) evolved to provide structural and informational support to cells. Within the ECM, numerous proteins, glycoproteins, and proteoglycans form a composite biomaterial that can differ significantly between tissues. While each ECM protein has its own unique set of functional properties, in most cases, it is the combinatorial signal produced by that component and its interacting partners that influence cell behavior. An example is the microfibril; ~12 nm fibers that provide strength to tissues, facilitate elastin assembly and regulate growth factor availability [1–5]. The major structural proteins of the

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microfibril are the fibrillins (FBN1, FBN2 & FBN3), which are large modular glycoproteins consisting of repeating calcium-binding epidermal growth factor-like domains interspersed between unique 8-cysteine sequences [4, 6, 7]. With the appearance of vertebrates, there emerged a unique set of microfibril-associated proteins that interact with fibrillin to facilitate elastin assembly (fibulin-4 & -5), growth factor signaling (emilins, LTBPs, and MAGPs), and microfibril assembly (ADAMTS) [8, 9]. Mutations in genes for fibrillin and these associated proteins all lead to known human diseases.

Microfibril-associated glycoprotein-1 and -2 (MAGP-1 & MAGP-2) form a unique two-member family of proteins that associate with fibrillin in vertebrate microfibrils (reviewed in [10]). MAGP-1 was first isolated from elastic tissues [11] and was initially suggested to be a bridging protein that facilitated elastin assembly on the microfibrillar bed. It is now clear from gene knockout studies that neither MAGP-1 nor MAGP-2 is required for elastin assembly; elastic fibers form normally when one or both MAGPs are deleted in mice [12, 13]. Abnormalities in MAGP-deficient animals appear in numerous organ systems, which reflects the wide-spread distribution of MAGPs and their importance to normal tissue function. A common mechanism underlying most of the traits associated with mutant MAGP phenotypes is altered TGFβ signaling, although the MAGPs can also influence cell signaling through the Notch pathway.

Both MAGPs covalently bind to fibrillin through their “matrix binding domains,” a cysteine-rich region near the C-terminus of each protein (Figure 1). Sequences near their amino terminus define areas for growth factor binding and interactions with other ECM proteins, including tropoelastin, collagen VI, decorin, and biglycan [14–16]. MAGP-2, but not MAGP-1, has an integrin recognition sequence (R-G-D) that enables its interaction with αvβ3 integrin [17]. MAGP-2 has a more restricted pattern of tissue expression than MAGP-1, being absent from the microfibrils of the ocular zonule, the peritubular matrix of fetal and mature kidney, and the medial layer of the aorta. High-level expression of MAGP-2 occurs in skeletal muscle, lung, mammary gland, thymus, uterus, and heart in human and mouse tissue whereas MAGP-1 is found in most tissues [18,19].

2. MAGP PROTEIN AND MFAP GENE FAMILY NOMENCLATURE

MAGP-1 was the first protein in this family to be characterized followed shortly thereafter by MAGP-2 [20]. Three other proteins, AMP [21], MFAP3 [22], and MAGP-36 [23], based on their small size and localization to microfibrils, were grouped together with the MAGPs as members of the MicroFibril-Associated Protein (MFAP) family. Even though these three proteins have no structural or sequence homology with MAGP-1 or MAGP-2 or with each other, they, together with MAGP-1 and MAGP-2, were assigned the gene family name *MFAP* and were numbered as their human gene was characterized (*MFAP1*=AMP [24], *MFAP2*=MAGP-1, *MFAP3*=MFAP3 [22], *MFAP4*=MAGP-36 [25], *MFAP5*=MAGP-2). The nomenclature is confusing with gene names often mixed up with protein names. For example, *MFAP2* is often mistaken for MAGP-2 in the literature and online databases. Therefore, caution must be exercised when interpreting gene names with these two proteins: *MFAP2* = MAGP-1 and *MFAP5* = MAGP-2.

3. MAGP-1 REGULATION OF GROWTH FACTOR SIGNALING

3.1 TGFβ and BMP

A function of microfibrils outside of providing structural support to tissues is to control the availability of growth factors—particularly members of the TGFβ superfamily [26]. Fibrillin is considered the primary functional protein in this regard, but the MAGPs also have a role to play in controlling growth factor signaling [13, 27]. All three TGFβs are secreted as an inactive latent dimer bound to a member of the latent TGFβ-binding protein (LTBP) family. This large latent complex (TGFβ-LLC) binds to fibrillin molecules in microfibrils, thereby creating a growth factor reserve in the ECM that can be activated and mobilized when needed (Figure 2). Many BMPs (BMP2, 4, 5, 7 & GDF5) also interact with fibrillins via a prodomain that confers latency to the growth factor [28, 29]. The latent form of TGFβ that binds to fibrillin requires activation and release through interactions with proteases or specific cell-surface integrins [30] (Figure 2, red arrow). The active TGFβ that is released is then free to interact with TGFβ receptors on the cell membrane (Figure 2, green arrow).

Binding studies with fibrillin fragments have mapped a high affinity-binding site for pro-BMPs and TGFβ-LLC to the N-terminal region of fibrillin-1 encoded by exons 4–7 [31–33]. Because MAGP-1 also binds to this region of fibrillin-1 [34] (as well as to a second site defined by exon 24 [35]), a possible mechanism whereby MAGP-1 influences growth factor activity is by blocking binding of latent growth factors to the microfibril [33] (Figure 2, top). Dysregulation of TGFβ activation and signaling would then occur because TGFβ-LLC would no longer be sequestered in the matrix away from the cell. It should be noted that whether loss of LLC binding to the matrix results in decreased as opposed to increased TGFβ signaling is not yet clear.

Another scenario has fibrillin and MAGP-1 working together in synergistic ways to influence growth factor availability. Because the MAGPs can directly bind active, but not latent forms of TGFβ and BMPs [13, 35], MAGP could serve to subdue or terminate TGFβ signaling by removing excess TGFβ from the immediate membrane microenvironment analogous to a decoy receptor (Figure 2, bottom). It is interesting in this regard that TGFβ binds to MAGP-1 and to its receptor with equal affinity. Because the form of TGFβ bound to MAGP is already activated and noncovalently bound, MAGP could harbor a store of active growth factor that could be easily mobilized if needed. In this scenario, it is apparent how the absence of MAGP-1 could account for increased TGFβ signaling.

3.2 MAGP and notch signaling

The matrix-binding domain of both MAGP-1 and MAGP-2 has a strong binding preference for tandem EGF-like motifs and has been shown using direct binding assays or yeast two-hybrid screens to interact with fibulins, Jagged1, Jagged2, Delta1, Notch1, and multiple EGF-like domain protein 6 (MEGF6), all of which contain EGF domain repeats [35–39]. MAGP-1 & MAGP-2 interaction with Notch1 [36] leads to release of the Notch1 extracellular domain and subsequent activation of Notch signaling. Also, by interacting with Jagged1, MAGP-2 induces its shedding from the cell surface and regulates the activity of the

shed Jagged1 fragment [39]. As will be shown below, MAGP's ability to influence Notch signaling is important in angiogenesis, cancer, and inflammation.

4. MAGP AND CLINICAL PHENOTYPES

The complexity of microfibrils makes discerning the primary function of its component proteins (particularly the MAGPs) extremely difficult. The application of gene targeting to inactivate microfibril-associated genes in model organisms, combined with human genetic studies that have linked specific diseases to mutations in genes for microfibrillar proteins, are beginning to unlock the complexity of microfibril function. MAGP-1 and MAGP-2, singly and together, have been inactivated in mice and zebrafish, and recent studies have linked several human diseases to MAGP mutations. Below is a summary of our current understanding of how abnormal MAGP expression influences development and disease.

4.1. Cardiovascular system

4.1.1 Thoracic aortic aneurysms and vascular integrity—*MFAP5*(MAGP-2) loss-of-function mutations in humans have recently been linked to thoracic aortic aneurysms and dissection (TAAD) [40]. Two mutations in different parts of the *MFAP5* gene segregated with the disease in mutation screens of a large number of TAAD-affected individuals. Functional analysis showed that the two *MFAP5* mutations resulted in MAGP-2 haploinsufficiency. Increased nuclear phosphorylated Smad2/3 was identified in the aorta from the affected individuals, suggesting TGFβ misregulation [40]. These findings are in agreement with MAGP-2's ability to bind and sequester TGFβ and with increased TGFβ signaling when MAGP-2 is deleted in mice [12]. Interestingly, no mutations in the gene for MAGP-1 *(MFAP2)* were found to track with the disease, indicating that MAGP-1 does not contribute to TAAD in this group.

Vascular disease associated with *MFAP5* heterozygosity in humans is different from what was found in mice. No vascular phenotype was identified in mice heterozygous for MAGP-1 ($Mfap2^{+/-}$) or MAGP-2, ($Mfap5^{+/-}$) or in mice lacking either protein ($Mfap2^{-/-}$ or $Mfap5^{-/-}$). However, aortic dilation was observed when MAGP-1 and MAGP-2 were both deleted ($Mfap2^{-/-}$; $Mfap5^{-/-}$). Vascular changes in double knockout mice occurred between the 4^{th} and 6^{th} month of age, suggesting that deterioration of vessel wall integrity is age-dependent in these animals [12].

In zebrafish, morpholino knockdown of MAGP-1 resulted in dilated vessels in the brain and the eyes, irregular lumens in axial vessels, and a dilated caudal vein with altered venous plexus formation. The fish were viable with these defects, and the phenotypes were incompletely penetrant [41]. Transiently expressed MAGP-1 protein rescued the vascular abnormalities, confirming that the vascular phenotypes are specific to the loss of the MAGP-1. Similar phenotypes (vessel dilation) were observed when MAGP-1 was overexpressed in zebrafish embryos indicating that a critical balance of MAGP-1 protein level is required for proper vascular morphogenesis [41]. Interestingly, fibrillin-1 and MAGP-1 morphant embryos exhibited overlapping vascular defects, supporting synergistic effects of these microfibrillar proteins.

4.1.2 MAGPs and angiogenesis—Knockdown of MAGP-1 in zebrafish resulted in the arrested development of the hyaloid vasculature characterized by defects in angiogenic remodeling and failure of vessels to coalesce into defined branches [42]. A characteristic of the phenotype was an aggregate of vascular endothelial cells at the posterior lens and a remarkably reduced number of branches that appear thicker poorly patterned and stagnated compared to hyaloid vasculature of wild-type (WT) larvae or control morphants [42, 43]. These branching defects in MAGP-1 morphant embryos suggests a role for MAGPs in vascular patterning. Whether MAGP works through direct cell-matrix interaction or by regulating growth factor signaling pathways is not known. However, a screen of differentially expressed genes associated with endothelial cell angiogenesis identified MAGP-2 as a pro-angiogenic factor [44]. Functional studies showed that MAGP-2 influenced endothelial cell sprouting by antagonizing Jagged1's ability to induce Notch1 cleavage and activation [38]. Notch signaling is known to antagonize angiogenic sprouting [45], which means that by interacting with Jagged1 and suppressing Notch signaling, MAGP-2 has the potential to serve as a novel activator of sprouting of endothelial cells and angiogenesis.

4.1.3 Overlapping function of MAGP-1 and MAGP-2 in maintaining vessel wall integrity—Disruption of vessel wall integrity through inactivation of the MAGP-2 gene in humans, the MAGP-1 gene in fish, and MAGP-1 and MAGP-2 together in mice suggests that MAGP-1 and MAGP-2 have shared, or overlapping, primary functions in maintaining vessel integrity. It is possible that the location or overall function of the two MAGPs within the vessel wall are different between fish, mice, and humans, or that observed differences in cardiovascular hemodynamics act as biological modifiers. In mouse and bovine aorta, MAGP-2 is highly expressed in the intima and adventitia whereas MAGP-1 is mostly expressed in the media associated with elastic fibers [12,18]. Hence, inactivation of both MAGP genes would have more pronounced effects on vessel wall integrity than the modification of each gene individually. While more studies are required to thoroughly understand the role that the MAGPs play in maintaining vessel patterning and maintenance of aortic wall integrity, zebrafish and mouse studies confirm that neither MAGP is required for the specification of vascular cell fate.

4.2. MAGP-2 in cancer and inflammation

Elevated MAGP-2 expression has been linked to poor outcome in neck squamous cell carcinomas [46] and in ovarian cancer [47–49], and has been proposed as an independent prognostic biomarker of survival and chemosensitivity [49]. MAGP-2 is expressed at high levels by cancer stromal fibroblasts and promotes tumor cell survival as well as endothelial cell motility and survival via the $\alpha v\beta 3$ integrin receptor. *In vitro* studies demonstrated a significant increase in ovarian cancer cell motility and invasion potential but not cell proliferation after treatment with recombinant MAGP-2. These properties were associated with intracellular calcium release that influenced cytoskeletal changes and mediated MAGP-2's motility and invasion-promoting effects [49]. There was also a correlation between increased MAGP-2 expression and microvessel density, suggesting that the proangiogenic role of MAGP-2 may lead to increased tumor growth *in vivo* [47].

MAGP-2 has also been shown to have anti-inflammatory activity. When administered to mice subjected to endotoxemic shock, MAGP-2 reversed the cytokine storm and provided a significant survival benefit comparable to treatment with anti-inflammatory anti-TNF-α [50]. MAGP-2 attenuated inflammation by stimulating endogenous secretion of IL-10. IL-10 is a pleiotropic cytokine that down-regulates Th1 cytokine expression and macrophage activation, and is the master suppressor cytokine secreted by regulatory T cells.

4.3. Lung disease

The MAGPs play little, if any role, in lung development since mouse lungs develop normally when one or both proteins are deleted. There is genetic evidence, however, that MAGP-2 is regulated by FGF signaling, and in turn, serves as a likely mediator of FGFR3 and FGFR4 control of alveologenesis [51]. In adult humans, a single nucleotide polymorphism in an intron of the MAGP-1 gene (*MFAP2*, rs2284746) was associated with lung function in a genome-wide association study (GWAS). Of the 16 loci showing an association with pulmonary function, the gene for MAGP-1 gave the most statistically significant signal for FEV1/FVC [52]. When the SNP for *MFAP2* and the other loci identified in this study were tested for interaction with smoking versus never smoking, none of the loci showed a significant interaction. Thus, the genetic effect associated with the MAGP-1 gene variant underlies lung function variability irrespective of smoke exposure. One of the other genes identified in the GWAS study as being associated with decreased lung function was TGFβ2 [52]. This places TGFβ signaling as a plausible mechanism for altered lung function and would be consistent with MAGP-1's ability to regulate TGFβ availability.

In mice, MAGP-1-deficient animals exposed to cigarette smoke for six months developed less emphysema than wild-type animals [53]. When the extent of inflammation was characterized, the number of monocytes/macrophages was lower in both the lung tissue and lavage of the smoke-exposed MAGP-1-knockout animals. Macrophages secrete potent proteolytic enzymes that lead to the development of emphysema through the destruction of the lung ECM [54]. Hence, the modest lung damage in the MAGP-1-deficient mice following cigarette smoke exposure is consistent with the lower macrophage burden in these animals.

4.4. Skeletal system

To date, no known skeletal diseases have been definitively linked to mutations in the human MAGP-1 gene. The MAGP-1 gene polymorphism rs2284746, discussed above, was associated with increased height in the GIANT consortium database [55], which is in agreement with skeletal studies in mice showing increased bone length in animals lacking MAGP-1 [56]. MAGP-1 deficiency in mice (*Mfap2*$^{-/-}$) results in numerous bone defects, supporting a connection between MAGP-1 and skeletal homeostasis. MAGP-1 mRNA levels are abundant in flushed bone samples and calvaria osteoblasts and osteoblasts derived from differentiated bone marrow stromal cells. Undifferentiated bone marrow stromal cells, in contrast, express low levels of MAGP-1 until stimulated to differentiate [57]. Loss of MAGP-2 (*Mfap5*$^{-/-}$) does not significantly alter bone mass or architecture, and the absence of MAGP-2 on a MAGP-1-deficient background (*Mfap5*$^{-/-}$; *Mfap2*$^{-/-}$) does not exacerbate changes in bone mass architecture associated with MAGP-1 loss-of-function [12].

A common trait in MAGP-1-deficient mice is the appearance of lesions on adult animal hind and forelimbs, which were determined to be abnormal healing fractures [13]. These bone lesions, along with increased overall body length, persisted in the MAGP-1-deficient animals through several back-crosses into inbred and outbred mouse strains [56, 57]. The prevalence of bone fractures suggested that bone strength and overall bone quality were compromised in the MAGP-1-deficient background. DEXA scans confirmed that MAGP-1-deficient animals have age-dependent osteopenia associated with a reduction in whole-body bone mineral content as early as eight weeks of age and decreased bone mineral density by 12 weeks. μCT identified narrowing of the marrow cavity despite normal cortical bone thickness, and examination of cancellous and cortical bone identified the cancellous as most affected by MAGP-1 deficiency [56–58].

Cancellous bone is characterized by osteoblast (bone forming) and osteoclasts (bone resorbing) cells lining the bone surface. Osteoblasts produce the highest level of MAGP-1 protein in bone, yet osteoblasts from MAGP-1-deficient animals show normal differentiation and function *in vitro* and *in vivo*. Thus, defective bone composition and strength in these animals are not due to an osteoblast defect. Instead, these mice have ~60% more osteoclasts compared to WT mice, suggesting that increased bone resorption is responsible for the osteopenia and bone weakening that is seen in MAGP-1 deficiency [57]. Osteoblasts and osteoclasts are coupled through the production of RANKL by osteoblasts and RANK receptor on osteoclast precursors (bone marrow macrophages). Signaling through the RANKL-RANK pathway is a primary mechanism for inducing osteoclastogenesis. MAGP-1-deficient mice have elevated serum RANKL protein levels due to increased expression by mutant osteoblasts. This increased RANKL environment helps drive osteoclastogenesis and accounts for accelerated bone resorption in these animals [27]. Experiments with cultured cells showed that RANKL expression was normalized when MAGP-1-deficient osteoblasts were treated with a neutralizing antibody targeting free active TGFβ, thereby establishing a link between RANKL expression, compromised bone integrity, and altered TGFβ signaling in MAGP-1 deficiency [27].

A potential contributor to the bone fragility that develops in the absence of MAGP-1 is bone marrow fat accumulation, which correlates with increased body adiposity and insulin resistance in MAGP-1-deficient mice (see below) [58]. In these animals, fatty marrow correlated with reduced white blood cell counts but there was no change in hematopoietic progenitor cell frequency. There was, however, an accumulation of macrophages consistent with an ‘inflamed’ state [58]. Pathologic fat accumulation in the bone marrow is often considered detrimental to skeletal health and can lead to reduced mechanical properties [59]. It is, therefore, possible that the skeletal fragility observed in older MAGP-1 -deficient mice is linked to excessive marrow fat content.

4.5. Metabolic disease

Both MAGP-1 and MAGP-2 have been linked directly and indirectly to obesity and metabolic disease in humans. Obesity and diabetes traits are associated with a locus on chromosome 1p36 that includes the gene for MAGP-1 [60–63]. Consistent with the genetic data that suggests a role in adipose tissue dysfunction, MAGP-1 expression was shown in

humans to be elevated in adipose tissues from obese individuals [64]. Like MAGP-1, MAGP-2 is highly expressed in human adipose tissue and is correlated with markers of insulin resistance. Obesity-related inflammation either directly or indirectly increases MAGP-2 (and most likely MAGP-1) expression in adipose tissue [65, 66], but how MAGP-2 influences adipose function is still unclear. Elevated TGFβ levels correlate with obesity in humans and mice, and suggests a mechanistic link between MAGPs, TGFβ, and metabolism.[67, 68].

Mouse models have been particularly useful in elucidating mechanisms whereby the MAGPs influences adipose tissue function. Increased body weight and fat pad mass (epididymal) are common phenotypes in MAGP-1-knockout mice [13, 56, 69]. MAGP-1 mRNA expression is detectable in RNA preps from whole white adipose tissue, and the protein has been visualized by immunohistochemistry [64]. Fractionation of fat pads into the stromal versus adipocyte compartments reveals that MAGP-1 mRNA is largely derived from the stromal fraction and not adipocytes (unpublished results). In the absence of MAGP-1, adipocytes are hypertrophic, but adipocyte number (adipogenesis) is unaffected [64].

Characterization of the metabolic phenotypes in MAGP-1-deficient animals determined that the excessive adiposity leads to the development of a pre-diabetic state (insulin resistance and glucose intolerance) which was exacerbated by high-fat diet feeding [56, 58, 64]. The defect leading to increased lipid accumulation when MAGP-1 is absent is related to elevated fatty acid uptake in a background of normal lipolysis or lipid catabolism [64]. Energy expenditure was found to be reduced in MAGP-1-deficient mice, and these animals were maladaptive to cold challenge, indicating reduced thermogenic potential. Impaired thermogenesis was not the result of reduced mitochondrial number, but instead, was associated with reduced expression of uncoupling protein-1 (UCP1), the protein responsible for proton leaks across the mitochondrial membrane and subsequent heat generation. Reduced core body temperature, abnormal lipid accumulation in the thermogenic-brown adipose tissue, and reduced UCP1 expression was apparent at around five weeks of age, preceding changes in lipid accumulation in white fat, and providing a rationale for inappropriate fat accumulation in MAGP-1-deficient mice [64]. Interestingly, MAGP-1 transcript in white adipose tissue (epididymal) was elevated in obese mice (high-fat diet fed). Similarly, MAGP-1 transcript in human subcutaneous white adipose tissue positively correlated with BMI. Together this suggests that MAGP-1 is expressed as a compensatory protective response to pathologic fat accumulation.

A comparison of TGFβ activity in white adipose tissue from MAGP-1-deficient and WT mice showed Smad2 phosphorylation to be significantly increased in the MAGP-1 knockout animals, indicative of elevated TGFβ activity [56, 64]. Fibrosis and inflammation, down-stream consequences of increased TGFβ signaling, were also elevated in white adipose tissue of knockout animals [64]. A role for TGFβ in orchestrating the MAGP-1-obesity phenotype was confirmed by treating MAGP-1-deficient and WT mice with a neutralizing antibody to TGFβ. After five weeks of treatment, adiposity and body temperature of MAGP-1 knockout animals were near WT levels [64]. Thus, MAGP-1 supports energy expenditure and protects against excess lipid accumulation by regulating the availability of TGFβ. These findings are in agreement with numerous studies showing that TGFβ has an

adverse effect on thermogenesis while supporting white adipose tissue expansion and insulin resistance [70].

5. COMPARISON OF DISEASE PHENOTYPES: MAGP-1 VERSUS FIBRILLIN-1

Multiple reports have shown similar skeletal phenotypes in both fibrillin-1 mutant mice and mice deficient in MAGP-1. Mice with 50% fibrillin-1 expression (MgN), ~30% fibrillin-1 expression (MgR), fibrillin-1 C1039G point mutation (*Fbn1*$^{C1039G/+}$), and loss of fibrillin-1's exon 7 (H1) have increased bone length and reduced bone mass, similar to MAGP-1-deficient animals [56]. Characterization of MgR mice and mice lacking fibrillin-1 in bone (*Prx1-Cre, Fbn1*$^{f/f}$) demonstrated increased bone resorption driven by dramatic increases in RANKL expression and osteoclast number with minor changes in osteoblasts and bone formation [71]. Similar to *Mfap2*$^{-/-}$ mice, RANKL and osteoclast number in fibrillin-1 mutant animals could be normalized by TGFβ inhibition. Thus, MAGP-1 and fibrillin-1 appear to have overlapping functions in the regulation of osteoclast number and bone resorption, mediated through control of TGFβ bioavailability.

There are, however, distinctions in how MAGP-1 and fibrillin-1 influence bone mesenchymal stem cell (MSC) differentiation. Loss of MAGP-1 appears to have no consequence on osteoblastogenesis, and bone loss in these mice occurs before marrow fat accumulation [56, 57]. In contrast, MSC differentiation frequency is reduced with fibrillin-1 loss and therefore both osteoblast and adipocyte numbers are reduced [72]. Interestingly, TGFβ inhibition was able to restore MSC frequency suggesting that while MAGP-1 and fibrillin-1 both contribute to TGFβ regulation, MAGP-1 and fibrillin-1 do not have overlapping functions regarding MSC maintenance.

MAGP-1 and fibrillin-1 also have distinct roles in influencing metabolic health. Marfan syndrome individuals with mutations in fibrillin-1 are often excessively lean and have elevated TGFβ signaling levels. This is a bit of a paradox since elevated TGFβ signaling is positively correlated with obesity, not leanness [67, 70]. As described above, loss of MAGP-1 in mice leads to the development of metabolic syndrome (obesity, insulin resistance, hyperlipidemia, and hepatic steatosis) whereas fibrillin-1 mutant mice revealed only modest metabolic changes, which included slightly increased adiposity and insulin resistance [56]. When TGFβ signaling was assessed, MAGP-1-deficient mice show a dramatic increase in Smad phosphorylation within adipose tissue [56, 69]. In contrast, fibrillin-1 perturbation in the MgN, *Fbn1*$^{C1039G/+}$, and H1 mice had little effect on TGFβ activity in the adipose tissue despite a clear increase in Smad phosphorylation in the bones of the same mice [56]. These studies show that perturbation of mature fibrillin-1, in mice, fails to capture the metabolic phenotypes typical of Marfan Syndrome. Because fibrillin-associated TGFβ-LLC must undergo some form of processing to generate active TGFβ, it is not clear whether loss of TGFβ-LLC binding to fibrillin, as has been proposed for Marfan syndrome mutations, results in increased as opposed to decreased TGFβ signaling. Accumulating evidence suggests that increased TGFβ activity seen in many connective tissue diseases may be unrelated to TGFβ sequestration issues but occurs as a standard

response associated with (sometimes unproductive) matrix remodeling [73, 74]. Similarly, genetic disruption of TGFβ signaling pathways in mice suggests that elevated TGFβ signaling may not be responsible for the adverse vascular phenotype associated with Marfan syndrome and related conditions [75–77].

A non-TGFβ-related pathway that links metabolic disease with fibrillin was proposed by Romere et al [78]. The authors present data suggesting that a C-terminal cleavage product of fibrillin-1, termed asprosin, has biological activity as a glucogenic protein hormone and is released from adipose tissue under fasting conditions to stimulate hepatic glucose production. *In vitro* studies with cultured adipocytes are suggestive of fibrillin-1 production [79], but others have shown significant downregulation of fibrillin-1 upon differentiation of adipocyte precursors [80, 81]. Current evidence indicates that MAGP-1 in adipose tissue is derived from the stromal-vascular compartment, not the adipocytes. Because MAGP-1 and fibrillin are both components of the microfibril, it is likely that fibrillin is produced by cells in the stromal-vascular compartment as well. Whether adipocytes can produce sufficient quantities of fibrillin-1 to account for the effects attributed to asprosin is unclear, but the idea that soluble fibrillin fragments might have biological activity outside of the protein's structural role in the microfibril is intriguing.

6. CONCLUSIONS AND FURTHER READING

The close relationship between the MAGPs and fibrillins raises the interesting question of how much of the pathology associated with fibrillin mutations are directly linked to changes in MAGP-fibrillin interactions and subsequent MAGP-dependent function. Conversely, do mutations that result in the absence of MAGP from the microfibril impart gain-of-function properties to fibrillin? These are complex questions that are difficult to answer when dealing with a polymeric structure comprising multiple components that influence each other in defining overall fiber function.

Readers interested in an in-depth discussion of microfibril structure and the role of microfibrils in growth factor signaling should consult reviews by Cleary and Gibson [2], Sengle and Sakai [82, 83], and Ramirez et al [72, 84]. Reviews focused on the MAGPs, and other microfibrillar proteins can be found in [10, 20, 85, 86].

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ABBREVIATIONS

ADAMTS	a disintegrin-like and metalloproteinase domain with thrombospondin-type 1 motifs
BMP	bone morphogenic growth factor
DEXA	longitudinal dual energy x-ray absorptiometry

ECM	extracellular matrix
FBN	fibrillin
LTBP	latent TGFβ binding proteins
MAGP	microfibril-associated glycoprotein
MFAP	microfibril-associated proteins
RANK	receptor activator of nuclear factor κB
RANKL	receptor activator of nuclear factor κB ligand
TβR	transforming growth factor-beta receptor I and II
TGFβ	transforming growth factor-beta
TGFβ-LLC	transforming growth factor-beta large latent complex
μCT	microcomputed tomography

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Highlights

MAGR-1 and MAGP-2 are microfibril-associated proteins that work with fibrillin to define microfibril function.

Both MAGPs bind active forms of TGFβ and BMPs, and are capable of modulating Notch signaling.

Neither protein appears to be required for normal development, but mutations in the MAGP genes lead to defects in multiple organ systems.

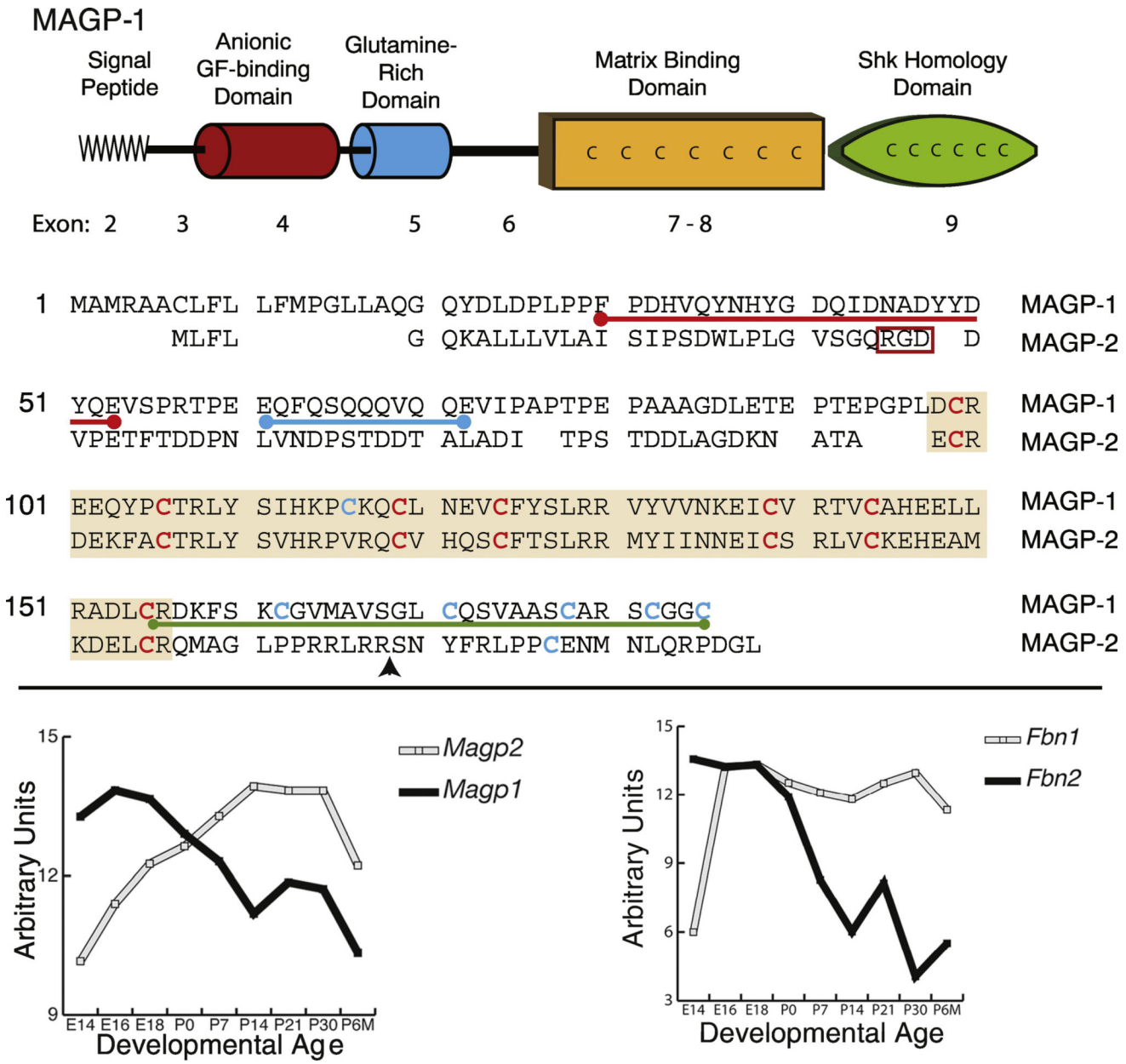

Figure 1.

Top) Functional domain diagram of MAGP-1 and sequence comparison of MAGP-1 and MAGP-2. The highly acidic growth factor binding domain of MAGP-1 (red underline) near the amino terminus is the site for TGFβ interaction. The adjacent glutamine-rich motif (blue underline) mediates self-interaction between MAGP-1 monomers through the formation of an extremely stable parallel β-pleated “tape” structure. Cysteine residues are contained in the C-terminal half of both proteins where seven cysteine residues contribute to the conserved matrix binding domain (shaded region). The last five cysteines in MAGP-1 suggest a ShK motif at the C-terminal end of the protein (green underline, MAGP-1). The arrowhead indicates a furin cleavage site in MAGP-2 that is absent in MAGP-1. While there is relatively high sequence homology within the matrix binding domain of MAGP-1 and

MAGP-2, sequences outside of this region share little homology. Other than the matrix binding domain, functional sequences in MAGP-2 have not yet been characterized. Bottom: MAGP and fibrillin gene expression in developing mouse aorta as determined by gene array analysis. Expression values in arbitrary units are plotted against developmental age, which begins at embryonic day 14 (E14) through 6 months (P6M). Expression of MAGP-1 is highest in the fetal and neonatal period and lowest in the adult. MAGP-2 shows the opposite expression pattern, being lowest in the fetal period and rising throughout the neonatal period to highest levels in adult tissue. Interestingly, fibrillin-1 has an expression pattern similar to MAGP-2 whereas fibrillin-2 follows the pattern of MAGP-1. It should be noted, however, that expression of both MAGPs is relatively high compared to other proteins at all stages of development.

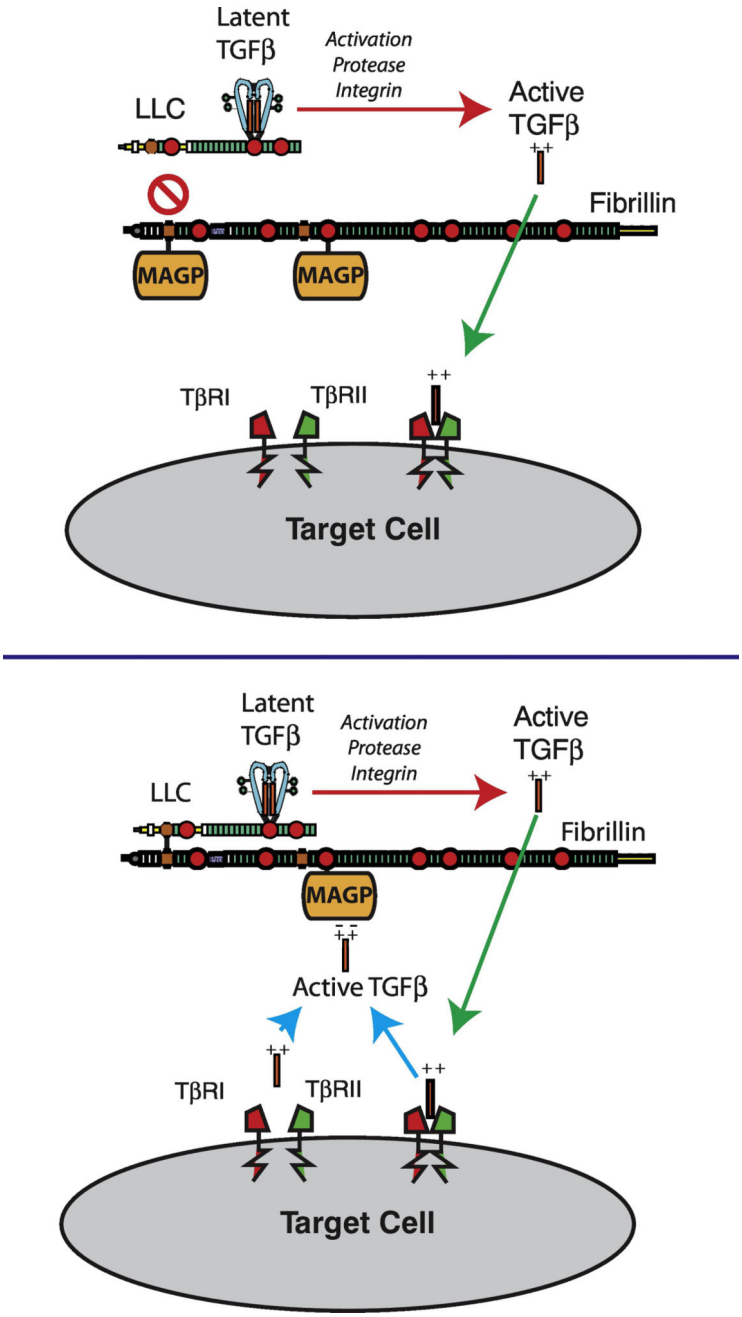

Figure 2.

Possible mechanisms for TGF growth factor regulation by MAGP. Top) MAGP blocks binding of the TGFβ-LLC to the microfibril by competing for a common interaction site on fibrillin. Bottom) MAGP serves to subdue TGFβ signaling by binding active growth factor after its release from the LLC. See text for details