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Progranulin: A growth factor, a novel TNFR ligand and a drug target

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Abstract

Progranulin (PGRN) is abundantly expressed in epithelial cells, immune cells, neurons, and chondrocytes, and reportedly contributes to tumorigenesis. PGRN is a crucial mediator of wound healing and tissue repair. PGRN also functions as a neurotrophic factor and mutations in the *PGRN* gene resulting in partial loss of the PGRN protein cause frontotemporal dementia. PGRN has been found to be a novel chondrogenic growth factor and to play an important role in cartilage development and inflammatory arthritis. Although research has shown that PGRN exhibits anti-inflammatory properties, the details about the exact molecular pathway of such effects, and, in particular, the PGRN binding receptor, have not been identified so far. Recently, researchers have shown that PGRN binds to tumor necrosis factor (TNF)-receptors (TNFR), interfering with the interaction between TNF α and TNFR. They further demonstrated that mice deficient in PGRN are susceptible to collagen-induced arthritis, an experimental model of rheumatoid arthritis, and that administration of PGRN reversed the arthritic process. An engineered protein made of three PGRN fragments (Atsttrin), displayed selective TNFR binding and was more active than natural PGRN. Both PGRN and Atsttrin prevented inflammation in various arthritis mouse models and inhibited TNF α -induced intracellular signaling pathways. Thus, PGRN is a key regulator of inflammation and it may mediate its anti-inflammatory effects, at least in part, by blocking TNF binding to its receptors. As we discuss here, TNFR-based interventions may both stimulate and suppress the growth of cancer cells, and the same may be true in analogy for Atsttrin as a new player.

Keywords

Progranulin; TNFR; TNF α ; Frontotemporal dementia; Cancer; Chondrogenesis

1. Introduction

Progranulin (PGRN), also referred to as granulin-epithelin precursor (GEP), proepithelin, PC cell derived growth factor (PCDGF), or acrogranin, was first purified as a growth factor from conditioned tissue culture media (Wright et al., 1989; Zhou et al., 1993). It has been identified from different sources by several independent laboratories (Anakwe & Gerton, 1990; Baba et al., 1993; Zanicco-Marani et al., 1999; Daniel et al., 2000). It is a 68.5-kDa secreted growth factor (Shoyab et al., 1990; Plowman et al., 1992; Baba et al., 1993; Zhou et al., 1993; Bateman & Bennett, 2009). PGRN is heavily glycosylated and appears as a ~90-kDa protein on sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. Structurally, it belongs to none of the well-established growth factor families. PGRN is secreted in an intact form (Wright et al., 1989; Zhou et al., 1993) and undergoes proteolysis, leading to the release of its constituent peptides, the granulins (Zanicco-Marani et al., 1999; Lu & Serrero, 2000; Davidson et al., 2004). Individual granulins have an approximate molecular weight of 6 kDa and are structurally defined by the presence of 12 cysteines arranged in a characteristic motif: $X\ 2-3CX5-6CX5CCX8CCX6CCX5CCX4CX5-6CX2$ (Hrabal et al., 1996).

PGRN is abundantly expressed in epithelial cells, in immune cells, in neurons, and in chondrocytes (Anakwe & Gerton, 1990; Baba et al., 1993; Daniel et al., 2000; Lu & Serrero, 2000; Xu et al., 2007; Feng et al., 2010; Guo et al., 2010). High levels of PGRN expression have been reported in human cancers, and PGRN is strongly believed to contribute to tumorigenesis (Bateman et al., 1990; Zhang & Serrero, 1998; Gonzalez et al., 2003; He & Bateman, 2003; He et al., 2003; Jones et al., 2003; Wang et al., 2003; Davidson et al., 2004). Although PGRN mainly functions as a secreted growth factor, it was also found to be localized inside cells and to directly modulate intracellular activities (Daniel et al., 2000; Hoque et al., 2003; Thornburg et al., 2004; Hoque et al., 2005). The role of PGRN in the regulation of cellular proliferation has been well characterized. Mouse embryo fibroblasts derived from mice with a targeted deletion of the insulin-like growth factor receptor gene ($R^{-/-}$ cells) are unable to proliferate in response to insulin-like growth factor and other growth factors [epidermal growth factor (EGF) and platelet-derived growth factor] that are necessary for cell cycle progression (Sell et al., 1994). In contrast, PGRN allows the cell to bypass the requirement for the insulin-like growth factor receptor, thus promoting the growth of $R^{-/-}$ cells (Xu et al., 1998; Zanicco-Marani et al., 1999). PGRN has been isolated as a differentially expressed growth factor in mesothelial differentiation (Sun et al., 2004), sexual differentiation of the brain (Suzuki & Nishihara, 2002), and macrophage development (Barreda et al., 2004). PGRN is a crucial mediator of wound healing and tissue repair (Zhu et al., 2002; He et al., 2003). PGRN also functions as a neurotrophic factor (Van Damme et al., 2008; Yin et al., 2010b) and mutations in the *PGRN* gene resulting in partial loss of the PGRN protein cause frontotemporal dementia (Baker et al., 2006; Cruts et al., 2006; Van Deerlin et al., 2010). Recently, PGRN has been found to be a novel chondrogenic growth factor and to play an important role in cartilage development and inflammatory arthritis (Bai et al., 2009; Feng et al., 2010; Guo et al., 2010; Tang et al., 2011).

Several PGRN-associated proteins have been reported to affect PGRN growth factor action in various processes. PGRN directly binds to the cartilage oligomeric matrix protein

(COMP) (Xu et al., 2007), a prominent noncollagenous component of cartilage that plays an important role in stabilizing the cartilage matrix (Briggs et al., 1995; Hecht et al., 1995) and is heavily degraded in arthritis (Liu et al., 2006a; Liu et al., 2006b; Luan et al., 2008).

Overexpression of PGRN stimulates the proliferation of chondrocytes and this stimulation is enhanced by COMP. Perlecan, a heparan sulfate proteoglycan important for chondrocyte differentiation and function (Nicole et al., 2000; French et al., 2002), was also shown to bind to PGRN (Gonzalez et al., 2003). During inflammation, neutrophils and macrophages release serine proteases that digest PGRN into individual 6 kDa granulin units, which are actually pro-inflammatory and can neutralize the anti-inflammatory effects of intact PGRN (Zhu et al., 2002; Kessenbrock et al., 2008). Both neutrophil elastase and proteinase 3 are known to digest PGRN at its linker regions, resulting in the liberation of individual granulin units (Zhu et al., 2002; Kessenbrock et al., 2008), and are involved in the PGRN conversion during neutrophil activation (Kessenbrock et al., 2008; Kessenbrock et al., 2011). PGRN's anti-inflammatory actions are protected by its binding proteins, which include the secretory leukocyte protease inhibitor (Zhu et al., 2002) and apolipoprotein A1 (Okura et al., 2010), both of which bind to PGRN and protect it against proteolytic degradation. In addition to serine proteases, several metalloproteinases were also found to associate with and degrade PGRN (Butler et al., 2008; Bai et al., 2009a; Bai et al., 2009b; Feng et al., 2010; Guo et al., 2010). A Disintegrin And Metalloproteinase with Thrombospondin Motifs (ADAMTS)-7 acts as a PGRN-convertase and neutralizes PGRN-stimulated endochondral bone formation (Bai et al., 2009a). More significantly, PGRN binds directly to ADAMTS-7 and ADAMTS-12 and inhibits their degradation of COMP (Liu, 2009; Guo et al., 2010). In addition, PGRN was also isolated as a substrate of the membrane type 1 matrix metalloproteinase (MMP-14) (Butler et al., 2008) and MMP-9 (Xu et al., 2008).

2. Progranulin is a novel ligand of tumor necrosis factor receptors

2.1. Progranulin binds to tumor necrosis factor receptors

Although PGRN plays crucial roles in multiple physiological and pathological conditions, efforts to exploit the actions of PGRN and understand the mechanisms involved have been hampered by our inability to identify its binding receptor(s) (Bateman & Bennett, 2009). To address this issue, a global genetic screen was performed that led to the identification of TNFR2 as the PGRN-associated receptor (Tang et al., 2011). The interaction between PGRN and TNFR2 in yeast was verified using a co-immunoprecipitation assay. In addition, a solid-phase binding assay showed that PGRN demonstrated dose-dependent binding and saturation to liquid-phase TNFR1 and TNFR2. Analytical surface plasmon resonance revealed that PGRN exhibited higher affinity for TNF receptors, especially TNFR2 when compared with TNF α . In contrast to TNF α , which demonstrated higher affinity for TNFR1 than TNFR2, PGRN exhibited comparable binding affinity for TNFR1 and TNFR2 (Tang et al., 2011).

In order to identify the domains of PGRN required for its interaction with TNF receptors, a series of PGRN mutants were constructed and analyzed for their interactions with TNFR2. No single granulin unit (A, B, C, D, E, F, or G) or linker region was able to bind to TNFR2, suggesting that the binding domain of PGRN may span granulin unit and linker. To examine

this hypothesis, each granulin with its immediately adjacent downstream linker was tested, and granulin F plus linker P3 was found to exhibit a weak interaction with TNFR2. Next, each granulin was linked to its immediately adjacent upstream linker; P4-granulin A and P5-granulin C both demonstrated weak binding affinity to TNFR2. Finally, all three fragments identified above were lined together to generate an engineered mutant (referred to as FAC) (Tang et al., 2011). These data are in accordance with the findings that granulin F, A, and C are the granulin domains most capable of independent folding and that these granulin domains have N and C terminal subdomains that are structurally independent by high resolution nuclear magnetic resonance (Tolkatchev et al., 2008). Interestingly, FAC exhibited an even stronger binding affinity to TNFR2 than PGRN (Tang et al., 2011). To identify the minimal engineered mutant protein with retained binding affinity, the mutant 2/3(FAC), which is identical to FAC except that only 2/3 of each granulin unit was included, was generated. 2/3(FAC) was found to bind to TNFR2 with a lower affinity than FAC. A further reduction from 2/3 to 1/2 of each granulin unit did not alter the binding affinity; however, a reduction to 1/4 completely abolished the interaction with TNFR2. Taken together, these results suggest that a mutant composed of half units of granulins A, C, and F plus linkers P3, P4, and P5 appears to be the “minimal” engineered molecule that retains affinity to TNFR2. This molecule was referred to as Atsttrin (A ntagonist of *T* NF/*T* NFR *S* ignaling via *T* argeting to *T* NF *R* eceptors) (Tang et al., 2011). When compared with TNF α , recombinant Atsttrin exhibited higher binding affinity for TNFR2, but lower affinity for TNFR1 (Tang et al., 2011).

The extracellular regions of TNF receptors are elongated structures with multiple cysteine-rich repeat domains (CRDs). TNF family ligands bind to receptors in a heterohexameric 3:3 complex in which each receptor subunit contacts two adjacent ligand subunits typically via CRD2 and CRD3, the “stalk” of the receptor (Wu & Siegel, 2011). By contrast, the opposite side of the CRD1 contains the preligand association domain, which mediates homotypic interactions among receptor chains and may also stabilize and propagate ligand–receptor contacts (Wu & Siegel, 2011). Deletion mutants of TNFR1 and TNFR2 used to map the binding of PGRN revealed that CRD3 from TNFR1 and TNFR2 was essential for TNFR binding to PGRN, because deletion mutants lacking CRD3 failed to interact with PGRN (Tian & Liu, unpublished data).

In addition to TNF receptors, sortilin was identified as a neuronal receptor for PGRN that regulates PGRN levels in vitro and in vivo (Hu et al., 2010), suggesting that sortilin-mediated PGRN endocytosis may play a role in the pathophysiology of the neurodegenerative disease frontotemporal lobar degeneration (Hu et al., 2010; Lewis & Golde, 2010). Sortilin is known to be a multi-ligand neural receptor that also interacts with other neural growth factors, including nerve growth factor (NGF) (Nykjaer et al., 2004) and brain-derived neurotrophic factor (BDNF) (Chen et al., 2005). Recently, PGRN was reported to associate with Toll-like receptor 9 (TLR9) and to contribute to innate immunity by acting as an essential secreted cofactor for TLR9 signaling that potentiates TLR9-driven responses to CpG-oligodeoxynucleotides (Moresco & Beutler, 2011; Park et al., 2011a).

2.2. Progranulin antagonizes tumor necrosis factor α

The finding that PGRN directly binds to TNFR raised the possibility that PGRN affects the TNF α /TNFR interaction. Indeed, PGRN demonstrated dose-dependent inhibition of TNF α binding to TNF receptors in a solid-phase binding assay and a flow cytometry assay with Raw 264.7 (Tang et al., 2011). PGRN potently inhibited TNF-mediated neutrophil activation (Zhu et al., 2002) and cartilage degradation (Feng et al., 2010). The deletion of PGRN led to a significant increase in hydrogen peroxide in neutrophils and nitric oxide in bone marrow derived macrophages (Tang et al., 2011). In addition, knockout of PGRN resulted in a marked increase in TNF α -induced COMP degradation (Tang et al., 2011). Furthermore, PGRN significantly protected regulatory T cells (Treg) from a negative regulation by TNF α in a dose-dependent manner (Zanin-Zhorov et al., 2010; Tang et al., 2011).

TNF-transgenic (Tg) mice are known to develop an inflammatory arthritis phenotype spontaneously (Li & Schwarz, 2003; Thwin et al., 2004). The deletion of PGRN markedly hastened the onset of arthritis. Twelve-week-old TNF-Tg/PGRN^{-/-} and TNF-Tg/PGRN^{+/-} mice developed severe swelling and joint deformation, which contributed to a significant loss of mobility. In contrast, TNF-Tg mice developed only mild signs of inflammation. TNF-Tg/PGRN^{-/-} and TNF-Tg/PGRN^{+/-} mice demonstrated significantly increased synovitis, pannus formation, destruction of the ankle joints, and loss of cartilage matrix. Treatment of TNF-Tg mice with recombinant PGRN resulted in the elimination of any visual signs of arthritis and a dramatically reduced arthritis severity score. Interestingly, at 7 days following the cessation of PGRN treatment, signs of arthritis began to develop (Tang et al., 2011). Taken together, these data suggest that PGRN is a naturally-occurring antagonist of TNF α -mediated inflammatory signaling.

2.3. Progranulin activates tumor necrosis factor receptor 2

Growing evidences indicate that TNFR2 signaling has a protective role in inflammatory arthritis and joint erosion (Blum et al., 2010; Faustman & Davis, 2010). It appears that PGRN is the optimal ligand for TNFR2, in terms of both biochemical (PGRN exhibits ~600-fold higher binding affinity to TNFR2 than TNF α) and functional evidences (PGRN and TNFR2 mediate beneficial and protective roles in the inflammatory processes) (Zhu et al., 2002; Kessenbrock et al., 2008; Blum et al., 2010; Faustman & Davis, 2010).

Approximately 40 genes are up-regulated (over 2-fold) following PGRN treatment as determined by a genome-wide DNA chip analysis (Feng et al., 2010). Interestingly, PGRN-inducible genes, including growth arrest and DNA damage gene (Gadd) 45 β and JunB, are also known to be activated by the transforming growth factor (TGF) β subfamily (Zavadil et al., 2001; Yang et al., 2003; Zavadil et al., 2004). PGRN-mediated induction of its target genes, JunB and Gadd45 β , primarily depends on TNFR2, since anti-TNFR2, but not anti-TNFR1, completely or largely abolishes PGRN-mediated transactivation in chondrocytes (Liu et al., unpublished data). TNF α directly up-regulates, whereas PGRN down-regulates, IFN γ secretion in effector T cells (Teff) (Tang et al., 2011). Furthermore, the TNFR1 blocking antibody largely inhibits TNF α -induced up-regulation of IFN γ secretion, but does not affect PGRN-mediated suppression; in contrast, the TNFR2 blocking antibody abolishes PGRN-mediated down-regulation of IFN γ production (Tang et al., 2011). TNFR2^{-/-} collagen-induced arthritis (CIA) mice are less sensitive to PGRN-derived Atsttrin treatment

(Tang et al., 2011). Collectively, PGRN, and probably its derived Atsttrin, exerts its protective effects in the pathogenesis of inflammatory arthritis through multiple mechanisms, such as acting as an antagonist of TNF-mediated inflammatory response and regulating the functions of Treg and Teff in a TNFR2-dependent manner.

3. Progranulin is a therapeutic target

3.1. Progranulin in autoimmune diseases

Since PGRN and Atsttrin modulate TNF/TNFR signaling (Liu, 2011; Tang et al., 2011; Wu & Siegel, 2011), which is the cornerstone of human inflammatory responses, they carry broad potential applicability to a host of autoimmune diseases, including rheumatoid arthritis (RA) (Liu, 2011; Tang et al., 2011). PGRN^{-/-} mice immunized with collagen II developed higher severity inflammatory arthritis and increased bone and joint destruction as compared with their control littermates. A significant increase in the arthritis severity score and a 100% incidence of arthritis in PGRN^{-/-} mice were observed, which was dramatically higher when compared with the 40% arthritis incidence of wild-type control CIA C57B6 mice. Importantly, the administration of recombinant PGRN completely blocked disease progression in PGRN-deficient CIA mice. Collectively, these data suggest that the loss of PGRN expression in vivo results in hyper-susceptibility to collagen induced arthritis, which can be entirely reversed by the administration of recombinant PGRN (Tang et al., 2011).

Administration of either PGRN or Atsttrin resulted in reduced disease severity in the collagen antibody induced arthritis (CAIA) model, and both agents significantly delayed the progression of arthritis. In the CIA model, DBA/1 mice treated with PGRN or Atsttrin demonstrated markedly reduced pathology, with Atsttrin-treated mice bearing marked similarity to normal mice. In addition, both PGRN- and Atsttrin-treated mice were found to have decreased circulating levels of fragmentary COMP, a marker for cartilage breakdown (Tang et al., 2011). The therapeutic efficacy of PGRN and Atsttrin was also confirmed in the TNF transgenic mouse model. Consistent with the results observed in the CAIA and CIA models, the administration of PGRN or Atsttrin markedly suppressed arthritis progression and notably eliminated signs of inflammation. In addition, signs of inflammation returned following the cessation of Atsttrin treatment (Tang et al., 2011).

Since tissue destruction in RA is caused by inflammatory mediators, the currently approved biological therapies for RA treatment primarily target cytokines such as TNF α . Although treatment with these agents is highly effective in ameliorating disease and improving quality of life in some patients with moderate-to-severe disease, current TNF α inhibitors fail to provide effective treatment for up to 50% of RA patients (Nurmohamed & Dijkmans, 2005). PGRN, especially its derived Atsttrin, demonstrates features that suggest it may compare favorably to these established agents. For example, all currently marketed anti-TNF therapies bind to the TNF α ligand, while, in contrast, PGRN and Atsttrin bind to TNFR and not to TNF α itself (Fig. 1). Due to this unique mechanism of action, PGRN and Atsttrin may be effective for the patients who fail to respond to current TNF α blockers (Nurmohamed & Dijkmans, 2005). The potential advantages of a 'targeting TNFR2' approach (in contrast to an 'anti-TNF' approach) to the treatment of human chronic inflammatory and autoimmune conditions have been previously reviewed (Kollias & Kontoyiannis, 2002; Faustman &

Davis, 2010). In addition, drugs such as Anakinra and Actemra, which target the IL-1 receptor and IL-6 receptor, have demonstrated that the selective targeting of cytokine receptors can deliver a highly effective clinical outcome (Rothe et al., 2008). Given that granulins produced by protease digestion of PGRN are highly proinflammatory (Zhu et al., 2002; Kessenbrock et al., 2008), whereas Atsttrin contains only partial granulin units and would not be expected to release any intact pro-inflammatory granulin units upon exposure to PGRN-converting enzymes such as elastase (Zhu et al., 2002), proteinase-3 (Kessenbrock et al., 2008) and ADAMTS-7 (Bai et al., 2009a), it is conceivable that Atsttrin, rather than PGRN, may be a promising molecule for clinical applications. Indeed, Atsttrin has been found to surpass PGRN itself in ameliorating inflammatory arthritis in mice, although both of them exhibit anti-inflammatory activity in vivo (Tang et al., 2011).

3.2. Progranulin in cartilage repair

Chondrogenesis is fundamentally involved in osteogenesis and joint development, and is closely controlled by cellular interactions with growth factors, nearby matrix proteins, and other environmental factors that mediate cellular signaling pathways and the transcription of specific genes (Colnot, 2005; Goldring et al., 2006; Feng et al., 2010). The role of the tumor growth factor (TGF)-beta superfamily, especially the bone morphogenic proteins is central. PGRN, which is richly expressed in chondrocytes (Xu et al., 2007; Feng et al., 2010), has also been shown to be a significant regulator of cartilage formation and degradation (Xu et al., 2007; Liu, 2009; Guo et al., 2010).

Human cartilage oligomeric matrix protein (COMP) mutations are known to be associated with multiple epiphyseal dysplasia and pseudoachondroplasia, but the role of COMP in skeletogenesis was unclear. In 2007, Xu et al. identified PGRN as a COMP-associated companion (Xu et al., 2007). COMP binds directly to PGRN both in vitro and in vivo. Specifically, PGRN selectively interacts with the COMP epidermal growth factor repeat domain. PGRN overexpression stimulates chondrocyte proliferation, which is enhanced by COMP. COMP also seems to be necessary for PGRN-mediated chondrocyte proliferation, since an anti-COMP antibody radically inhibits PGRN-induced chondrocyte proliferation. This is the first evidence of an association between COMP and PGRN and of PGRN in cartilage as a COMP-binding protein, and shows that PGRN-stimulated chondrocyte proliferation is interceded by COMP. The study enlarges our understanding of the actions of growth factors in cartilage biology.

ADAMTS-7 has been shown to interact with PGRN and inactivate PGRN-mediated chondrogenesis (Bai et al., 2009a). ADAMTS-7 and ADAMTS-12, two members of the ADAMTS family, associate with and degrade COMP by binding to the epidermal growth factor COMP domain. A novel protein–protein interaction network between PGRN, ADAMTS-7 and ADAMTS-12 metalloproteinases, and COMP extracellular matrix protein, has been found by a recent study that revealed that PGRN is a novel specific inhibitor of ADAMTS-7/-12-mediated COMP degradation (Guo et al., 2010). PGRN inhibits TNF α -induced ADAMTS-7 and ADAMTS-12 expression and disrupts COMP binding and cleavage by ADAMTS-7 and ADAMTS-12 through direct protein–protein interactions.

These results might, in the future, result in new therapeutic targets, such as PGRN or its derivatives, to prevent and treat diseases involving cartilage destruction.

Feng et al., in a study that included testing the ex vivo roles of recombinant PGRN in a rabbit cartilage repair model, demonstrated that chondrocyte differentiation is activated by PGRN through extracellular regulated kinase (Erk)1/2 signaling and that the JunB transcription factor is one of the key downstream molecules of PGRN in this differentiation (Feng et al., 2010). These results suggest conceivable molecule targets, especially recombinant PGRN protein, for the treatment of cartilage destruction and arthritic disorders.

3.3. Progranulin in tumorigenesis

Recent studies have shown that actions of PGRN on both growth modulation and inflammation may be due to the negative regulation of TNF- α signaling by PGRN.

TNF- α , the most-studied TNFR ligand, is a trimeric protein that is encoded within the major histocompatibility complex and that shares various common functions with PGRN: both are produced by macrophages, and both have pleiotropic inflammatory functions and growth modulating functions and are involved in wound healing and cancer. The anti-inflammatory effects of PGRN are exerted through inhibition of TNF-TNFR-mediated NF- κ B and MAPK signaling by competitively binding to TNFR, especially TNFR-2, which is found in bone marrow-derived macrophages (Tang et al., 2011). The growth modulating properties of PGRN may also be due to negative regulation of TNF- α signaling with both growth-enhancing and growth inhibitory properties depending upon the cellular context (Kumar-Singh, 2011). Therefore, TNFR-based interventions may both stimulate and suppress the growth of cancer cells, and the same may be true in analogy for Atsttrin as a new player.

PGRN is highly expressed in various forms of human cancer, and, as we discuss below, contributes to tumorigenesis in breast and ovarian cancer and hepatocellular carcinoma (HCC) and, probably, gastric cancer.

Initial evidence for PGRN as a functional growth factor originated from studies on cancer, when it was shown to act as an autocrine growth stimulus in aggressive murine teratoma (Zhou et al., 1993; Bateman & Bennett, 2009). Decreasing PGRN mRNA expression deeply abridged tumor formation by teratoma cells (Zhang & Serrero, 1998) and in in vivo breast cancer (Lu & Serrero, 2000), liver cancer (Cheung et al., 2004) and squamous esophageal cancer cell lines (Chen et al., 2008), demonstrating that tumor genesis in these cells requires PGRN. In addition, it has been demonstrated not only that PGRN is required for tumor growth, but also that it actively confers malignancy, as overexpression of PGRN in a normally weakly tumor-forming line of SW13 adenocarcinoma cells prompted substantial tumors in a murine model (He & Bateman, 1999).

PGRN aids tumor growth through increased proliferation (He & Bateman, 1999; Lu & Serrero, 2001), reduced apoptosis (Tangeangsirisin et al., 2004; Kim & Serrero, 2006; Pizarro et al., 2007) and greater invasiveness through the extracellular matrix (ECM) (He et al., 2002; Tangeangsirisin et al., 2004). All these actions involve the activity of the ERK

and phosphatidylinositol 3-kinase (PI3K) signal transduction pathways, although the contribution of either pathway may vary (Zanocco-Marani et al., 1999).

Studies have shown that PGRN is expressed in human breast cancer cells and, in patients with early stage breast cancer, overexpression of PGRN in tumors is associated with poorer disease-free and overall survival rates. Moreover, a correlation has been found between tumor genesis and estradiol-stimulated PGRN expression transcriptionally in estrogen receptor-positive cells (Lu & Serrero, 2001; Abrhale et al., 2011). Aromatase inhibitors, which inhibit the growth of breast cancer cells by blocking estrogen synthesis, are the treatment of choice in post-menopausal, estrogen-receptor positive breast cancer, but some patients present de novo or acquired aromatase inhibitor resistance. Interactions between estrogen and growth factor signaling pathways in estrogen-responsive cells have been identified as one possible factor in the acquisition of resistance. Abrhale et al., who studied the role of PGRN in the acquisition of resistance to the aromatase inhibitors letrozole in estrogen-receptor positive breast cancer cells, found overexpression of PGRN in invasive ductal carcinoma (Abrhale et al., 2011).

The study, which used two aromatase-overexpressing human breast cancer cell lines, MCF-7-CA cells and AC1 cells, and their letrozole-resistant counterparts, as study models, found that PGRN induced time- and dose-dependent cell proliferation and produced letrozole resistance (Abrhale et al., 2011).

There was a 10-fold increase in PGRN expression in breast cancer cells that were naturally letrozole-resistant compared to letrozole-sensitive cells. The overexpression or exogenous addition of PGRN blocked the inhibitory effect of letrozole on proliferation, and stimulated survival and colony formation. In letrozole-resistant cells, silencing PGRN by small interfering RNA (siRNA) inhibited cell proliferation and reinstated letrozole sensitivity (Abrhale et al., 2011).

Elucidation of the role and mechanisms of PGRN in resistance to anti-hormonal treatments might help to improve breast cancer therapies. For example, the study speculates that it would be of interest to examine whether PGRN overexpression in tumor tissue is associated with a poor response to aromatase inhibitors. Likewise, drug combinations that include growth factor and anti-hormone inhibition might provide new therapeutic pathways for patients with breast cancer and overcome resistance.

A study recently successfully measured blood levels of PGRN and found significant differences in serum PGRN levels between subjects with no history of breast cancer and breast cancer patients of all stages. This suggests that testing PGRN serum levels could be used as to screen for breast cancer in apparently healthy women. Likewise, the results suggest other useful lines of research, including studies in patients with breast cancer to determine the correlation between serum PGRN levels and disease-free and overall survival parameters and other tumor characteristics in patients enrolled at diagnosis on the one hand, and the correlation between PGRN and responses to therapy, progression and survival in patients with advanced breast cancer on the other hand (Tkaczuk et al., 2011).

In ovarian cancer, PGRN is also overexpressed and has been implicated in the stimulation of cell proliferation, malignancy, and chemoresistance, and identified as an autocrine growth factor. Kamrava et al.(2005) studied PGRN production and functions and found that, in ovarian cancer cells: (a) PGRN production is regulated by endothelin-1 (ET-1), lysophosphatidic acid (LPA), and cAMP; (b) cAMP signals PGRN production through the exchange protein activated by cAMP (EPAC); (c) ET-1 and cAMP/EPAC induce PGRN through extracellular-signal-regulated kinases (ERKs) (or classical MAP kinases) 1 and 2; and (d) PGRN neutralization results in apoptosis. Exposure of HEY-A8 and OVCAR3 ovarian cancer cells to LPA and ET-1 resulted in dose- and time-dependent PGRN production and secretion. Stimulation of cAMP production induced PGRN in a protein kinase A (PKA)-independent fashion. EPAC, an intracellular cAMP receptor, is specifically activated by the cAMP analog 8-CPT-20-OMe- cAMP (8-CPT); 8-CPT treatment stimulated PGRN production and secretion. The mitogen-activated protein (MAP) kinase kinase (MEK) inhibitor U0126 annulled PGRN production in response to ET-1 and 8-CPT, corroborating MAPK involvement. When cells were treated with BAPTA, an internal calcium chelator, partial inhibition of basal and stimulated PGRN production was found. Neutralizing anti-PGRN antibody reversed basal as well as LPA, ET-1 and 8-CPTinduced ovarian cancer cell growth and induced apoptosis, as confirmed by caspase-3 and poly (ADP-ribose) polymerase (PARP) cleavage, nuclear condensation and DNA fragmentation.

These findings suggest that PGRN is a growth and survival factor for ovarian cancer, induced by LPA and ET-1 and cAMP/EPAC through ERK1/2. The expression of PGRN and, therefore, PGRN-mediated cell proliferation and survival are controlled by both the cAMP and MAPK-mediated signaling pathways. Thus, inhibition of these two pathways in combination might represent an effective approach for the treatment of ovarian cancer. Combining cAMP and MAPK pathway inhibitors might result in increased blockade of PGRN production together with inhibition of another growth factor pathway in ovarian cancer. The possible applications of this approach might also include antibody blockade of PGRN in combination with endothelin receptor small molecule antagonists.

Expression of low levels of PGRN has recently been found in normal ovarian tissue and benign ovarian tumors. The trend toward higher levels of expression of PGRN in patients with advanced International Federation of Gynaecology and Obstetrics (FIGO) stages and the correlation of high PGRN mRNA levels with poorer overall survival suggests that overexpression of PGRN may contribute to growth, invasion, and metastasis in ovarian cancer (Cuevas-Antonio et al., 2010).

A pilot study also found that PGRN is a useful biomarker in plasma for patients in the advanced stages of epithelial ovarian cancer. At three months, PGRN was the only biomarker independently associated with progression-free and overall survival in comparison with secretory leukocyte protease inhibitor and markers HE4 and cancer antigen 125. The sensitivity and specificity in predicting progression at 18 months were 93% and 100%, respectively (Han et al., 2011).

PGRN has also been identified as a potential therapeutic target in HCC (Ho et al., 2008). Cheung et al.(2004) showed that PGRN controls HCC proliferation, invasion, and

tumorigenicity, and these biological findings are supported by clinical results showing that PGRN expression is associated with aggressive HCC features such as large tumors, venous infiltration, and prompt post-surgical recurrence. Tellingly, overexpression of PGRN was found in >70% of HCCs studied (Cheung et al., 2004).

Ho et al. studied the effects of the anti-PGRN monoclonal antibody (mAb) A23 on hepatoma cells and normal liver cells in vitro (Ho et al., 2008). Using a nude mouse model transplanted with human HCC, they determined whether anti-PGRN mAb inhibits tumor growth in vivo, and found that while anti-PGRN mAb inhibited hepatoma cell growth, it had no substantial effect on normal liver cells. Anti-PGRN mAb reduced levels of PGRN in serum and inhibited established tumor growth in a dose-dependent fashion. Anti-PGRN mAb abridged tumor cell proliferation through the phospho-p44/42 MAPK (Erk1/2) and Akt pathways, and reduced tumor angiogenesis to deprive the nutrient supply, with a decrease in microvessel density and levels of tumor vascular endothelial growth factor.

In summary, the anti-PGRN monoclonal antibody A23 inhibited the growth of two HCC cell lines in a dose-dependent fashion, regardless of endogenous PGRN levels, suggesting that PGRN is a therapeutic goal in HCC. The evidence suggests that A23 could inhibit tumor growth and stabilize disease in cases of large HCCs not suitable for surgery.

More recently, Park et al. designed three siRNAs targeting the PGRN gene (GEP-siRNA1, 2 and 3) and tested their tumor regression and suppression effects on cell proliferation (Park et al., 2011b). They found that PGRN-siRNA1 had the greatest time-dependent anti-proliferative effect of the three. In addition, they constructed a short hairpin RNA (shRNA) using an H1/TO promoter with the same sequence of PGRN-siRNA1 (PGRN-shRNA) in order to increase siRNA biostability. PGRN-shRNA reduced the expression of PGRN and tumor cell growth via cell cycle arrest at the G2/M stage and down-regulation of the cell proliferation proteins cyclin D1 and α -tubulin. PGRN-shRNA also significantly inhibited tumor growth after intratumoral injection of tumor-bearing Balb/C nude mice. These findings are the first known therapeutic application of RNA interference to PGRN with the aim of suppressing HCC cell proliferation.

Finally, although a high expression of PGRN in gastric carcinoma cells has been reported, the relationship between PGRN and potentially-carcinogenic *Helicobacter pylori* infection-induced cellular responses remains unclear. A recent study found that infection of gastric epithelial cells by *H. pylori* upregulated PGRN, and that the induction of PGRN by *H. pylori* infection stimulated gastric epithelial cell proliferation and migration, possibly contributing to oncogenic transformation. The study found that *H. pylori* infection activated the p38 and MAPK kinase (MEK) 1/2 signal pathway, resulting in PGRN overexpression in gastric epithelial cells. Likewise, gastric epithelial cells infected with *H. pylori* responded by upregulating PGRN mRNA and protein production (Wang et al., 2011).

3.4. Progranulin in neuronal degeneration diseases

PGRN is a neurotrophic factor (Van Damme et al., 2008; Yin et al., 2010b), with PGRN gene mutations that result in partial loss of the PGRN protein causing frontotemporal dementia, also known as frontotemporal lobar degeneration (FTLD) (Baker et al., 2006; Van

Deerlin et al., 2010). FTLT, the second most-frequent type of presenile dementia, is characterized by a gradual worsening of decision-making, and behavior and language control, with early memory impairment; around a quarter of cases are hereditary (Cenik et al., 2011).

PGRN mutations are the most-frequent known inherited cause of FTLT, and exhibit TAR DNA-binding protein 43 (TDP-43) plus ubiquitin aggregates (Baker et al., 2006; Cruts et al., 2006; Gass et al., 2006; Hu et al., 2010). In these families, there is autosomal-dominant FTLDTDP-43 protein inheritance, with the PGRN mutations resulting in loss of function. PGRN levels are some 50% lower in cases with PGRN mutant FTLT-TDP (Baker et al., 2006; Cruts et al., 2006; Gass et al., 2006; Ghidoni et al., 2008; Finch et al., 2009; Sleegers et al., 2009). However, despite the causal role of PGRN haploinsufficiency in FTLT-TDP, its neurobiology remains unknown.

More than 60 pathogenic PGRN mutations have now been reported in patients with FTLT, all of which are thought to cause a PGRN haploinsufficiency (Cenik et al., 2011). PGRN-deficient mice exhibit dysregulated cerebral immune responses and recapitulate phosphorylated cytoplasmic TDP-43 aggregates seen in FTLT brains (Yin et al., 2010a; Yin et al., 2010b). Likewise, serum PGRN levels are reported to be lower in FTLT patients and carriers of the mutation than in normal controls (Finch et al., 2009; Sleegers et al., 2009), suggesting that decreased expression of PGRN causes FTLT. Thus, increasing the expression of PGRN might prevent or retard disease progression. Interestingly, Capell et al. recently demonstrated that alkalizing drugs and vacuolar ATPase inhibitors increase PGRN expression via a post-transcriptional mechanism (Capell et al., 2011).

Taking this evidence that loss-of-function mutations in one PGRN allele cause familial and sporadic FTLT, Cenik et al. recently searched for small molecule enhancers of PGRN transcription by high-throughput screening (HTS) of chemical libraries (Cenik et al., 2011). Based on the assumption that identifying a Food and Drug Administration (FDA)-approved compound would hasten the search for a cure for FTLT, they initially selected the Prestwick Chemical Library® of 1200 FDA approved drugs, which thus presents the highest degree of drug-likeness [the library was designed to decrease the risk of low quality hits and the cost of initial screening, and to accelerate novel discoveries (<http://www.prestwickchemical.com/index.php?pa=26>)]. The authors identified many compounds that increased PGRN promoter activity by more than three standard deviations above the mean.

Screening showed that suberoylanilide hydroxamic acid (SAHA), an FDA-approved histone deacetylase (HDAC) inhibitor currently used clinically to treat cutaneous T-cell lymphoma (Mann et al., 2007), enhances PGRN expression in various types of cells in cultures. SAHA increased PGRN mRNA and protein levels in cultured cells in a dose-dependent manner and restored near-normal PGRN expression in human haploinsufficient cells. These results provide the first evidence for a small molecule enhancer of PGRN transcription. However, the study was unable to determine the details of the specific mechanism of the individual HDACs responsible for the increase in the expression of PGRN found after SAHA therapy. In addition, both chemically similar (trichostatin A) and structurally unrelated (M344) HDAC inhibitors, and sodium butyrate, also increased PGRN expression, further evidence

for the theory that inhibition of HDAC is critical to the fundamental mechanism. In summary, PGRN expression at both the mRNA and protein levels is significantly enhanced by SAHA in murine and human models.

As discussed above, Hu et al. recently demonstrated that PGRN endocytosis mediated by sortilin, a protein encoded by the *SORT1* gene, may play an essential role in the pathophysiology of FTLN-TDP (Hu et al., 2010; Zheng et al., 2011). They found that PGRN binds to cortical neurons through its C terminus, and unbiased expression cloning identified sortilin as a binding site and also that sortilin^{-/-} neurons showed reduced PGRN binding. Sortilin is expressed by neurons in the central nervous system, and PGRN was most strongly expressed by activated microglial cells after injury. Sortilin suffered rapid endocytosis and delivered PGRN to lysosomes. Mice lacking sortilin had raised brain and serum PGRN levels of 2.5- to 5-fold. Interestingly, the 50% reduction in PGRN which acts as a causal factor in cases of FTLN-TDP is mirrored in PGRN^{+/-} mice, and was entirely normalized by sortilin ablation. The interaction between PGRN and sortilin-mediated endocytosis notably decreased steady state PGRN levels by at least as much as the reduction produced by the PGRN mutations causing FTLN-TDP. Thus, sortilin binding seems to have therapeutic potential as a means of altering PGRN-dependent pathways and alleviating TDP-43 disorders. The study also showed that microglial cells are a chief source of PGRN production, with activated microglia in the neighborhood of axotomized motoneurons strongly inducing PGRN after sciatic nerve damage. Therefore, characterization of the mechanisms of microglial PGRN induction or the selectivity of PGRN for microglia subsets could also play a potential role in modulating the course of FTLN-TDP.

Other FTLN phenotypes may be explained by competitive binding of PGRN to TNFR. For example, TNF- α with a biphasic diurnal pattern helps to regulate bodily circadian rhythm and modulate sleep (Young et al., 1995): significant circadian rhythm and sleep disorders have been reported in patients with FTLN (Anderson et al., 2009). Studies now in progress will show which TNFR-PGRN interactions occur in frontocortical brain regions, but it is probable that future studies will identify more multi-ligand cell-surface receptors for PGRN, thereby supplying a better overall vision of PGRN cell-signaling pathways which are abrogated in FTLN-TDP (Kumar-Singh, 2011).

Furthermore, determining how PGRN is regulated by sortilin and how some PGRN functions, especially the inflammatory functions, may be mediated by the interaction of PGRN with TNF- α will be aided by the recent identification of two putative PGRN receptors. Although the role of these putative receptors in disease pathogenesis requires intensive study, the search for other cell-surface proteins, with which PGRN or its peptides interact with intermediate-to-high affinities, are also a research priority as they may supply a more complete picture of disease pathogenesis (Kumar-Singh, 2011).

4. Conclusions and future directions

Using a functional genomic approach combined with biochemistry, cellular biology, and molecular biology techniques, PGRN growth factor has been identified as a previously unrecognized ligand for TNF receptors (TNFR1 and TNFR2) (Tang et al., 2011). In

addition, PGRN and its derivative, Atsttrin, effectively prevent the onset and progression of inflammatory arthritis in several preclinical animal models (Tang et al., 2011).

We envisage potential applications of PGRN, and its derivative, Atsttrin, in other autoimmune diseases, such as systemic lupus erythematosus, inflammatory bowel disease, ankylosing spondylitis, plaque psoriasis, and psoriatic arthritis, on the grounds that TNF inhibitors have been used for treating such diseases. PGRN and Atsttrin might also be useful in neuronal degenerative diseases in which TNF are believed to play an important role in disease pathogenesis — notably FTLN, which is considered a chronic inflammatory disease. Likewise, the molecule may represent an attractive therapeutic approach in cancers in which PGRN is highly expressed and known to be responsible for tumor cell growth, such as breast cancer, ovarian cancer, and HCC; of note, in addition to its blockade of TNF binding to TNFR in inflammatory diseases, Atsttrin inhibits PGRN-mediated cancer cell proliferation through competing with PGRN for binding to TNFR (Liu, 2011).

It may be worth exploring the potential therapeutic application of monoclonal antibodies targeting PGRN as well as RNA interference with PGRN with the aim of suppressing cancer cell proliferation, taking advantage of the recent results in HCC.

The therapeutic possibilities of SAHA have been demonstrated in other neurodegenerative disorders and it could potentially be a first generation drug for FTLN prevention and treatment. As SAHA is already licensed for other indications, human trials in patients with FTLN are greatly facilitated.

Finally, as long as these molecules prove useful and safe in humans, we also envisage potential combinations with currently available drugs for treating these diseases.

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Abbreviations

PGRN	progranulin
TNF	tumor necrosis factor
TNFR	tumor necrosis factor receptors
PCDGF	PC cell derived growth factor
GEP	granulin-epithelin precursor
COMP	cartilage oligomeric matrix protein
ADAMTS	A Disintegrin And Metalloproteinase with Thrombospondin Motifs
MMP	matrix metalloproteinase
CRDs	cysteine-rich repeat domains

TLR	Toll-like receptor
TNF-Tg mice	TNF-transgenic mice
TGF	transforming growth factor
IFN	interferon
RA	rheumatoid arthritis
CIA	collagen-induced arthritis
IL	interleukin
LPA	lysophosphatidic acid
ET-1	endothelin-1
ERK	extracellular regulated kinase
EPAC	exchange protein activated by cAMP
MAP	mitogen-activated protein
MAPK	mitogen-activated protein kinase
mRNA	messenger RNA
HCC	hepatocellular carcinoma
mAb	monoclonal antibody
siRNA	small interfering RNA
shRNA	short hairpin RNA
<i>H. pylori</i>	<i>Helicobacter pylori</i>
MEK	mitogen-activated protein kinase kinase
FTLD	frontotemporal lobar degeneration
TDP-43	TAR DNA-binding protein 43
SAHA	suberoylanilide hydroxamic acid
HDAC	histone deacetylase
FDA	Food and Drug Administration

References

- Abrhale T, Brodie A, Sabnis G, Macedo L, Tian C, Yue B, et al. GP88 (PC-Cell Derived Growth Factor, progranulin) stimulates proliferation and confers letrozole resistance to aromatase overexpressing breast cancer cells. *BMC Cancer*. 2011; 11:231. [PubMed: 21658239]
- Anakwe OO, Gerton GL. Acrosome biogenesis begins during meiosis: evidence from the synthesis and distribution of an acrosomal glycoprotein, acrogranin, during guinea pig spermatogenesis. *Biol Reprod*. 1990; 42(2):317–328. [PubMed: 1692485]
- Anderson KN, Hatfield C, Kipps C, Hastings M, Hodges JR. Disrupted sleep and circadian patterns in frontotemporal dementia. *Eur J Neurol*. 2009; 16(3):317–323. [PubMed: 19170747]

- Baba T, Hoff HB III, Nemoto H, Lee H, Orth J, Arai Y, et al. Acrogranin, an acrosomal cysteine-rich glycoprotein, is the precursor of the growth-modulating peptides, granulins, and epithelins, and is expressed in somatic as well as male germ cells. *Mol Reprod Dev.* 1993; 34(3):233–243. [PubMed: 8471244]
- Bai XH, Wang DW, Kong L, Zhang Y, Luan Y, Kobayashi T, et al. ADAMTS-7, a direct target of PTHrP, adversely regulates endochondral bone growth by associating with and inactivating GEP growth factor. *Mol Cell Biol.* 2009a; 29(15):4201–4219. [PubMed: 19487464]
- Bai XH, Wang DW, Luan Y, Yu XP, Liu CJ. Regulation of chondrocyte differentiation by ADAMTS-12 metalloproteinase depends on its enzymatic activity. *Cell Mol Life Sci.* 2009b; 66(4): 667–680. [PubMed: 19151918]
- Baker M, Mackenzie IR, Pickering-Brown SM, Gass J, Rademakers R, Lindholm C, et al. Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. *Nature.* 2006; 44(7105):916–919. [PubMed: 16862116]
- Barreda DR, Hanington PC, Walsh CK, Wong P, Belosevic M. Differentially expressed genes that encode potential markers of goldfish macrophage development in vitro. *Dev Comp Immunol.* 2004; 28(7–8):727–746. [PubMed: 15043942]
- Bateman A, Belcourt D, Bennett H, Lazure C, Solomon S. Granulins, a novel class of peptide from leukocytes. *Biochem Biophys Res Commun.* 1990; 173(3):1161–1168. [PubMed: 2268320]
- Bateman A, Bennett HP. The granulin gene family: from cancer to dementia. *Bioessays.* 2009; 31(11): 1245–1254. [PubMed: 19795409]
- Bluml S, Binder NB, Niederreiter B, Polzer K, Hayer S, Tauber S, et al. Antiinflammatory effects of tumor necrosis factor on hematopoietic cells in a murine model of erosive arthritis. *Arthritis Rheum.* 2010; 62(6):1608–1619. [PubMed: 20155834]
- Briggs MD, Hoffman SM, King LM, Olsen AS, Mohrenweiser H, Leroy JG, et al. Pseudoachondroplasia and multiple epiphyseal dysplasia due to mutations in the cartilage oligomeric matrix protein gene. *Nature.* 1995; 10(3):330–336.
- Butler GS, Dean RA, Tam EM, Overall CM. Pharmacoproteomics of a metalloproteinase hydroxamate inhibitor in breast cancer cells: dynamics of membrane type 1 matrix metalloproteinase-mediated membrane protein shedding. *Mol Cell Biol.* 2008; 28(15):4896–4914. [PubMed: 18505826]
- Capell A, Liebscher S, Fellerer K, Brouwers N, Willem M, Lammich S, et al. Rescue of progranulin deficiency associated with frontotemporal lobar degeneration by alkalizing reagents and inhibition of vacuolar ATPase. *J. Neurosci.* 2011; 31(5):1885–1894. [PubMed: 21289198]
- Cenik B, Sephton CF, Dewey CM, Xian X, Wei S, Yu K, et al. Suberoylanilide hydroxamic acid (vorinostat) up-regulates progranulin transcription: rational therapeutic approach to frontotemporal dementia. *J Biol Chem.* 2011; 286(18):16101–16108. [PubMed: 21454553]
- Chen ZY, Ieraci A, Teng H, Dall H, Meng CX, Herrera DG, et al. Sortilin controls intracellular sorting of brain-derived neurotrophic factor to the regulated secretory pathway. *J Neurosci.* 2005; 25(26): 6156–6166. [PubMed: 15987945]
- Chen XY, Li JS, Liang QP, He DZ, Zhao J. Expression of PC cell-derived growth factor and vascular endothelial growth factor in esophageal squamous cell carcinoma and their clinicopathologic significance. *Chin Med J.* 2008; 121(10):881–886. [PubMed: 18706200]
- Cheung ST, Wong SY, Leung KL, Chen X, So S, Ng IO, et al. Granulin-epithelin precursor overexpression promotes growth and invasion of hepatocellular carcinoma. *Clin Cancer Res.* 2004; 10(22):7629–7636. [PubMed: 15569995]
- Colnot C. Cellular and molecular interactions regulating skeletogenesis. *J Cell Biochem.* 2005; 95(4): 688–697. [PubMed: 15880692]
- Cruts M, Gijselinck I, van der Zee J, Engelborghs S, Wils H, Pirici D, et al. Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. *Nature.* 2006; 442(7105):920–924. [PubMed: 16862115]
- Cuevas-Antonio R, Cancino C, Arechavaleta-Velasco F, Andrade A, Barron L, Estrada I, et al. Expression of progranulin (Acrogranin/PCDGF/Granulin-Epithelin Precursor) in benign and malignant ovarian tumors and activation of MAPK signaling in ovarian cancer cell line. *Cancer Inves.* 2010; 28(5):452–458.

- Daniel R, He Z, Carmichael KP, Halper J, Bateman A. Cellular localization of gene expression for progranulin. *J Histochem Cytochem*. 2000; 48(7):999–1009. [PubMed: 10858277]
- Davidson B, Alejandro E, Florenes VA, Goderstad JM, Risberg B, Kristensen GB, et al. Granulin-epithelin precursor is a novel prognostic marker in epithelial ovarian carcinoma. *Cancer*. 2004; 100(10):2139–2147. [PubMed: 15139056]
- Faustman D, Davis M. TNF receptor 2 pathway: drug target for autoimmune diseases. *Nat Rev Drug Discov*. 2010; 9(6):482–493. [PubMed: 20489699]
- Feng JQ, Guo FJ, Jiang BC, Zhang Y, Frenkel S, Wang DW, et al. Granulin epithelin precursor: a bone morphogenic protein 2-inducible growth factor that activates Erk1/2 signaling and JunB transcription factor in chondrogenesis. *FASEB J*. 2010; 24(6):1879–1892. [PubMed: 20124436]
- Finch N, Baker M, Crook R, Swanson K, Kuntz K, Surtees R, et al. Plasma progranulin levels predict progranulin mutation status in frontotemporal dementia patients and asymptomatic family members. *Brain*. 2009; 132(Pt 3):583–591. [PubMed: 19158106]
- French MM, Gomes RR Jr, Timpl R, Hook M, Czymmek K, Farach-Carson MC, et al. Chondrogenic activity of the heparan sulfate proteoglycan perlecan maps to the N-terminal domain I. *J Bone Miner Res*. 2002; 17(1):48–55. [PubMed: 11771669]
- Gass J, Cannon A, Mackenzie IR, Boeve B, Baker M, Adamson J, et al. Mutations in progranulin are a major cause of ubiquitin-positive frontotemporal lobar degeneration. *Hum Mol Genet*. 2006; 15(20):2988–3001. [PubMed: 16950801]
- Ghidoni R, Benussi L, Glionna M, Franzoni M, Binetti G. Low plasma progranulin levels predict progranulin mutations in frontotemporal lobar degeneration. *Neurology*. 2008; 71(16):1235–1239. [PubMed: 18768919]
- Goldring MB, Tsuchimochi K, Ijiri K. The control of chondrogenesis. *J Cell Biochem*. 2006; 97(1):33–44. [PubMed: 16215986]
- Gonzalez EM, Mongiat M, Slater SJ, Baffa R, Iozzo RV. A novel interaction between perlecan protein core and progranulin: potential effects on tumor growth. *J Biol Chem*. 2003; 278(40):38113–38116. [PubMed: 12900424]
- Guo F, Lai Y, Tian Q, Lin EA, Kong L, Liu C. Granulin-epithelin precursor binds directly to ADAMTS-7 and ADAMTS-12 and inhibits their degradation of cartilage oligomeric matrix protein. *Arthritis Rheum*. 2010; 62(7):2023–2036. [PubMed: 20506400]
- Han JJ, Yu M, Houston N, Steinberg SM, Kohn EC. Progranulin is a potential prognostic biomarker in advanced epithelial ovarian cancers. *Gynecol Oncol*. 2011; 120(1):5–10. [PubMed: 20950846]
- He Z, Bateman A. Progranulin gene expression regulates epithelial cell growth and promotes tumor growth in vivo. *Cancer Res*. 1999; 59(13):3222–3229. [PubMed: 10397269]
- He Z, Bateman A. Progranulin (granulin-epithelin precursor, PC-cell-derived growth factor, acrogranin) mediates tissue repair and tumorigenesis. *J Mol Med*. 2003; 81(10):600–612. [PubMed: 12928786]
- He Z, Ismail A, Kriazhev L, Sadvakassova G, Bateman A. Progranulin (PC-cell-derived growth factor/acrogranin) regulates invasion and cell survival. *Cancer Res*. 2002; 62(19):5590–5596. [PubMed: 12359772]
- He Z, Ong CH, Halper J, Bateman A. Progranulin is a mediator of the wound response. *Nat Med*. 2003; 9(2):225–229. [PubMed: 12524533]
- Hecht JT, Nelson LD, Crowder E, Wang Y, Elder FF, Harrison WR, et al. Mutations in exon 17B of cartilage oligomeric matrix protein (COMP) cause pseudoachondroplasia. *Nat Genet*. 1995; 10(3):325–329. [PubMed: 7670471]
- Ho JC, Ip YC, Cheung ST, Lee YT, Chan KF, Wong SY, et al. Granulin-epithelin precursor as a therapeutic target for hepatocellular carcinoma. *Hepatology Baltimore, Md*. 2008; 47(5):1524–1532.
- Hoque M, Tian B, Mathews MB, Pe'ery T. Granulin and granulin repeats interact with the Tat.P-TEFb complex and inhibit Tat transactivation. *J Biol Chem*. 2005; 280(14):13648–13657. [PubMed: 15653695]
- Hoque M, Young TM, Lee CG, Serrero G, Mathews MB, Pe'ery T. The growth factor granulin interacts with cyclin T1 and modulates P-TEFb-dependent transcription. *Mol Cell Biol*. 2003; 23(5):1688–1702. [PubMed: 12588988]

- Hrabal R, Chen Z, James S, Bennett HP, Ni F. The hairpin stack fold, a novel protein architecture for a new family of protein growth factors. *Nat Struct Biol.* 1996; 3(9):747–752. [PubMed: 8784346]
- Hu F, Padukavidana T, Vaegter CB, Brady OA, Zheng Y, Mackenzie IR, et al. Sortilin-mediated endocytosis determines levels of the frontotemporal dementia protein, progranulin. *Neuron.* 2010; 68(4):654–667. [PubMed: 21092856]
- Jones MB, Spooner M, Kohn EC. The granulin-epithelin precursor: a putative new growth factor for ovarian cancer. *Gynecol Oncol.* 2003; 88(1 Pt 2):S136–S139. [PubMed: 12586105]
- Kamrava M, Simpkins F, Alejandro E, Michener C, Meltzer E, Kohn EC. Lysophosphatidic acid and endothelin-induced proliferation of ovarian cancer cell lines is mitigated by neutralization of granulin-epithelin precursor (GEP), a prosurvival factor for ovarian cancer. *Oncogene.* 2005; 24(47):7084–7093. [PubMed: 16044162]
- Kessenbrock K, Dau T, Jenne DE. Tailor-made inflammation: how neutrophil serine proteases modulate the inflammatory response. *J Mol Med.* 2011; 89(1):23–28. [PubMed: 20809089]
- Kessenbrock K, Frohlich L, Sixt M, Lammernann T, Pfister H, Bateman A, et al. Proteinase 3 and neutrophil elastase enhance inflammation in mice by inactivating antiinflammatory progranulin. *J Clin Invest.* 2008; 118(7):2438–2447. [PubMed: 18568075]
- Kim WE, Serrero G. PC cell-derived growth factor stimulates proliferation and confers Trastuzumab resistance to Her-2-overexpressing breast cancer cells. *Clin Cancer Res.* 2006; 12(14 Pt 1):4192–4199. [PubMed: 16857791]
- Kollias G, Kontoyiannis D. Role of TNF/TNFR in autoimmunity: specific TNF receptor blockade may be advantageous to anti-TNF treatments. *Cytokine Growth Factor Rev.* 2002; 13(4–5):315–321. [PubMed: 12220546]
- Kumar-Singh S. Progranulin and TDP-43: mechanistic links and future directions. *J Mol Neurosci.* 2011
- Lewis J, Golde TE. Sorting out frontotemporal dementia? *Neuron.* 2010; 68(4):601–603. [PubMed: 21092851]
- Li P, Schwarz EM. The TNF-alpha transgenic mouse model of inflammatory arthritis. *Springer Semin Immunopathol.* 2003; 25(1):19–33. [PubMed: 12904889]
- Liu CJ. The role of ADAMTS-7 and ADAMTS-12 in the pathogenesis of arthritis. *Nat Clin Pract Rheumatol.* 2009; 5(1):38–45. [PubMed: 19098927]
- Liu CJ. Progranulin: a promising therapeutic target for rheumatoid arthritis. *FEBS Lett.* 2011 May 4. (Epub ahead of print).
- Liu CJ, Kong W, Ilalov K, Yu S, Xu K, Prazak L, et al. ADAMTS-7: a metalloproteinase that directly binds to and degrades cartilage oligomeric matrix protein. *FASEB J.* 2006a; 20(7):988–990. [PubMed: 16585064]
- Liu CJ, Kong W, Xu K, Luan Y, Ilalov K, Sehgal B, et al. ADAMTS-12 associates with and degrades cartilage oligomeric matrix protein. *J Biol Chem.* 2006b; 281(23):15800–15808. [PubMed: 16611630]
- Lu R, Serrero G. Inhibition of PC cell-derived growth factor (PCDGF, epithelin/ granulin precursor) expression by antisense PCDGF cDNA transfection inhibits tumorigenicity of the human breast carcinoma cell line MDA-MB-468. *Proc Natl Acad Sci U S A.* 2000; 97(8):3993–3998. [PubMed: 10760271]
- Lu R, Serrero G. Mediation of estrogen mitogenic effect in human breast cancer MCF-7 cells by PC-cell-derived growth factor (PCDGF/granulin precursor). *Proc Natl Acad Sci U S A.* 2001; 98(1): 142–147. [PubMed: 11134521]
- Luan Y, Kong L, Howell DR, Ilalov K, Fajardo M, Bai XH, et al. Inhibition of ADAMTS-7 and ADAMTS-12 degradation of cartilage oligomeric matrix protein by alpha-2-macroglobulin. *Osteoarthritis Cartil.* 2008; 16(11):1413–1420. [PubMed: 18485748]
- Mann BS, Johnson JR, Cohen MH, Justice R, Pazdur R. FDA approval summary: vorinostat for treatment of advanced primary cutaneous T-cell lymphoma. *Oncologist.* 2007; 12(10):1247–1252. [PubMed: 17962618]
- Moresco EM, Beutler B. Special delivery: granulin brings CpG DNA to tolllike receptor 9. *Immunity.* 2011; 34(4):453–455. [PubMed: 21511177]

- Nicole S, Davoine CS, Topaloglu H, Cattolico L, Barral D, Beighton P, et al. Perlecan, the major proteoglycan of basement membranes, is altered in patients with Schwartz-Jampel syndrome (chondrodystrophic myotonia). *Nat Genet.* 2000; 26(4):480–483. [PubMed: 11101850]
- Nurmohamed MT, Dijkmans BA. Efficacy, tolerability and cost effectiveness of disease-modifying antirheumatic drugs and biologic agents in rheumatoid arthritis. *Drugs.* 2005; 65(5):661–694. [PubMed: 15748099]
- Nykjaer A, Lee R, Teng KK, Jansen P, Madsen P, Nielsen MS, et al. Sortilin is essential for proNGF-induced neuronal cell death. *Nature.* 2004; 427(6977):843–848. [PubMed: 14985763]
- Okura H, Yamashita S, Ohama T, Saga A, Yamamoto-Kakuta A, Hamada Y, et al. HDL/apolipoprotein A-I binds to macrophage-derived progranulin and suppresses its conversion into proinflammatory granulins. *J Atheroscler Thromb.* 2010; 17(6):568–577. [PubMed: 20215705]
- Park B, Buti L, Lee S, Matsuwaki T, Spooner E, Brinkmann MM, et al. Granulin is a soluble cofactor for toll-like receptor 9 signaling. *Immunity.* 2011a; 34(4):505–513. [PubMed: 21497117]
- Park MY, Park YS, Nam JH. RNA interference against granulin-epithelin precursor prevents hepatocellular carcinoma growth: its application as a therapeutic agent. *Int J Oncol.* 2011b; 39(4):853–861. [PubMed: 21701774]
- Pizarro GO, Zhou XC, Koch A, Gharib M, Raval S, Bible K, et al. Prosurvival function of the granulin-epithelin precursor is important in tumor progression and chemoresponse. *Int J Cancer.* 2007; 120(11):2339–2343. [PubMed: 17266030]
- Plowman GD, Green JM, Neubauer MG, Buckley SD, McDonald VL, Todaro GJ, et al. The epithelin precursor encodes two proteins with opposing activities on epithelial cell growth. *J Biol Chem.* 1992; 267(18):13073–13078. [PubMed: 1618805]
- Rothe A, Power BE, Hudson PJ. Therapeutic advances in rheumatology with the use of recombinant proteins. *Nat Clin Pract Rheumatol.* 2008; 4(11):605–614. [PubMed: 18813220]
- Sell C, Dumenil G, Deveaud C, Miura M, Coppola D, DeAngelis T, et al. Effect of a null mutation of the insulin-like growth factor I receptor gene on growth and transformation of mouse embryo fibroblasts. *Mol Cell Biol.* 1994; 14(6):3604–3612. [PubMed: 8196606]
- Shoyab M, McDonald VL, Byles C, Todaro GJ, Plowman GD. Epithelins 1 and 2: isolation and characterization of two cysteine-rich growth-modulating proteins. *Proc Natl Acad Sci U S A.* 1990; 87(20):7912–7916. [PubMed: 2236009]
- Slegers K, Brouwers N, Van Damme P, Engelborghs S, Gijssels I, van der Zee J, et al. Serum biomarker for progranulin-associated frontotemporal lobar degeneration. *Ann Neurol.* 2009; 65(5):603–609. [PubMed: 19288468]
- Sun X, Gulyas M, Hjerpe A. Mesothelial differentiation as reflected by differential gene expression. *Am J Respir Cell Mol Biol.* 2004; 30(4):510–518. [PubMed: 14551161]
- Suzuki M, Nishihara M. Granulin precursor gene: a sex steroid-inducible gene involved in sexual differentiation of the rat brain. *Mol Genet Metab.* 2002; 75(1):31–37. [PubMed: 11825061]
- Tang W, Lu Y, Tian QY, Zhang Y, Guo FJ, Liu GY, et al. The growth factor progranulin binds to TNF receptors and is therapeutic against inflammatory arthritis in mice. *Science.* 2011; 332(6028):478–484. [PubMed: 21393509]
- Tangkeangsirisin W, Hayashi J, Serrero G. PC cell-derived growth factor mediates tamoxifen resistance and promotes tumor growth of human breast cancer cells. *Cancer Res.* 2004; 64(5):1737–1743. [PubMed: 14996734]
- Thornburg NJ, Kusano S, Raab-Traub N. Identification of Epstein–Barr virus RK-BARF0-interacting proteins and characterization of expression pattern. *J Virol.* 2004; 78(23):12848–12856. [PubMed: 15542637]
- Thwin MM, Douni E, Aidinis V, Kollias G, Kodama K, Sato K, et al. Effect of phospholipase A2 inhibitory peptide on inflammatory arthritis in a TNF transgenic mouse model: a time-course ultrastructural study. *Arthritis Res Ther.* 2004; 6(3):R282–R294. [PubMed: 15142275]
- Tkaczuk KR, Yue B, Zhan M, Tait N, Yarlagadda L, Dai H, et al. Increased circulating level of the survival factor GP88 (Progranulin) in the serum of breast cancer patients when compared to healthy subjects. *Breast Cancer (Auckl).* 2011; 5:155–162. [PubMed: 21792312]

- Tolkatchev D, Malik S, Vinogradova A, Wang P, Chen Z, Xu P, et al. Structure dissection of human progranulin identifies well-folded granulin/epithelin modules with unique functional activities. *Protein Sci.* 2008; 17(4):711–724. [PubMed: 18359860]
- Van Damme P, Van Hoecke A, Lambrechts D, Vanacker P, Bogaert E, van Swieten J, et al. Progranulin functions as a neurotrophic factor to regulate neurite outgrowth and enhance neuronal survival. *J Cell Biol.* 2008; 181(1):37–41. [PubMed: 18378771]
- Van Deerlin VM, Sleiman PM, Martinez-Lage M, Chen-Plotkin A, Wang LS, Graff-Radford NR, et al. Common variants at 7p21 are associated with frontotemporal lobar degeneration with TDP-43 inclusions. *Nat Genet.* 2010; 42(3):234–239. [PubMed: 20154673]
- Wang W, Hayashi J, Kim WE, Serrero G. PC cell-derived growth factor (granulin precursor) expression and action in human multiple myeloma. *Clin Cancer Res.* 2003; 9(6):2221–2228. [PubMed: 12796389]
- Wang H, Sun Y, Liu S, Yu H, Li W, Zeng J, et al. Upregulation of progranulin by *Helicobacter pylori* in human gastric epithelial cells via p38MAPK and MEK1/2 signaling pathway: role in epithelial cell proliferation and migration. *FEMS Immunol Med Microbiol.* 2011; 63(1):82–92. [PubMed: 21707777]
- Wright WE, Sassoon DA, Lin VK. Myogenin, a factor regulating myogenesis, has a domain homologous to MyoD. *Cell.* 1989; 56(4):607–617. [PubMed: 2537150]
- Wu H, Siegel RM. Progranulin resolves inflammation. *Science.* 2011; 332(6028):427–428. [PubMed: 21512023]
- Xu D, Suenaga N, Edelmann MJ, Fridman R, Muschel RJ, Kessler BM. Novel MMP-9 substrates in cancer cells revealed by a label-free quantitative proteomics approach. *Mol Cell Proteomics.* 2008; 7(11):2215–2228. [PubMed: 18596065]
- Xu SQ, Tang D, Chamberlain S, Pronk G, Masiarz FR, Kaur S, et al. The granulin/epithelin precursor abrogates the requirement for the insulin-like growth factor 1 receptor for growth in vitro. *J Biol Chem.* 1998; 273(32):20078–20083. [PubMed: 9685348]
- Xu K, Zhang Y, Ilalov K, Carlson CS, Feng JQ, Di Cesare PE, et al. Cartilage oligomeric matrix protein associates with granulin-epithelin precursor (GEP) and potentiates GEP-stimulated chondrocyte proliferation. *J Biol Chem.* 2007; 282(15):11347–11355. [PubMed: 17307734]
- Yang YC, Piek E, Zavadil J, Liang D, Xie D, Heyer J, et al. Hierarchical model of gene regulation by transforming growth factor beta. *Proc Natl Acad Sci U S A.* 2003; 100(18):10269–10274. [PubMed: 12930890]
- Yin F, Banerjee R, Thomas B, Zhou P, Qian L, Jia T, et al. Exaggerated inflammation, impaired host defense, and neuropathology in progranulin-deficient mice. *J Exp Med.* 2010a; 207(1):117–128. [PubMed: 20026663]
- Yin F, Dumont M, Banerjee R, Ma Y, Li H, Lin MT, et al. Behavioral deficits and progressive neuropathology in progranulin-deficient mice: a mouse model of frontotemporal dementia. *FASEB J.* 2010b; 24(12):4639–4647. [PubMed: 20667979]
- Young MR, Matthews JP, Kanabrocki EL, Sothorn RB, Roitman-Johnson B, Scheving LE. Circadian rhythmometry of serum interleukin-2, interleukin-10, tumor necrosis factor-alpha, and granulocyte-macrophage colony-stimulating factor in men. *Chronobiol Int.* 1995; 12(1):19–27. [PubMed: 7750154]
- Zanin-Zhorov A, Ding Y, Kumari S, Attur M, Hippen KL, Brown M, et al. Protein kinase C-theta mediates negative feedback on regulatory T cell function. *Science.* 2010; 328(5976):372–376. [PubMed: 20339032]
- Zanocco-Marani T, Bateman A, Romano G, Valentinis B, He ZH, Baserga R. Biological activities and signaling pathways of the granulin/epithelin precursor. *Cancer Res.* 1999; 59(20):5331–5340. [PubMed: 10537317]
- Zavadil J, Bitzer M, Liang D, Yang YC, Massimi A, Kneitz S, et al. Genetic programs of epithelial cell plasticity directed by transforming growth factor-beta. *Proc Natl Acad Sci U S A.* 2001; 98(12):6686–6691. [PubMed: 11390996]
- Zavadil J, Cermak L, Soto-Nieves N, Bottinger EP. Integration of TGF-beta/Smad and Jagged1/Notch signalling in epithelial-to-mesenchymal transition. *EMBO J.* 2004; 23(5):1155–1165. [PubMed: 14976548]

- Zhang H, Serrero G. Inhibition of tumorigenicity of the teratoma PC cell line by transfection with antisense cDNA for PC cell-derived growth factor (PCDGF, epithelin/granulin precursor). *Proc Natl Acad Sci U S A*. 1998; 95(24):14202–14207. [PubMed: 9826678]
- Zheng Y, Brady OA, Meng PS, Mao Y, Hu F. C-terminus of progranulin interacts with the Beta-propeller region of sortilin to regulate progranulin trafficking. *PLoS One*. 2011; 6(6):e21023. [PubMed: 21698296]
- Zhou J, Gao G, Crabb JW, Serrero G. Purification of an autocrine growth factor homologous with mouse epithelin precursor from a highly tumorigenic cell line. *J Biol Chem*. 1993; 268(15): 10863–10869. [PubMed: 8496151]
- Zhu J, Nathan C, Jin W, Sim D, Ashcroft GS, Wahl SM, et al. Conversion of proepithelin to epithelins: roles of SLPI and elastase in host defense and wound repair. *Cell*. 2002; 111(6):867–878. [PubMed: 12526812]

Figure 1.

A model for illustrating distinct anti-inflammatory mechanisms of PGRN and current anti-TNF α therapy. TNF α binds to TNFR1 and mediates inflammation. Currently-marked anti-TNF α therapies, exemplified by infliximab (Remicade), bind to TNF α ligand and in turn block TNF α -mediated inflammation; however, PGRN and its derived Atsttrin exert their anti-inflammatory action via direct binding to and by occupying TNFR1. Note that PGRN-mediated activation of the protective PGRN/TNFR2 pathway is not included in this model.