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Progranulin: At the interface of neurodegenerative and metabolic diseases

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Abstract

Progranulin is a widely expressed, cysteine-rich, secreted glycoprotein originally discovered for its growth factor-like properties. Its subsequent identification as a causative gene for frontotemporal dementia (FTD), a devastating early-onset neurodegenerative disease, has catalyzed a surge of new discoveries about progranulin's function in the brain. More recently, progranulin was recognized as an adipokine involved in diet-induced obesity and insulin resistance, revealing its metabolic function. Here, we review progranulin biology in both neurodegenerative and metabolic diseases. In particular, we highlight progranulin's growth factor-like, trophic, and anti-inflammatory properties as potential unifying themes in these seemingly divergent conditions. We also discuss potential therapeutic options for raising progranulin levels to treat progranulin-deficient FTD, as well as the possible consequences of such treatment.

A Progranulin Primer

Progranulin is one in a growing list of proteins with roles in both neurodegenerative and metabolic diseases. Deficiency of the secreted protein progranulin in the central nervous system (CNS) causes neurodegeneration: the neurodegenerative disease frontotemporal dementia (FTD, see glossary) with partial progranulin deficiency and neuronal ceroid lipofuscinosis with total deficiency [1]. In peripheral tissues, progranulin excess is linked to obesity and insulin resistance [2, 3] (Figure 1). It is unclear how alterations in progranulin levels result in such diverse disease mechanisms. Progranulin has been linked to angiogenesis, wound repair, cell proliferation, and inflammation [4]. We suggest that the processes necessary for tissue remodeling and injury repair, such as vascularization, cell proliferation, and inflammation, represent a common theme that connects progranulin with neurologic and metabolic diseases. During neurodegeneration, the brain must deal with the burden of neuronal death and cellular debris. Likewise, the progression of obesity, which is

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central to metabolic diseases, requires continual tissue remodeling as the adipose tissue expands.

Here, we review recent studies linking progranulin to neurodegenerative and metabolic diseases. Where appropriate, we speculate on functions of progranulin that might lead to common disease mechanisms. Additionally, we point out the many areas where our knowledge is incomplete and requires further investigation. Essential background information on progranulin is presented in Box 1.

Progranulin Modulates Growth Factor Signaling

In the 1990s, high levels of progranulin were shown to be associated with tumorigenesis (see Box 2), suggesting that progranulin functions as an autocrine growth factor [4]. Progranulin may promote cell proliferation and survival by activating proteins in multiple extracellular signaling pathways, including the MAPK extracellular signal-related kinase (Erk1/2) and the PI-3 kinase effectors Akt1 and p70-S6 kinase [4]. Progranulin may also act as an atypical growth factor. Progranulin promotes the passage of fibroblasts lacking insulin-like growth factor (IGF-1) receptor through all stages of the cell cycle, as opposed to just the S-phase or the M-phase usually seen with other growth factors [4]. This finding suggests that progranulin and IGF-I signaling pathways share common downstream mechanisms. The relevance of these signaling pathways to neural and metabolic cell types is unclear. The impact of progranulin cleavage to granulins for signaling is also unclear.

The Search for Progranulin Receptors and Binding Proteins

The recent identification of progranulin receptor(s) was a much-anticipated first step towards understanding the signaling cascade(s) that mediate progranulin function. In 2010, the extreme C-terminal three amino acids of progranulin were reported as being necessary for progranulin to bind sortilin [5, 6], a multi-ligand type-I receptor that regulates intracellular protein trafficking in the Golgi and acts as a clearance receptor on the cell surface. Sortilin-deficient (*Sort1*^{-/-}) mice exhibited fivefold more circulating progranulin [6], and serum progranulin levels in humans were influenced by sortilin expression [7], suggesting that sortilin is involved in progranulin's endocytic processing and/or degradation. Indeed, in addition to its localization in the ER and Golgi of microglia, progranulin was detected in the lysosomes of astrocytes grown in mixed cultures with neurons [8]. Inasmuch as astrocytes do not express progranulin [9], this might reflect sortilin-mediated uptake of neuron-derived progranulin. Nonetheless, in the absence of sortilin, exogenous progranulin promotes neurite outgrowth [10] and enhances survival of progranulin knockout cells [11], suggesting other receptors mediate these functions of progranulin. The role of sortilin-binding may be limited to sorting and recycling or possibly to acting as a co-receptor with an unidentified partner [12]. Indeed, progranulin binds to *Sort1*^{-/-} neurons [6], further suggesting the existence of additional neuronal progranulin receptors.

In 2011, progranulin was reported to bind the tumor necrosis factor receptors (TNFRs), thereby blocking receptor activation by binding of TNF and initiation of downstream signaling cascades [13]. Although involvement of this pathway is appealing given the strong evidence that progranulin modulates inflammation, a recent study did not detect this TNFR interaction [14], thus more studies are needed to clarify if progranulin is a TNFR ligand. Nevertheless, many studies agree that progranulin has anti-inflammatory properties [15]. Progranulin robustly suppresses chronic inflammation in mouse models of arthritis [13] and may have significant implications for treating progranulin-deficient inflammatory disease, such as FTD. In addition, progranulin acts on neurons to promote neurite outgrowth, a process that is regulated by TNFRs [10]. Understanding whether these actions of progranulin occur via the TNFRs or other receptors is important.

There is little information on receptors for granulins. Granulins act as cofactors for the binding of CpG oligonucleotides, common in bacterial DNA, to toll-like receptor 9 in the endolysosome [16], an event critical for initiating the innate immune response. Since the sequence homology of granulins is mostly conserved cysteines, (see Box 1), it will be interesting to see if all granulins bind the same receptor(s) or if different receptors exist for different granulins.

Circulating progranulin interacts with soluble binding partners (see [15] for a comprehensive table), including secretory leukocyte protease inhibitor (SLPI) [17]. SLPI binding to progranulin blocks elastase-mediated cleavage into granulins, resulting in blunted activation of cultured neutrophils and improved wound healing in *Slpi*^{-/-} mice [17]. These results are consistent with granulins' pro-inflammatory properties and with progranulin's ability to promote tissue repair. Recently, circulating progranulin was shown to exist as homodimers [18]. It remains to be determined how dimerization affects progranulin's binding to SLPI, susceptibility to cleavage, or overall function.

Progranulin Deficiency Causes Frontotemporal Dementia

FTD is the most common neurodegenerative disease in individuals under the age of 60 [19]. It typically strikes in late middle age and is distinct from Alzheimer disease and psychiatric disorders, although its symptoms are often confused with the latter. Affected individuals display personality, behavior, and/or language abnormalities associated with atrophy of the frontal and temporal brain lobes [20]. Memory is typically spared. Approximately 40% of cases have family histories with dominantly inherited genetic mutations that cause FTD; of these, approximately 5–20% are caused by progranulin gene (*GRN*) mutations [21].

To date, 69 *GRN* mutations, spanning the entire gene, have been found to cause FTD (see <http://www.molgen.vib-ua.be/FTDMutations>) [21]. *GRN* mutations are inherited in an autosomal dominant manner with high penetrance [1]. Most mutations result in a premature termination codon in one allele, causing the mutant mRNA to be targeted for nonsense-mediated decay and creating a functionally null allele [22]. Other mutations are predicted to produce a nonfunctional or unstable protein [22]. Therefore, progranulin-deficient FTD is a disease of haploinsufficiency caused by progranulin loss of function [22]. In mutation carriers, serum progranulin levels are often reduced by 70–80% compared with controls [23] rather than the 50% expected by loss of one allele, suggesting additional regulation of progranulin's expression. In human lymphoblasts, methylation of CpG units 1–2 kb distal to progranulin's transcription start site is inversely correlated with its expression, suggesting altered epigenetic modifications partially explains the lower-than-expected expression of progranulin in mutation carriers [24]. In agreement with this, *GRN* mRNA levels in fibroblasts from *GRN* mutation heterozygous carriers are less than 50% of control subjects. However, when reprogrammed to pluripotent stem cells, which removes most epigenetic modifications, *GRN* mRNA levels are restored to ~50% [25]. These latter studies suggest that epigenetic mechanisms influence progranulin levels in mutation carriers and that better understanding of these mechanisms might be useful for therapies aimed at raising their progranulin levels. Reduced progranulin levels may also be a risk factor for Alzheimer disease [26] and possibly autism [27], adding additional import to understanding epigenetic modifiers of progranulin expression.

Genetic modifiers of progranulin expression, which are possible targets for disease intervention, include several microRNAs and TMEM106b, a lysosomal transmembrane glycoprotein. A single nucleotide polymorphism (SNP) in the 3' UTR of progranulin affects a predicted binding site for miR-659 and alters gene expression [28]. Similarly, miR-29b [29] and miR-107 [30] also regulate progranulin expression. Genome-wide association studies

(GWAS) of progranulin mutation carriers identified SNPs in or near TMEM106b that impacted the age of onset of FTD [31, 32]. Specifically, a protective SNP, which correlated with a delayed age of onset and relatively mild symptoms in the small sample size of progranulin mutation carriers with this protective allele, was identified. The function of TMEM106b is unclear, but it may be required for endolysosomal homeostasis [33], which, as is discussed below, is an emerging theme in FTD research.

Neuropathological hallmarks of progranulin-deficient FTD include dystrophic neurites, inflammation, neuronal and microglial ubiquitin-positive inclusions, and severe neuron loss in the frontal and temporal lobes. Pathologic changes are also observed in the striatum, thalamus, substantia nigra, and hippocampus [20, 34]. Intracellular inclusions do not contain progranulin, but instead contain hyperphosphorylated, ubiquitinated, and proteolytically cleaved Tau DNA-binding protein-43 (TDP-43) [22]. TDP-43 is a highly conserved, ubiquitous DNA- and RNA-binding protein that binds many mRNAs, including *GRN*, and functions as a transcriptional repressor and inhibitor of exon splicing [35]. TDP-43 aggregates are also found in the motor neurons of amyotrophic lateral sclerosis (ALS) patients, and mutations in the TDP-43 gene *TARDBP* cause ALS, suggesting common pathological etiologies for FTD and ALS [35]. Indeed, progranulin mutations modify the course of ALS [36] and, in rare cases, cause ALS [37], and FTD and ALS both result in the loss of large neurons: von Economo neurons in the frontal lobes in FTD [38] and motor neurons in ALS [39].

Recently, two individuals with homozygous *GRN* mutations were reported. These individuals had undetectable levels of circulating progranulin, progressive vision loss, retinal dystrophy, ataxia, and seizures in their third decade of life [40]. Interestingly, while loss of one functional *GRN* allele leads to FTD, loss of both alleles results in neuronal ceroid lipofuscinosis (NCL). NCLs are a group of lysosomal storage disorders characterized by progressive degeneration of the brain and retina, with accelerated intracellular accumulation of lipofuscin [41], a lipid-containing pigment related to lysosomal degradation dysfunction and associated with aging. It remains to be seen whether progranulin-deficient NCL patients will also exhibit FTD-like pathology in their frontal and temporal lobes with age.

The link of progranulin to lysosomal disease suggests that progranulin and/or granulin may have a distinct function in the lysosomal compartment, possibly as activators of transporters of lysosomal enzymes [42]. This discovery therefore significantly altered the FTD field by implying that a key function of progranulin may reside in the lysosome. The notion that altered vesicle trafficking and protein homeostasis may be central to FTD pathogenesis is supported by the alterations observed in cells with mutations in two other genes that more rarely cause FTD [1]: charged multivesicular body 2B (CHMP2B) and valosin-containing protein (VCP). CHMP2B encodes a subunit of the ESCRTIII complex involved in endosomal trafficking and degradation, and mutations result in impaired fusion of endosomes with lysosomes [43]. VCP is an ATPase with multiple functions, including endoplasmic reticulum-associated degradation of misfolded proteins. VCP mutations result in vacuolization and impaired protein degradation, autophagy, and mitochondrial function [44]. The convergence of similar biological pathways underlying three genes associated with inherited FTD suggests a common mechanism, and therefore, a common therapeutic strategy may be possible.

Modeling CNS Progranulin Deficiency and FTD in Mice

Progranulin knockout (*Grn*^{-/-}) mouse models [45-49] accelerated our understanding of progranulin's function in the CNS. Each lab's mouse line exhibited abnormalities in social

behavior without gross deficiencies in overall health. In the brain, *Grn*^{-/-} mice exhibit pathology reminiscent of FTD, with gliosis in the cortex, hippocampus and thalamus, and accumulation of ubiquitin-positive aggregates [47-52]. Aggregates also contain lipofuscin [49] and phosphorylated TDP-43 [48, 52], although mislocalized TDP-43 has not been found in cortical neurons. Unlike human FTD patients, *Grn*^{-/-} mice do not show neuronal death and brain-mass loss [49]. However, *Grn*^{-/-} mice exhibit increased susceptibility to neuroinflammation and neuron loss after 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxin-induced injury [45], social deficits [47, 51, 52], impaired learning and memory in older (13–18 mo) mice [51, 52], depression-like behaviors [52], and anxiety alterations [46, 47, 52], consistent with various behavioral symptoms associated with human FTD. This modeling of FTD-like behavior defects is quite remarkable, given the considerable differences between the frontal lobes of mice and humans.

Interestingly, neuroinflammation and gliosis are absent in *Grn*^{+/-} mice up to 12 months of age, yet these animals still exhibit some social and emotional changes reminiscent of FTD as early as 6 months [53]. These changes may result from reduced neuronal activation in the amygdala, a region of the brain critical for fear and social behaviors [53]. Why loss of only one *GRN* allele results in robust neuropathological changes in humans, including neuroinflammation and neuronal death, yet only localized neuronal changes in mice, is unknown. Although the relationship of neuronal injury and neuroinflammation in FTD mouse models is unclear, these findings suggest neuronal effects of progranulin deficiency might be a primary cause of FTD, with increased inflammation as a secondary confounder.

Progranulin-Deficient Mice Also Exhibit Peripheral Phenotypes

In the periphery, *Grn*^{-/-} mice display alterations in bone, adipose tissue, and the immune system. Chondrocyte-specific knockdown of *Grn* leads to reduced cartilage mass and decreased femur growth-plate size [54], consistent with progranulin's function as a growth factor. As discussed in detail below, *Grn*^{-/-} mice are also protected from high-fat diet-induced obesity and insulin resistance [3]. In addition, *Grn*^{-/-} mice have increased susceptibility to collagen-induced arthritis [13], contact dermatitis [55] and infection with *Listeria monocytogenes* [48], providing evidence of multiple deficiencies in the immune response.

Progranulin Is Linked to Metabolic Diseases, including Type 2 Diabetes

Progranulin and metabolic disease were linked when individuals with visceral obesity and type 2 diabetes (T2D) were shown to have 1.4-fold increased serum progranulin levels [56]. Additionally, circulating progranulin levels were positively correlated with body mass index (BMI), fat mass, fasting glucose and insulin levels, and insulin resistance, a hallmark of T2D [57]. Circulating progranulin levels are higher in insulin-resistant, compared with insulin-sensitive, obese individuals [58], and, most recently, increased plasma progranulin levels were reported in individuals with T2D, as well as in obese individuals [2]. Other recent studies reported that circulating progranulin levels are independent of BMI and/or insulin, but these studies included subjects with complicating conditions, such as gestational diabetes [59], renal failure [60], or post-nutritional weight loss [61]. Thus, in the absence of specific comorbid conditions, circulating progranulin levels appear to be a useful biomarker for inflammation and T2D related to obesity. Further studies are needed to determine if measuring progranulin levels will be useful as a biomarker of metabolic syndrome.

In contrast to other studies showing progranulin has anti-inflammatory actions, recent studies in mice identified progranulin as a proinflammatory adipokine that is expressed in adipocytes and macrophages in white adipose tissue [3]. *Grn*^{-/-} mice consuming a high-fat diet (HFD) were protected from HFD-induced obesity, adipocyte hypertrophy, and insulin

resistance [3]. Knockdown of progranulin in 3T3-L1 adipocytes abolished TNF α -induced expression of IL-6, an inflammatory adipokine involved in the development of insulin resistance, and insulin resistance induced by systemic injections of recombinant murine progranulin was blocked by administering neutralizing antibodies against IL-6 [3]. Thus, progranulin may promote insulin resistance by increasing IL-6 expression.

Progranulin might also promote insulin resistance by directly inhibiting the insulin-signaling cascade. Treating 3T3-L1 adipocytes with exogenous progranulin inhibited insulin-induced phosphorylation of insulin receptor substrate (IRS)-1 and Akt in a dose-dependent manner, while knockdown of progranulin in 3T3-L1 adipocytes enhanced insulin sensitivity [3]. The phosphorylation of insulin receptor in response to insulin was not affected, suggesting that progranulin inhibits insulin signaling at an early step between insulin receptor phosphorylation and IRS-1 phosphorylation. Thus, progranulin is an important adipokine that promotes insulin resistance by increasing levels of IL-6 and inhibiting the insulin-signaling cascade [3].

However, the situation for progranulin and insulin resistance may be more complex. The studies in mice did not examine the effects of progranulin processing to granulin peptides, and so, it is unclear whether progranulin or granulins were responsible for the observed findings. For example, progranulin-cleaving proteases, such as elastase and proteinase 3, may cleave exogenously added progranulin, thereby shifting the progranulin/granulin balance toward pro-inflammatory granulins. In this case, granulins, rather than progranulin, would be pro-inflammatory under conditions of HFD-induced diabetes. Further studies are needed to unravel the relative roles of progranulin and granulins in obesity and insulin resistance.

Tissue Repair and Remodeling May Explain Progranulin's Role in Metabolic Disease

Why are progranulin levels elevated in obesity and T2D? What are the functional consequences of this elevation? Tissue repair and remodeling through inflammation and vascularization are central to metabolic disease. Chronic inflammation is widely recognized as a key consequence of obesity and T2D [62] and is characterized by increased secretion of cytokines and chemokines and the recruitment and activation of innate immune cells, such as monocytes and neutrophils to adipose tissue. As a result, obese and T2D individuals have increased plasma levels of pro-inflammatory markers, including C-reactive protein (CRP), TNF α , and IL-6 [63]. In addition, TNF α treatment increases progranulin mRNA levels in cultured macrophages [64] and adipocytes [3]. Therefore, the increase in circulating progranulin in individuals with obesity and T2D is likely a consequence of increased inflammatory adipokines and cytokines.

Whether the upregulation of progranulin promotes or quells obesity-induced inflammation is under debate. In support of the notion that progranulin suppresses the inflammatory response, progranulin has been reported to suppress arthritis in animal models [13]. Consistent with this idea, progranulin-deficient macrophages exhibit an exaggerated response to pro-inflammatory stimuli [45, 48]. Indeed, with the exception of obesity-induced insulin resistance, the bulk of evidence suggests progranulin is anti-inflammatory. However, in a chronic disease setting, progranulin may promote inflammation through monocyte recruitment. Progranulin promotes monocyte chemotaxis *in vitro* as effectively as the potent chemotactic molecule macrophage chemoattractant protein (MCP)-1, and its levels significantly correlate with macrophage infiltration of omental adipose tissue *in vivo* [56]. Thus, increased progranulin levels in obesity and T2D may contribute to the chronic inflammatory state by increasing macrophage recruitment to adipose tissue.

The upregulation of progranulin levels in obesity might also reflect tissue remodeling. Increased vascularization is a contributing factor to the growth and remodeling of adipose tissue in obesity [65]. Progranulin is detected in proliferating blood vessels [66], and transgenic mice overexpressing progranulin in endothelial cells exhibited vascular abnormalities in late gestation [67]. In cancers, progranulin stimulates the expression of vascular endothelial growth factor (VEGF) [68], a major angiogenic factor, although a similar effect was not observed in the mice with endothelial overexpression [67]. Finally, progranulin stimulates migration and invasiveness in breast cancer cells [68]. Since progranulin expression in adipose tissue positively correlates with BMI in humans [56] and is increased in a mouse model of diet-induced obesity [3], elevated progranulin levels may contribute to the development of obesity by stimulating angiogenesis and promoting growth of adipose tissue. Collectively, these studies suggest the elevated circulating progranulin levels in obese and diabetic states result from the upregulation of progranulin expression in response to adipose tissue repair and remodeling.

Progranulin Regulates Energy Balance—Further Links between Metabolism and the Brain?

Mice lacking progranulin are lean and protected from obesity, suggesting that progranulin also regulates energy balance. Although a small decrease was detected in respiratory exchange rate in *Gpn*^{-/-} mice during the dark cycle, overall, neither their food intake nor energy expenditure appears to differ from wild-type mice [3]. However, assessing energy balance in mice is challenging [69], and changes in energy expenditure might have been missed.

The hypothalamus coordinates glucose sensing and appetite control, and several hypothalamic cell types express progranulin, including neurons, microglia, and epithelial-like cells called tanycytes (specialized ependymal cells believed to transfer chemical signals from the CNS to the bloodstream). Administration of purified progranulin into the intraventricular space in mice suppressed nocturnal feeding, while injection of progranulin siRNA into the hypothalamus increased food intake and promoted weight gain [70]. Interestingly, dysregulated feeding behavior, such as overeating and a preference for sweets, together with concomitant hypothalamic atrophy, is commonly noted in FTD patients [71]. Mechanistically, progranulin upregulated the anorexigenic factor proopiomelanocortin and downregulated the orexigenic factors neuropeptide Y and Agouti-related peptide; based on these data, Kim *et al.* concluded that progranulin in the hypothalamus acted as an anorexigenic factor that decreased appetite in the fed state. However, the progranulin concentrations that elicited effects are 3,000–10,000 times higher than those reported in human CSF. Further, since food intake was not altered in *Gpn*^{-/-} mice fed a regular or high-fat diet [3], it remains unclear whether progranulin plays a role in the physiological control of food intake. Adding to the uncertainty, human studies of progranulin and energy balance are limited, but surprisingly, circulating progranulin levels may positively predict resting metabolic rate when normalized for body weight [57].

Progranulin May Modulate Processes Involved in Atherosclerosis

Recent studies indicate a functional link of progranulin with atherosclerosis, another metabolic disease involving the inflammatory process. Atherosclerosis results from the accumulation of cholesterol in macrophages of the arterial wall that leads to formation of atherosclerotic plaques. Critical to the initiation and progression of atherosclerosis are inflammation and proliferation and migration of vascular smooth muscle cells (VSMC) toward the intima, the innermost arterial layer. Progranulin modulates both of these processes. Indeed, progranulin has been detected in human atherosclerotic plaques by

immunohistochemistry, specifically in the VSMC of the intima and in macrophages [72]. Moreover, progranulin modulates migration of human aortic smooth muscle cells in culture, suggesting its trophic properties may influence atherosclerotic plaque formation [72]. Full-length progranulin is expected to be anti-atherogenic whereas granulins might be pro-atherogenic, owing to their purported respective anti- and pro-inflammatory properties. Indeed, recently progranulin deficiency was shown to exacerbate atherosclerosis in apolipoprotein (apo-) E knockout mice fed a high fat diet, with increased inflammatory cytokine production and enhanced cholesterol accumulation in macrophages [73]. Interestingly, in humans, elevated serum progranulin levels were reported to be an independent positive predictor of atherosclerosis, based on measurements of carotid intima-media thickness [74].

Progranulin might also affect plasma lipoprotein metabolism and atherosclerosis through sortilin [6]. Sortilin reduces circulating LDL levels by both increasing sortilin-mediated uptake and catabolism of LDL [75, 76] and decreasing secretion of apoB-containing very-low-density lipoprotein (VLDL) [76, 77]. It remains to be determined whether progranulin binding to sortilin has functional consequences for LDL metabolism.

High plasma levels of high-density lipoproteins (HDL) are inversely correlated with atherosclerosis, and progranulin is a component of human HDL as shown by binding the major HDL protein apo-A-I [64]. Moreover, in cultured macrophages, HDL blocked cleavage of progranulin into granulins and inhibited granulin-mediated inflammation. These findings sparked considerable interest since HDL's beneficial effects are believed to be, in part, due to its anti-inflammatory properties [78]. However, subsequent studies using several methods of HDL isolation [18] and two proteomic studies [79, 80] did not detect progranulin in human or mouse HDL. Thus, a direct link of progranulin to HDL seems unlikely.

Progranulin and Therapeutics

There is considerable interest in therapeutic approaches focused on increasing progranulin levels in FTD linked to progranulin haploinsufficiency [81, 82]. Additionally, if progranulin suppresses neuroinflammation, therapies that increase levels of progranulin in the CNS might be useful for several neurodegenerative diseases. Table 1 lists compounds and approaches that increase progranulin levels *in vitro* or, based on experimental evidence, are likely to do so.

In theory, restoring progranulin levels in the brain by increasing expression from the remaining functional allele or promoting progranulin secretion should provide therapeutic benefit for progranulin-deficient FTD and possibly other neurodegenerative diseases characterized by impaired tissue remodeling or inflammation. However, therapies aimed at increasing progranulin levels should be approached cautiously as, in addition to promoting insulin resistance in obesity [3], progranulin can promote tumor growth [4] (see Box 2). Furthermore, our understanding of the relationship between anti-inflammatory progranulin and its processing to the pro-inflammatory granulins remains limited. If FTD and associated neuroinflammation exacerbates the cleavage of progranulin into granulins, and granulins promote disease via their pro-inflammatory properties, then increasing progranulin levels could paradoxically be counter-therapeutic. A better understanding of the relationship between progranulin and granulin biology, and between progranulin and angiogenesis, remains an important challenge.

Concluding Remarks and Future Perspectives

Progranulin's importance in neurodegeneration is well established, but the links of progranulin to diseases of metabolism and inflammation are more recent, and information is

limited. Paradoxically, progranulin suppresses neuroinflammation and neuronal death in the CNS and appears to suppress atherosclerosis, but is apparently a pro-inflammatory molecule in the context of diet-induced obesity (see Box 3). Resolving this paradox will require a better understanding of progranulin's tissue- and cell-specific actions, its proteolytic processing, and its receptor(s) and signaling pathways. In particular, further unraveling of progranulin and granulin peptides effects on growth and inflammation and as modulators of tissue repair in response to injury may provide a useful framework for unraveling the complex biology of progranulin. Such insights into the basic biology of progranulin will undoubtedly help to guide therapeutic strategies.

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GLOSSARY

Adipokine	A signaling molecule secreted by adipose tissue that acts on the immune system.
Atherosclerosis	A progressive disease, with a strong inflammatory component, in which an artery wall thickens as a result of the accumulation of lipids and cell proliferation.
Body mass index (BMI)	An individual's body mass divided by the square of their height. Obesity is often defined as BMI ≥ 30. In general, obese BMI values are associated with an increased risk of disease.
C-reactive protein (CRP)	A serum protein synthesized and secreted by the liver in response to inflammatory molecules released by macrophages and adipocytes. It functions during the acute phase response to activate the complement system.
Energy balance	The biological homeostasis of energy in living systems measured by the equation Energy intake = Internal heat produced + External work + Energy stored.
Frontotemporal dementia (FTD)	A group of complex neurodegenerative diseases characterized by progressive deterioration of the frontotemporal lobes resulting in behavioral and/or speech changes. FTD generally begins in the mid-50s and is more common than Alzheimer disease in this age group, with a prevalence of 3–15 cases per 100,000. Patients with pathogenic mutations in one copy of progranulin develop FTD.
Gliosis	The proliferation of glial cells (microglia, astrocytes, and oligodendrocytes) in response to brain insults. Gliosis is believed to have both beneficial and destructive consequences. FTD patients exhibit pronounced gliosis.
Hypothalamus	A region in the ventral part of the brain, above the brain stem, that coordinates with the endocrine system to regulate and/or respond to hunger, thirst, body temperature, sleep, circadian cycles, and fatigue.

Microglia	Resident immune cells of the brain, analogous in function to their peripheral counterparts, macrophages. Microglia, oligodendrocytes, and astrocytes are the main glial cell types in the brain.
Neuronal ceroid lipofuscinosis (NCL)	A group of neurodegenerative disorders characterized by the accumulation of oxidized lipids and proteins in the brain, liver, spleen, and kidneys. Patients with pathogenic mutations in two copies of progranulin develop NCL.
Secretory leukocyte protease inhibitor (SLPI)	An inhibitor of elastase, a protease that cleaves progranulin into granulins. When progranulin is complexed with SLPI, proteolytic processing by elastase is prevented.
Sortilin	A transmembrane sorting receptor mainly expressed in the nervous system that mediates trafficking of numerous unrelated neuropeptides, including progranulin, to endosomes and lysosomes.
Type 2 diabetes (T2D)	Also known as adult-onset diabetes; a common metabolic disorder characterized by high blood glucose levels in the context of insulin resistance and relative insulin deficiency.

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BOX 1

Background on Progranulin

Progranulin is a secreted, ~80-kDa glycoprotein expressed in many cell types, including epithelial cells, neurons, myeloid cells, immune cells, and adipocytes [83]. Progranulin's broad expression profile suggests a role in basic cellular functions, such as survival and proliferation. Indeed, it promotes motor neuron survival and neurite outgrowth, tumor cell growth, wound healing, vascularization, and cell migration [42]. Progranulin levels in human and murine plasma are ~200 ng/ml [23, 56] and ~550 ng/ml [45], respectively. In cerebrospinal fluid (CSF), its concentration is much lower: ~6 ng/ml [84, 85] in humans and ~2 ng/ml in mice [86]. Although progranulin is expressed by most neuronal populations in the brain [9, 87], neurons of the frontal and temporal lobes appear to be most sensitive to alterations in its expression, as these are the regions primarily affected in FTD. The expression of progranulin is dramatically upregulated in microglia after injury and during repair [30, 45, 88, 89], underscoring progranulin's involvement in the inflammatory response. In adipose tissue, progranulin is expressed by both adipocytes and cells of the stromal vascular fraction [3] and acts as an adipokine.

The *GRN* gene (which encodes progranulin) contains 12 coding exons and is translated into a protein containing 7.5 tandem repeats called granulins (see Figure I). The granulin repeat is structurally defined by the cysteine-rich sequence X₂₋₃CX₅₋₆CX₅CCX₈CCX₆CCDXXXHCCPX₄CX₅₋₆CX^[90]. The divergent linker sequences that separate granulin repeats can be cleaved by extracellular and intracellular proteases, including neutrophil elastase, proteinase 3, ADAMTS-7, and matrix metalloproteinase 12 (MMP-12), to release individual ~6-kDa granulins or, possibly, linked combinations of granulins^[91]. Progranulin lacks clear consensus sequences for protease cleavage, suggesting a susceptibility to cleavage by multiple proteases.

Unraveling the relative functions of progranulin and granulins has so far been challenging, and most studies have not addressed this distinction. Is progranulin the main actor, or a precursor protein for potent granulin peptides? Both full-length progranulin and individual granulins exist *in vivo* [4] and are anti-inflammatory [13] and pro-inflammatory, respectively, suggesting opposing functions [90]. The processing of progranulin to granulins resembles that of prosaposin and saposins. Saposins are 8–11 kDa polypeptides that activate lysosomal enzymes, and are processed from the precursor prosaposin [42]. Although the absence of granulin-specific reagents has limited our understanding of the biological significance of progranulin cleavage, new techniques that make tissue-specific profiling of granulin species possible [92] will shed light on their functional importance.

BOX 2

Progranulin and cancer

Early work demonstrated progranulin's ability to stimulate cell division in cultured cell lines and promote anchorage independent growth [90]. For example, knockdown of progranulin expression reduced tumorigenicity of a breast cancer-derived cell line, and overexpression of progranulin conferred robust tumor formation properties onto previously weak tumor forming cells [4]. Subsequent studies identified progranulin's role as an enhancer of key steps in tumorigenesis, including proliferation, survival, migration, invasion and angiogenesis [4]. High levels of progranulin correlate with increased tumor severity, increased risk of reoccurrence, and chemoresistance, making progranulin expression a potentially valuable prognostic indicator [90]. Indeed, progranulin neutralizing antibodies are in preclinical development for the treatment of breast and lung cancer (www.agpharma.com). Progranulin's role in the progression of cancer must be carefully considered when evaluating potential progranulin-elevating therapeutics for the treatment of progranulin-deficient FTD (see Table 1). Ideal candidates might restore progranulin to normal levels.

BOX 3

Outstanding questions

- What are the molecular regulators of progranulin and granulins? What are their respective receptors and signaling pathways and how do they interact?
- What are the relative functions of progranulin versus granulin peptides? Do different granulins have similar or opposing activities?
- Is the cleavage of progranulin into granulins altered in disease states?
- Is progranulin's primary FTD-relevant function in neurons or microglia? Similarly, is progranulin's primary metabolic disease-relevant function in adipocytes, monocytes, endothelial cells, or vascular smooth muscle cells?
- How does progranulin regulate energy balance in the CNS?
- Which of progranulin's functions (e.g., trophic factor, anti-inflammatory molecule, granulin precursor, growth factor) are key to the development of neurodegenerative disease or metabolic disease?

Highlights

- Progranulin is a secreted glycoprotein found in plasma and CSF
- Progranulin has growth factor-like and inflammation-related properties
- Progranulin deficiency causes a neurodegenerative disease – frontotemporal dementia
- Obese and type 2 diabetic individuals have increased serum progranulin levels
- *Grn*^{-/-} mice are protected from diet-induced obesity and insulin resistance

CONSEQUENCES OF REDUCED PROGRANULIN LEVELS
Cause NEUROLOGICAL DISEASE	Modulate susceptibility to METABOLIC DISEASE
	Affected Cell types: Neurons and microglia	Adipocytes and macrophages
	<ul style="list-style-type: none"> • Heterozygous deficiency causes frontotemporal dementia (FTD) • Homozygous deficiency causes neuronal ceroid lipofuscinosis (NCL) • Reduced progranulin levels might be a risk factor for Alzheimer disease (AD) 	<ul style="list-style-type: none"> • Homozygous deficiency protects against diet-induced obesity and insulin resistance in mice • Homozygous deficiency promotes atherosclerosis in genetic models

Figure 1. Genetic deficiency of progranulin influences neurological and metabolic diseases
Decreased progranulin levels cause neurodegenerative disease and exacerbate atherosclerosis, yet protect against some aspects of metabolic disease. Patients with loss of function mutations in one progranulin allele develop frontotemporal dementia, while loss of both progranulin alleles leads to neuronal ceroid lipofuscinosis. Further, reduced progranulin levels may also be a risk factor for Alzheimer disease. Conversely, deletion of progranulin in normal mice protects against diet-induced obesity. However, loss of progranulin in mice that lack apo-E promotes the development of atherosclerosis. Thus, loss of progranulin is apparently disease-causing in the brain and has mixed effects on metabolic disease in the periphery. Dysregulation of progranulin-mediated cell growth and inflammation may be involved in the pathogenesis of both subsets of disease.

Figure I. (for Box 1). Overview of progranulin biochemistry

Progranulin is composed of 7.5 highly homologous, cysteine-rich repeats called granulins, shown in blue, that are separated by linker regions, shown in white, which share less sequence homology. A signal sequence, shown in red, is required for secretion. Progranulin can be cleaved into granulins by proteases, including neutrophil elastase, proteinase 3, MMP-12, ADAMTS-7, and possibly others. Cleavage can be prevented by binding to the protease inhibitor secretory leukocyte protease inhibitor (SLPI). Progranulin's reported cell-surface receptors include sortilin and TNF receptors. The binding of progranulin to TNFRs is controversial (see text). Granulin receptors are unknown.

TABLE 1
Compounds or approaches with potential therapeutic utility by increasing progranulin levels or actions

Selected references are provided where applicable. These strategies are mostly directed toward FTD and neuroinflammation. Therapeutic strategies to lower progranulin are not available.

Drug or Approach	Known or proposed activity and use	Known or (proposed) effect on target	Cell and/or tissue type; organism	Ref
microRNA knockdown (miR-29b)	miR-29b is one of a cluster of 4 closely miRs which regulate diverse cellular processes. Reduction of miR-29b increased <i>GRN</i> mRNA in cultured cells	Increased progranulin protein	HEK293 and 3T3 cells; human	[29]
Progranulin C-terminal peptide	Progranulin's C-terminal 3 residues are required to bind sortilin. Circulating progranulin levels increase in sortilin-deficient mice. Exogenous peptide containing progranulin's C-terminus could compete for sortilin binding.	Increased circulating progranulin (<i>proposed</i>)	Neurons; human (<i>proposed</i>)	[5, 6]
Protein disulfide isomerase 4 (PDI4) overexpression	PDI4 is an ER-chaperone required for disulfide-mediated protein folding. Overexpression increases secreted progranulin in cultured cells	Increased progranulin protein	HEK293 cells; human	[8]
Recombinant progranulin (by pump)	Direct replacement of purified progranulin protein	Increased progranulin protein (<i>proposed</i>)	Nervous system; human (<i>proposed</i>)	n/a
Suberoylanilide hydroxamic acid (SAHA) or other HDAC inhibitors	SAHA is a histone deacetylase used to treat cancer. It upregulated progranulin expression in <i>GRN</i> ^{+/-} patient cells to nearly wild-type levels.	Increased <i>GRN</i> mRNA	N2A cells and primary dermal fibroblasts from progranulin mutation carriers; human and mouse	[82]
Upregulation of vacuolar ATPase	Selective inhibitor of vacuolar ATPase in cultured cells increased progranulin via a translational mechanism independent of lysosomal degradation, autophagy, or endocytosis	Increased progranulin secretion	HeLa and HEK293T cells and MEFs; human and mouse	[81]
Viral gene replacement therapy (<i>GRN</i> expression)	Increased expression of progranulin by introduction of exogenous transgene	Increased <i>GRN</i> mRNA	Primary neuronal and microglial cultures; mouse	n/a
WNT antagonists	The WNT signaling pathway is upregulated in progranulin-deficient human cells, mice and worms.	Block upregulation of WNT signaling pathway (<i>proposed</i>)	Nervous system; human (<i>proposed</i>)	[93]