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Invited review

Approaches to develop therapeutics to treat frontotemporal dementia

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HIGHLIGHTS

- Frontotemporal dementia is the second prevalent dementia after Alzheimer's disease.
- GRN mutations leading to PGRN deficiency are linked to frontotemporal dementia.
- PGRN deficiency causes lysosome defects, neurodegeneration, and neuroinflammation.
- Efforts to raise PGRN levels are a therapeutic strategy for frontotemporal dementia.

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ABSTRACT

Frontotemporal degeneration (FTD) is a complex disease presenting as a spectrum of clinical disorders with progressive degeneration of frontal and temporal brain cortices and extensive neuroinflammation that result in personality and behavior changes, and eventually, death. There are currently no effective therapies for FTD. While 60–70% of FTD patients are sporadic cases, the other 30–40% are heritable (familial) cases linked to mutations in several known genes. We focus here on FTD caused by mutations in the *GRN* gene, which encodes a secreted protein, progranulin (PGRN), that has diverse roles in regulating cell survival, immune responses, and autophagy and lysosome function in the brain. FTD-linked mutations in *GRN* reduce brain PGRN levels that lead to autophagy and lysosome dysfunction, TDP43 accumulation, excessive microglial activation, astrogliosis, and neuron death through still poorly understood mechanisms. PGRN insufficiency has also been linked to Alzheimer's disease (AD), and so the development of therapeutics for *GRN*-linked FTD that restore PGRN levels and function may have broader application for other neurodegenerative diseases. This review focuses on a strategy to increase PGRN to functional, healthy levels in the brain by identifying novel genetic and chemical modulators of neuronal PGRN levels.

1. Introduction

Frontotemporal dementia (FTD) is the most common age-related dementia in people under 60 years of age (Rabinovici and Miller, 2010; Rademakers et al., 2012; Riedl et al., 2014; Greaves and Rohrer, 2019). The clinical presentation of FTD is distinct from Alzheimer's Disease (AD), the most common age-related dementia, in that there is a major impairment of executive functioning in FTD rather than an impairment of memory functions as in AD. At onset, FTD patients can show changes in personality and social conduct, exhibited by social disinhibition and distractibility. Patients normally show lapses in judgment, loss of insight, and cognitive deficits in attention, abstraction, planning, and

problem solving and deficits in both expressive and receptive language.

The brains of FTD patients show loss of neurons in the frontal and temporal lobes. Spindle neurons, important for social behavior, are especially vulnerable (Seeley et al., 2006, 2007). The FTD brain can be classified by abnormal deposits of TAR DNA-binding protein 43 (TDP43) and tau. The most prevalent genetic cause of FTD is an abnormal hexanucleotide repeat expansion (HNE) in *C9ORF72* which is also associated with TDP43 pathology and comprises about 25% of familial FTD cases (Van Langenhove et al., 2012). The other frequent genetic causes of FTD are due to mutations in the microtubule associated protein tau (*MAPT*) gene that encodes tau (5–20% of familial cases) and result in abnormal tau deposits, or the *GRN* gene that

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encodes the progranulin (PGRN) protein (5–20% of familial cases) and result in PGRN insufficiency, and TDP43 pathology (Rademakers et al., 2012; Ghidoni et al., 2012; Baker et al., 2006; Cruts et al., 2006). PGRN has been shown to reduce TDP43 phosphorylation and pathology, so PGRN insufficiency may exacerbate TDP43 pathology in FTD (Beel et al., 2018; Chang et al., 2017; Fujita et al., 2018; Wils et al., 2012; Yin et al., 2010a, 2010b). The remaining familial FTD cases are linked to less frequent mutations in a handful of genes, including valosin-containing protein (*VCP*; ~1%), sequestosome 1 (*SQSTM1*; < 1%), ubiquilin 2 (*UBQLN2*; < 1%), optineurin (*OPTN*; < 1%), coiled-coil-helix-coiled-coil-helix domain containing 10 (*CHCHD10*; < 1%), TANK binding kinase 1 (*TBK1*; 1%–3%), charged multivesicular body protein 2B (*CHMP2B*; < 1%), and dynactin 1 (*DCTN1*; < 1%). We will focus this review on GRN-linked FTD (Ferrari et al., 2019; Deleon and Miller, 2018). Interestingly, a recent meta-analysis suggests that there may be gender differences in FTD, where there was a higher prevalence of *GRN* mutations in female FTD cases (Curtis et al., 2017). There were no sex differences observed for *C9ORF72*-related or *MAPT*-related FTD.

PGRN insufficiency may have a broader role in FTD beyond those patients with mutations in the *GRN* gene. A subset of FTD cases are linked to mutations in the *MAPT* gene, which encodes the protein tau, and are considered tauopathies as they result in the aggregation of phosphorylated tau (p-tau) forms that drive neurodegeneration (Fujita et al., 2018; Gasparini et al., 2007). Interestingly, PGRN levels can modulate the extent of the tauopathy: Hosokawa et al. (2015, 2017, 2018) showed that a transgenic mouse model of FTD expressing P301L tau (*MAPT P301L*) exhibited increased p-tau, neuropathology and cognitive deficits and that lowering PGRN levels in brain further exacerbated the tauopathy by augmenting the already increased levels of p-tau in brain (Hosokawa et al., 2015, 2018). Similarly, when the levels of a mutant form of tau (P301L tau) associated with increased risk of FTD were increased in the brains of *Grn*^{−/−} mice by AAV injection, there was greatly enhanced expression of p-tau compared to similar AAV injection into the brains of control mice (Takahashi et al., 2017). This suggests that PGRN deficiency results in increased expression of a highly pathogenic forms of tau believed to cause neurodegeneration in FTD.

PGRN insufficiency has also been linked to AD, a tauopathy that also presents with TDP43 pathology; 50% of AD patients have TDP43 pathology (Josephs et al., 2014; Wilson et al., 2011). A SNP (rs5848) in the *GRN* gene that leads to reduced PGRN levels is linked to increased risk of AD (Chen et al., 2015; Hsiung et al., 2011; Kamalainien et al., 2013; Perry et al., 2013; Xu et al., 2016). Reduced PGRN levels exacerbate pathologies in AD mouse models, including impaired microglial function and increased toxic accumulation of amyloid beta and tau proteins (Hosokawa et al., 2015, 2017, 2018; Minami et al., 2014; Pereson et al., 2009). Genetically increasing the levels of PGRN in mouse models of AD suppresses neuroinflammatory-, pathological-, and cognitive-based disease phenotypes (Hosokawa et al., 2015; Minami et al., 2014; Pereson et al., 2009).

In the brain, PGRN is expressed primarily by neurons and microglia, is involved in neuronal survival, neurite outgrowth, and synaptogenesis, and is a key regulator of neuroinflammation consistent with its neuroprotective role in models of FTD and AD (Ahmed et al., 2007; Cenik et al., 2012; Chitramuthu et al., 2010; Gass et al., 2012; Petkau and Leavitt, 2014; Petkau et al., 2012; Petoukhov et al., 2013). Little is known about how PGRN deficiency leads to neurodegeneration in FTD and AD. Complete loss of PGRN in the brain causes neuronal ceroid lipofuscinosis, a lysosomal storage disorder, characterized by severe lysosome dysfunction, and the abnormal accumulation of lipofuscin, a lipid-rich lysosome degradation residue associated with cell senescence, aging, and neurodegenerative diseases (Gotzl et al., 2014; Smith et al., 2012). Haploinsufficiency of PGRN in mouse models of FTD causes lysosomal defects leading to neurodegeneration and social behavior dysfunction. This suggests a key role for PGRN in lysosome function (Ahmed et al., 2010; Evers et al., 2017; Tanaka et al., 2017).

2013a, 2013b, 2014, 2017; Ward, 2017).

PGRN has also been suggested to regulate autophagy, a key cellular pathway directly linked to lysosomes and involved in the clearance of misfolded disease-causing proteins such as p-tau and TDP43. PGRN deficiency leads to dysregulated autophagy and the subsequent accumulation of toxic forms of TDP43 in neurons (Beel et al., 2018; Chang et al., 2017). Increasing PGRN levels reduced insoluble TDP43 levels *in vitro* (Beel et al., 2018; Chang et al., 2017) and corrected lysosomal deficits and reversed behavioral deficits in a mouse model of FTD (Arrant et al., 2017). Thus, reduced levels of PGRN that may occur in FTD and AD may impair protein clearance, causing a build-up in CNS of p-tau and TDP43, that are linked to neurodegeneration. This suggests that approaches to increase PGRN levels in brain to compensate for insufficiency could be therapeutically relevant in diminishing neurodegeneration in FTD and possibly AD.

However, PGRN is a protein that is unlikely to effectively cross the blood brain barrier to enter the brain, limiting its therapeutic use. Approaches to identify genetic modifiers of PGRN levels, that either increase *GRN* transcription, translation or processing, could provide a unique strategy to develop small molecule drugs that could increase PGRN levels in brain to treat FTD and AD. This review focuses on mechanisms that regulate PGRN levels in different brain cells, how PGRN regulates the clearance of disease-causing proteins from neurons to impact neurodegeneration and the identification of potential genetic modifiers of PGRN levels that could be translated into the development of novel small molecule regulators of PGRN levels to treat FTD and possibly other neurodegenerative diseases.

2. Cell type selective deficits of PGRN in FTD and disease

PGRN may exert different cell type-specific roles in FTD. For example, selective knockout of *Grn* in neurons in mice can induce changes in social dominance, a behavioral phenotype of FTD patients (Arrant et al., 2017). In contrast, selective knockout of *Grn* in microglia increases self-grooming, an OCD-like behavior in mice, and FTD patients with *GRN* mutations exhibit a similar behavior (Krabbe et al., 2017). The social dominance behavior deficits present in mice with selective neuronal *Grn* knockout were not observed in mice with depleted PGRN in microglia suggesting the behavioral abnormalities may have a cell autonomous basis.

Complete knockout of *Grn* in mice that induces OCD-like behavior can induce neuronal hyperactivity both in neurons in the nucleus accumbens (Krabbe et al., 2017), an area implicated in development of OCD and in thalamocortical neurons (Lui et al., 2016). In *Grn*^{−/−} mice, pathology, including microgliosis, astrogliosis and deposits of lipofuscin first occur in the thalamus before spreading to other regions of the brain (Wils et al., 2012; Yin et al., 2010a; Petkau et al., 2012, 2016; Ahmed et al., 2010; Ghoshal et al., 2012). The hyperexcitability in the thalamocortical neurons was associated with an increase in synaptic pruning that has been shown to produce a neurodegenerative phenotype in which inhibitory synapses in the ventral thalamus were eliminated (Lui et al., 2016). The hyperexcitability resulting from *Grn* knockout was related to microglia activation and an increase in innate immunity gene expression in microglia. Blocking complement production or reducing TNFα expression, a proinflammatory cytokine, abolished excessive self-grooming and the associated hyperexcitability in *Grn*^{−/−} knockout mice suggesting that PGRN in microglia normally suppresses microglial activation while loss of PGRN results in excessive microglial activation and neuronal hyperactivity which may be linked to neurodegeneration in FTD (Krabbe et al., 2017; Lui et al., 2016).

A number of studies have suggested neuronal hyperactivity may play a key role in neurodegeneration in FTD and AD (Vossel et al., 2013). Patients with mild cognitive impairment in the early stages of AD display hippocampal hyperactivity and epileptiform activity years before overt cognitive impairment or neuronal loss is detected (Vossel et al., 2013). Murine models of AD also show epileptic activity leading

to learning and memory loss prior to overt pathology (Sanchez et al., 2012). Furthermore, human iPSC-derived neurons from patients with FTD show hyperactivity that precedes neuronal death (Imamura et al., 2016). The potential role of early hyperactivity in neurodegeneration and disease progression is the reason why anti-epileptic agents are being tested for efficacy in patients with mild cognitive impairment (Sanchez et al., 2012).

While PGRN in neurons and microglia may contribute to different aspects of FTD, the role of neuronal and microglial PGRN in disease pathology is still unclear. Selective deletion of PGRN in either neurons or microglia produces little of the neuropathology seen in null *Grn*^{-/-} mice (Arrant et al., 2017; Petkau et al., 2017a, 2017b). Furthermore, patients with *GRN* mutations show heterogeneous loss of PGRN in brain, with levels of the protein decreased in unaffected brain regions, such as cerebellum and occipital cortex, while PGRN levels appear normal in brain regions showing neuronal loss, such as the frontal and temporal cortices (Chen-Plotkin et al., 2010). This may be due to microglia infiltration to those regions of the FTD brain most affected by disease suggesting that the PGRN in the infiltrating microglia may compensate for the loss of PGRN caused by neuronal death. Interestingly, Arrant et al. (2018) reported that expression of PGRN in the brains of *Grn*^{-/-} mice using an AAV delivery system reduced microgliosis and microglial activation and the PGRN was mainly delivered to neurons (Arrant et al., 2018). This suggests that PGRN secreted by neurons may keep microglia in check to reduce neurodegeneration and approaches to increase PGRN expression in neurons may be potentially useful in treating FTD. A better understanding of the mechanisms that control PGRN levels may be useful in developing such therapeutic approaches.

3. Regulation of PGRN levels

3.1. Regulation of PGRN uptake into cells

PGRN is a secreted protein and extracellular PGRN is neuroprotective (Elia et al., 2019). Multiple mechanisms can control the levels of extracellular PGRN including its uptake, degradation and secretion that can impact its protective function.

Despite being a protein, PGRN can be taken up into cells via an active process involving the neuronal cell membrane receptor sortilin. Sortilin is a lysosomal sorting receptor that traffics proteases to the lysosome (Canuel et al., 2008). It also regulates the uptake of extracellular PGRN and delivers it to lysosomes (Hu et al., 2010; Zheng et al., 2011). Reducing sortilin levels in neurons increases levels of extracellular PGRN and we showed that knocking down sortilin expression in the neuron-like Neuro2a (N2a) cells resulted in increased extracellular PGRN (Elia et al., 2019). Importantly, polymorphisms in the sortilin gene (*SORT1*) increase the risk of FTD (Philtjens et al., 2018) and polymorphisms near *SORT1* in FTD patients have been shown to increase *SORT1* gene expression and reduce extracellular levels of PGRN (Carrasquillo et al., 2010) suggesting that altered uptake of PGRN may be linked to FTD.

Prosaposin (PSAP) is a precursor of lysosomal saposin peptides, which are activators of lysosomal sphingolipid metabolizing enzymes, and recent work has shown that PSAP, via mannose-6-phosphate receptor (M6PR) and low density lipoprotein receptor-related protein 1 (LRP1), can facilitate the lysosomal trafficking of PGRN in both the biosynthetic and endocytic pathways (Zhou et al., 2015). PGRN directly interacts with PSAP via the granulin D and E motifs and the linker region between saposin B and C, and itself reciprocally facilitates PSAP trafficking to lysosomes (Zhou et al., 2017a, 2017b). Interestingly, a genome-wide association study (GWAS) to find novel regulators of PGRN levels identified the *PSAP* locus as significantly associated with plasma PGRN levels in humans (Nicholson et al., 2016).

3.2. PGRN and lysosomal function

Once it is taken inside cells, PGRN localizes to lysosomes and can play a direct role in the function of these organelles (Beel et al., 2017; Chang et al., 2017; Evers et al., 2017; Liu et al., 2015; Tanaka et al., 2013a, 2013b, 2014, 2017). Tanaka et al. (2017) suggested that PGRN regulates lysosome acidification and PGRN may directly bind to and modulate lysosomal enzymes, such as cathepsin D (Beel et al., 2017). Furthermore, lysosome activity regulates PGRN levels since inhibition of lysosome function with bafilomycin A1 increased PGRN levels in neuronal cells (Capell et al., 2011, 2014). This suggests that PGRN may have a reciprocal relationship with lysosomes: it can affect lysosome activity and, in turn, lysosomes can regulate PGRN levels by degrading the protein.

The relationship between PGRN and lysosomal function may have relevance in FTD since the neuropathology of FTD has been linked to lysosomal dysfunction (Ward et al., 2017). Increased lipofuscinosis, indicative of lysosome dysfunction, and other lysosomal abnormalities were found in postmortem cortex of FTD patients with heterozygous *GRN* mutations. In FTD patients, noninvasive retinal imaging revealed preclinical retinal lipofuscinosis in heterozygous *GRN* mutation carriers (Ward et al., 2017). Lymphoblasts from heterozygous *GRN* mutation carriers also accumulated prominent amounts of lipofuscin, which could be rescued by normalizing PGRN expression (Ward et al., 2017). Fibroblasts from heterozygous *GRN* mutation carriers also showed impaired lysosomal protease activity suggesting that FTD may be due to PGRN insufficiency associated with lysosomal dysfunction.

Mouse models of FTD (*Grn*[±] mice) also show lysosomal dysfunction in brain (Ahmed et al., 2010; Arrant et al., 2017, 2018). In fact, increasing brain PGRN levels *in vivo* in *Grn*[±] mice reversed the lysosomal neuropathology and social behavior deficits (Arrant et al., 2017b, 2018). We showed that reducing PGRN levels in mouse cortical neurons either by knockdown using *Grn* siRNA or in neurons from PGRN-deficient (*Grn*^{R493X/+}) mice, resulted in an increase in the size and number of lysosomes, which usually indicates dysfunction (Elia et al., 2019). Furthermore, PGRN-deficient lysosomes imaged by electron microscopy appear overloaded with aggregated undigested material suggesting impaired function, and iPSC-derived human cortical neurons from FTD patients harboring the *GRN*^{R493X/+} mutation also display enlarged lysosomes (unpublished results).

PGRN deficiency also leads to lysosome dysfunction in microglia, with both cell autonomous and nonautonomous consequences (Gotzl et al., 2018, 2019; Lui et al., 2016; Tanaka, 2017). Götzl et al. (2018) showed that loss of PGRN led to altered lysosome cathepsin levels and maturation in microglia which impaired lysosome function in that cell type but triggered enhanced lysosomal cathepsin maturation in other brain cell types (Gotzl et al., 2018). In addition, Götzl et al. (2019) showed that PGRN deficiency in microglia caused these cells to adopt a neurodegenerative disease-associated activation state compared to triggering receptor expressed on myeloid cells 2 (TREM2)-deficient microglia which adopted a homeostatic activation state, with the PGRN deficient microglia displaying altered phagocytosis, migration, and clustering around amyloid plaques (Gotzl et al., 2019). Finally, Lui et al., 2016 showed that PGRN deficiency led to lysosome defects and altered microglial complement production and activation that led to aberrant synaptic pruning in neurons and induced OCD behavior in mice (Lui et al., 2016). These findings indicate that a common neuropathological defect resulting from loss of PGRN in FTD patients and in FTD models is lysosomal dysfunction, with different functional consequences occurring in microglia vs. neurons.

3.3. PGRN and autophagy

In addition to directly regulating lysosomal function, PGRN may also have a role in modulating autophagy, the main neuronal pathway involved in clearance of misfolded, disease causing proteins such as p-

tau and TDP43. PGRN has been shown to inhibit autophagy (Chang et al., 2017), and the addition of extracellular PGRN to neurons repressed autophagy. In contrast, inhibiting *Grn* expression with siRNA caused an exaggerated increase in autophagy in hepatocytes (Liu et al., 2015).

To test if PGRN directly modulates autophagy in neurons, we employed a novel, cell imaging technology, optical pulse-labelling (OPL) to measure autophagic flux in single primary mouse cortical neurons using robotic microscopy and longitudinal imaging of a photoswitchable fluorescent protein reporter, EOS2-LC3⁶². Using this approach, we found that knockdown of PGRN levels by > 50% resulted in an upregulation of autophagic flux in mouse cortical neurons (Elia et al., 2019). We and others (Osaka et al., 2015) showed that pharmacological inhibition of autophagy with 3-methyladenine (3-MA) increased levels of extracellular PGRN, whereas overexpressing a genetic inducer of autophagy, Beclin1, decreased PGRN secretion. Thus, there appears to be a reciprocal relationship between PGRN and autophagy, with PGRN inhibiting autophagic flux and autophagy reducing excess secretion of PGRN from neurons.

Downregulating autophagy is counterintuitive to studies showing a beneficial role for autophagy in neurodegenerative diseases. However, excessive autophagy can lead to activation of alternative cell death pathways as seen in cerebral ischemia (Shi et al., 2012; Bialik et al., 2018; Jaeger and Wyss-Coray, 2009). Autophagic stress could also arise from inefficient autophagosome turnover due to lysosomal defects. Lee and Gao (2009) have shown that excess accumulation of autophagosomes due to dysfunction of the endosomal sorting complex required for transport III (ESCRT-III) contributed to neuronal cell loss in a mechanism that was uncoupled from the accumulation of ubiquitinated protein aggregates (Lee and Gao, 2009). Inhibiting autophagy by treatment with 3-MA or knocking-out *atg5* delayed this neuronal cell loss without affecting the endosomal accumulation of ubiquitinated proteins caused by the ESCRT-III dysfunction, suggesting that dysregulated autophagy can be a co-contributor to neurodegeneration in addition to the aberrant accumulation of toxic proteins (Lee and Gao, 2009).

Autophagy and lysosomal activity are linked in neurons since autophagy is responsible to transporting cargoes destined to degradation to lysosomes. The enhanced autophagy caused by PGRN insufficiency may also be linked to a progressive impairment of lysosome function because in essence, the lysosomes are overworked (Elrick and Lieberman, 2013; Elrick et al., 2012). If PGRN deficiency results in an impaired autophagy-lysosomal pathway (ALP) in neurons, then this may explain why in PGRN deficient neurons there is increased expression of aggregated cytoplasmic TDP43 which is normally cleared by the ALP.

3.4. PGRN and TDP43 pathology

Insufficiency of PGRN in brain results in the accumulation and aggregation of TDP43 in the cytoplasm of neurons and reduced transport of TDP43 to the nucleus, impairing its normal regulation of RNA. Inability of TDP43 to shuttle from the cytoplasm to the nucleus is a critical pathogenic mechanism in ALS and likely in FTD (Ayala et al., 2008; Neumann et al., 2006; Ward et al., 2014). We showed that cortical neurons from transgenic *Grn*^{493X/+} mice with PGRN haploinsufficiency exhibit TDP43 pathology; they express significant toxic cytoplasmic accumulation of TDP43⁶². We also found that *Grn* knockdown reduced the turnover of TDP43 in individual neurons nearly three-fold, monitored with OPL technology. This finding suggests that reduced turnover and increased accumulation and aggregation of cytoplasmic TDP43 in PGRN-deficient neurons may be caused by impaired clearance of TDP43 by the ALP. Furthermore, we found that cortical neurons overexpressing TDP43 have a significantly increased risk of death. Adding exogenous PGRN to these neurons reduced TDP43 cytoplasmic levels and increased neuronal survival. These findings are

consistent with PGRN restoring ALP functions to clear toxic forms of TDP43 to reduce neurodegeneration, since we know that TDP43 can be cleared via the ALP. As FTD and AD are associated with diminished levels of brain PGRN and TDP43 pathology, our findings suggest that PGRN **replacement therapy** may have disease-modifying consequences to treat FTD and AD.

4. Genetic regulators of neuronal PGRN expression

To better define the neuronal mechanisms that control PGRN expression and to identify potential novel molecular targets to develop drugs to increase PGRN expression to treat FTD, we employed an unbiased whole-genome RNAi screen on N2a cells, which endogenously express PGRN, to identify genes that control the extracellular levels of PGRN (Elia et al., 2019). The screen identified a select group of genes that have known roles in regulating ALP. The targets are druggable since small molecule inhibitors have been developed against the proteins encoded by these genes. Furthermore, reduction in expression of these potential genetic modifiers of PGRN levels increased expression and secretion of PGRN from N2a cells and mouse primary cortical neurons. It also raised PGRN levels in mouse *Grn*[±] haploinsufficient cortical neurons to levels found in wild-type neurons. This suggests that drugs targeting these gene products might be able to reverse PGRN insufficiency in humans such as FTD patients.

The cassette of potential genetic modifiers of PGRN levels included (*Gabarap1*, *Tom1*, *Tsg101*, *Foxo1*, *Sort1*, *Jmjd6*, *Elk3*, and *Trap1/HSP90L*). GABARAP1 is a member of the LC3 family of proteins that are essential for autophagy flux and mediate autophagosome formation and maturation and are also important in autophagosome-to-lysosome fusion (Albanesi et al., 2015; Jenzer et al., 2014; Martens, 2016; McEwan et al., 2015; Nguyen et al., 2016; Wang et al., 2015). TOM1 is an endosome protein that functions in autophagosome-to-lysosome fusion (Bond et al., 2011; Tumbarello et al., 2012, 2013; Wang et al., 2010). TSG101 is an ESCRT1 protein that is required for endosomal maturation, trafficking and exosome secretion (Babst et al., 2000; Colombo et al., 2013). FOXO1 is a forkhead O family transcription factor that has been shown to regulate autophagy in response to stress (He et al., 2018; Zhao et al., 2010). JMJD6 encodes a jumonji C-domain containing, bifunctional arginine demethylase and lysyl-hydroxylase (Chang et al., 2007; Webby et al., 2009; Boeckel et al., 2011; Heim et al., 2014; Mantri et al., 2011; Poulard et al., 2014; Unoki et al., 2013; Walport et al., 2016; Zhang et al., 2015) that has been shown to promote autophagy in triple negative breast cancer cells (Liu et al., 2018). Elk3 is an ETS transcription factor that functions as a transcriptional repressor and regulates gene expression during angiogenesis and hypoxia (Gross et al., 2007, 2008; Heo and Cho, 2014). Knockdown of *ELK3* in a triple negative breast cancer cell line led to a repression of autophagy via activation of the PI3K/Akt/mTOR pathway and, as a result, conferred sensitivity to the anticancer drug, doxorubicin (Park et al., 2016). TRAP1/HSP90L is a mitochondrial chaperone protein and a member of the HSP90 family of heat shock proteins (Altieri et al., 2012; Amoroso et al., 2012; Baldo et al., 2012; Hong et al., 2013; Matassa et al., 2012; Putcha et al., 2010; Takemoto et al., 2011). TRAP1/HSP90L has been shown recently to act as a regulator of autophagy in lung cancer cells (Barbosa et al., 2018).

To determine if the potential genetic modifiers may act via a common mechanism to increase PGRN levels, we tested if reducing expression of these genes affected transcriptional activity of *Grn* in N2a cells. siRNA knockdown of *Jmjd6* or *Foxo1* increased the relative abundance of *Grn* mRNA and PGRN levels. In contrast, siRNA knockdown of *Trap1/Hsp90L*, *Tom1*, or *Tsg101* did not affect *Grn* mRNA, despite increasing extracellular PGRN levels. Thus, these validated genes parse into two distinct mechanistic categories: those that regulate PGRN levels primarily at the transcriptional level (*Jmjd6*; *Foxo1*) and those that regulate PGRN levels primarily at a post-translational level (*Trap1/Hsp90L*; *Tom1*; *Tsg101*), suggesting that PGRN levels are

regulated by multiple mechanisms.

5. Small molecule enhancers of PGRN expression as potential drugs to treat FTD

Since PGRN deficiency is a potential disease mechanism in a significant population of FTD patients, then identifying drugs that safely increase PGRN levels in brain could be potentially developed as therapeutics to treat FTD. We found two genes, *Foxo1*, and *Trap1/Hsp90L* that are potentially interesting molecular targets to develop small molecule drugs to treat FTD since inhibiting the activity of their gene products resulted in an increase PGRN levels in neurons.

5.1. *Foxo1* inhibitors

Small molecule drugs inhibitors, PsammaplyseneA (PSA) (Mojsilovic-Petrovic et al., 2009) and AS1842856¹²⁶ have been developed targeting *Foxo1* gene product and we tested those drugs for efficacy in increasing PGRN levels in neurons. We were interested in *Foxo1* as a potential genetic modifier because reducing its expression resulted in increased levels of *Grn* mRNA, suggesting that small molecule inhibitors might produce a long-lasting increase in PGRN levels in brain neurons. Furthermore, knocking down expression of *Foxo1* in PGRN-deficient neurons not only increased PGRN levels but also suppressed the lysosome enlargement suggesting that *Foxo1* inhibitors might reverse the neuropathology caused by PGRN insufficiency.

Both PSA and AS1842856, which act through different mechanisms to block *Foxo1* activity, effectively and potently increased PGRN levels in N2A cells in a dose dependent manner. PSA produced a 2.5-fold increase in PGRN at concentrations as low as 10 nM AS1842856 at 10 nM increased PGRN levels by 50%, which would be sufficient to reverse PGRN haploinsufficiency. Both inhibitors showed a sustained increase in PGRN levels.

The efficacy of these two drugs to increase PGRN level is of potential therapeutic utility. PSA reduces neurodegeneration in models of ALS (Mojsilovic-Petrovic et al., 2009), which, like FTD, exhibit significant TDP43 pathology, suggesting that *Foxo1* inhibitors may be neuroprotective. AS1842856 is orally bioavailable and has been used to investigate the *in vivo* role of *Foxo1* in diabetes (Nagashima et al., 2010). Both compounds have been tested in animals and produce no overt side effects. If these drugs are effective in *in vivo* animal models of FTD and *in vitro* human models of FTD (iPSC derived neurons from FTD patients) then *Foxo1* may be a unique target for developing drugs to treat FTD.

5.2. TRAP1 inhibitors

TRAP1 is of interest because it mediates the functions of the proinflammatory cytokine TNF α (Altieri et al., 2012; Matassa et al., 2012), and prior evidence had already suggested an important role of TNF α in causing neurodegeneration in FTD. Thus, TRAP1 inhibition might be effective in blocking neurodegeneration in FTD through at least two mechanisms: by reducing the degenerative effects of TNF α and by reversing PGRN haploinsufficiency to return PGRN to normal levels. In fact, there is an interesting relationship between PGRN and TNF α since PGRN has been reported to inhibit downstream activity of TNF α by reducing expression of the TNF α -regulated cytokines CXCL9, CXCL10 and IL-10 (Mundra et al., 2016; Wei et al., 2014). Thus, by increasing PGRN levels, TRAP1 inhibitors may further block the proinflammatory actions of TNF α .

TRAP1 is a member of the HSP90 family and HSP90 inhibitors block TRAP1 function (Baldo et al., 2012; Hong et al., 2013; Matassa et al., 2012). We tested two HSP90 inhibitors, 17AAG and AUY922, for their ability to increase PGRN levels in N2a cells (Elia et al., 2019; Hong et al., 2013). We tested these compounds because they effectively inhibit TRAP1 and have also undergone multiple Phase 1 and 2 clinical trials as anticancer agents (Banerji et al., 2005; Goetz et al., 2005; Grem

et al., 2005; Heath et al., 2008; Nowakowski et al., 2006; Ramanathan et al., 2005, 2007; Ronnen et al., 2006; Solit et al., 2007, 2008; Kong et al., 2016), are well tolerated in humans, and produce no obvious limiting side effects even with chronic administration. Because the TRAP1 inhibitors are safe in humans, if they are found effective in preclinical models of FTD, they could eventually be transitioned for testing in humans to treat FTD. Both showed dose-dependent increases in PGRN (Elia et al., 2019). AUY922 increased PGRN levels by threefold at concentrations as low as 100 nM. Importantly, we also tested a third TRAP1 inhibitor, NVP-HSP990. NVP-HSP990 is being developed to treat cancer and has been shown to have good pharmacokinetics and pharmacodynamic properties after oral administration and has been shown to be safe in Phase 1 clinical trials (McBride et al., 2014; Spreafico et al., 2015). When administered intraperitoneally at a single dose (12 mg/kg) to either wild-type mice or an animal model of FTD with PGRN haploinsufficiency (*Grn*^{R493X/+}) it significantly increased PGRN levels in brain by more than 50% indicating it can reverse PGRN haploinsufficiency *in vivo* (unpublished results). We also showed target engagement of NVP-HSP990 *in vivo* in brain by measuring changes in ATPase activity (unpublished results). NVP-HSP990 has been reported to be a more potent inhibitor of TRAP1 ATPase activity than 17-AAG (Menezes et al., 2012). At the dose of NVP-HSP990 that increases PGRN levels in brain by at least 50%, the drug blocks TRAP1 ATPase activity by a similar amount indicating the drug increases PGRN levels by blocking TRAP1 activity in brain. This finding is important because it shows for the first time that a TRAP1 inhibitor that gets into the brain after peripheral administration increases PGRN in brain, which we can now use to test for preclinical efficacy in FTD animal models and possibly transition to development as a treatment of FTD.

5.3. Therapeutic considerations of small molecule PGRN inducers

While drug inhibitors targeting genetic modifiers of PGRN expression including Gabarap1, Foxo1 Tom1, Tsg101, TRAP1, Jmjd6, and Elk3 may have utility in treating FTD, caution may be necessary in their long term use because they may be expected to produce off-target actions, such as reducing autophagy, increasing their potential for inducing side effects. Little is known with regards to the role of each of these molecular targets in the control of autophagy in brain neurons. It is not known whether partial inhibition of individual targets may be compensated for and have minor overall effects on autophagy and protein clearance. In fact, there are few small molecule drugs that selectively inhibit specific proteins in the autophagy pathway, and those that inhibit TRAP1 and Foxo1, as described above, produce no overt side effects in animals or humans.

However, a more important question is how a drug that inhibits autophagy could be useful in treating a disease like FTD that is associated with increased levels of a toxic misfolded protein such as TDP43 which may build up in neurons in part because it is not effectively cleared from brain cells through the autophagic-lysosomal pathway. While a definitive answer to this question is not available, it is possible that the ameliorative effects of small molecule drug inhibitors of TRAP1 and Foxo1 may be due to their multiple mechanisms of actions as is the case for most drugs that treat brain disorders. For example, the ability of Foxo1 inhibitors to increase PGRN levels is due at least in part to their ability to increase PGRN mRNA levels which is not likely to be due to inhibiting autophagy. Also, TRAP1 inhibitors, as described above not only affect autophagy but also may reduce the degenerative effects of the proinflammatory cytokine TNF α . Thus, the potential therapeutic effects of TRAP1 and Foxo1 inhibitors may be primarily due to their ability to reverse the loss of PGRN in disease. The effects of these drugs on autophagy may have minor consequences in their overall therapeutic benefits in treating brain diseases.

6. Summary and future directions

GRN-linked FTD is a multifaceted disease with specific neuronal pathologies and neuroinflammation resulting from reduced PGRN levels in the brain. Understanding how low PGRN levels lead to neurodegeneration has been a central and important focus of current research to identify targets to treat this incurable and progressively fatal disease. This review has focused on the identification of novel genetic and chemical modulators that act through multiple mechanisms to restore neuronal PGRN to control levels in the brain, and reverse functional deficits in the ALP.

The complexity of FTD poses a unique challenge for drug development and so the identification of multiple strategies to increase PGRN levels will help to ensure a high likelihood of success to develop effective therapeutics. While this review has focused on a strategy to raise PGRN levels in the brain, additional strategies directly targeting neuroinflammation or TDP43 pathology can be envisioned. Directly targeting the neuroinflammation associated with reduced PGRN levels, might include identifying ways to dampen disease associated microglial inflammation or to mitigate the neurotoxic effects of proinflammatory cytokines. Directly targeting the toxic aggregation and/or mislocalization of TDP43 might include identifying ways to modulate autophagy and/or enhance lysosome function to help promote the efficient clearance of TDP-43 aggregates.

FTD is a rare disease and qualifies as an orphan indication. This opens up a number of grant and accelerated approval mechanisms through the Orphan Drug Act and FDA Office of Orphan Products Developments. There is broader potential for treating other neurodegenerative diseases, as a successful FTD drug that is rapidly approved will have been clinically validated. This can help offset or lower the potential financial risks for therapeutics development for AD, for instance, for which reduced PGRN levels have been identified as a known risk factor for this disease. However, there is a need to develop more rigorous preclinical models that faithfully and effectively recapitulate the human disease to ensure success in clinical trials and in translating potential drugs into approved treatments. New protocols to successfully differentiate human induced pluripotent stem cells (iPSC) into specific neuronal subtypes, microglia, or astrocytes are now available, opening up the potential of developing more effective human preclinical cell models. The ability to differentiate the key cell types affected in FTD will enable a better understanding of cell type specific autonomous as well as nonautonomous phenotypes. These cell models can complement the FTD animal models, which fail to recapitulate some of the key pathologies of the human disease, such as excessive neuroinflammation. Human iPSC-derived cell models may represent new potential to develop better *in vivo* animal models for FTD: a very recent publication from Hasselmann et al. (2019), showed that it is possible to develop a chimeric mouse using xenotransplantation of human iPSC-derived microglia (Hasselmann, 2019). Human iPSC-derived hematopoietic-progenitors transplanted into the postnatal brain of humanized, immune-compromised mice led to differentiation into microglia that can respond to inflammatory challenges and AD pathology (Hasselmann, 2019). This could enable the development of more rigorous FTD animal models that recapitulate the inflammatory pathology currently lacking from the existing models. Given the current advances in CRISPR technology, there is an exciting potential for new screening opportunities in human iPSC-derived FTD microglia that can be performed and functionally validated *in vivo* in chimeric animals, thus providing powerful new approaches to expedite drug development for FTD and AD.

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