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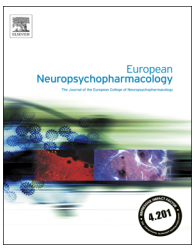

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Increasing progranulin levels and blockade of the ERK1/2 pathway: Upstream and downstream strategies for the treatment of progranulin deficient frontotemporal dementia

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Abstract
Frontotemporal lobar degeneration (FTLD) is a neurodegenerative disorder marked by mild-life onset and progressive changes in behavior, social cognition, and language. Loss-of-function *progranulin* gene (*GRN*) mutations are the major cause of FTLD with TDP-43 protein inclusions (FTLD-TDP). Disease-modifying treatments for FTLD-TDP are not available yet. Mounting evidence indicates that cell cycle dysfunction may play a pathogenic role in neurodegenerative disorders including FTLD. Since cell cycle re-entry of posmitotic neurons seems to precede neuronal death, it was hypothesized that strategies aimed at preventing cell cycle progression would have neuroprotective effects. Recent research in our laboratory revealed cell cycle alterations in lymphoblasts from FTLD-TDP patients carrying a null *GRN* mutation, and in PGRN deficient SH-SY5Y neuroblastoma cells, involving overactivation of the ERK1/2 signaling pathway. In this work, we have investigated the effects of PGRN enhancers drugs and ERK1/2 inhibitors, in these cellular models of PGRN-deficient FTLD. We report here that both restoring

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the PGRN content, by suberoylanilide hydroxamic acid (SAHA) or chloroquine (CQ), as blocking ERK1/2 activation by selumetinib (AZD6244) or MEK162 (ARRY-162), normalized the CDK6/pRb pathway and the proliferative activity of PGRN deficient cells. Moreover, we found that SAHA and selumetinib prevented the cytosolic TDP-43 accumulation in PGRN-deficient lymphoblasts. Considering that these drugs are able to cross the blood-brain barrier, and assuming that the alterations in cell cycle and signaling observed in lymphoblasts from FTLD patients could be peripheral signs of the disease, our results suggest that these treatments may serve as novel therapeutic drugs for FTLD associated to *GRN* mutations.

1. Introduction

The term frontotemporal lobar degeneration (FTLD) refers to a group of progressive brain rare diseases, which involve shrinkage of specific areas of the brain that regulate behavior, personality, and language. The onset of symptoms usually occurs before the age of 60 years, accounting for 5–10% of dementia patients (Graff-Radford and Woodruff, 2007; Ratnavalli et al., 2002). The course of this disease is progressive with mortality within 6–8 years. FTLD patients can be classified into three clinical syndromes depending on the early and predominant symptoms: a behavioural variant (bvFTD) and two language variants; semantic dementia (SD) and primary progressive non-fluent aphasia (PPNFA) (Neary et al., 1998; Rabinovici and Miller, 2010). Each clinical variant is associated with a distinct regional pattern of brain atrophy and, to a varying degree, a characteristic histopathology. Additionally, other neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS), progressive supranuclear palsy (PSP), parkinsonism with frontotemporal dementia (FTDP) or corticobasal degeneration syndrome (CBS) are closely related to FTLD (Burrell et al., 2011; Deng et al., 2011; Lomen-Hoerth, 2004; Van Langenhove et al., 2012).

Pathologically, these disorders share deposits of abnormal proteins in neuroectodermic cells, and severe cell loss with atrophy of vulnerable cortical and subcortical structures. Histochemically, FTLD can be categorized according to the major component of the celular inclusions deposited in the brain. In the majority of cases, the pathological protein is either the microtubule-associated protein tau or the transactive response DNA-binding protein TDP-43 (FTLD-tau, FTLD-TDP respectively), a small number of cases present inclusions of fused in sarcoma (FUS) protein (FTLD-FUS) (Cairns et al., 2007; Mackenzie et al., 2011a, 2011b).

A positive family history of FTLD is present in 25–50% of cases and the transmission is usually autosomal dominant (Goldman et al., 2007; Rademakers et al., 2012; Sieben et al., 2012). A few genes have been associated with familial FTLD including *microtubule-associated protein tau* (*MAPT*) (Poorkaj et al., 1998; Spillantini et al., 1998), *progranulin* (*GRN*) (Baker et al., 2006; Cruts et al., 2006), *transactive response (TAR) DNA-binding protein-43* (*TARDBP*) (Gitcho et al., 2009), *chromatin-modifying 2B protein* (*CHMP2B*) (Holm et al., 2007), *valosin-containing protein* (*VCP*) (Watts et al., 2004), and *chromosome 9 open reading frame 72* (*C9ORF72*) (DeJesus-Hernandez et al., 2011). Recentely, two new rare mutations associated with FTLD and ALS have been identified in the *sequestome1/p62* (*SQSTM1*) (Le Ber et al., 2013) and *Ubiquilin-2* (*UBQLN2*) (Deng et al., 2011; Vengoechea et al., 2013) genes.

A large number of FTLD-TDP patients have been identified to harbor loss-of-function mutations (including null mutations) in the gene encoding progranulin (*GRN*) (Baker et al., 2006; Cruts et al., 2006). Up to now more than 60 different mutations in *GRN* have been described associated with the etiology of the disease (www.molgen.ua.ac.be/FTDmutations/) (Gijselinck et al., 2008). Most of the pathogenic mutations result in null allele, suggesting that FTLD in these families results from progranulin (PGRN) haploinsufficiency (Cruts et al., 2006; Cruts and Van Broeckhoven, 2008).

Current treatment options for FTLD associated to PGRN deficiency remain very limited, mainly involving therapy for the mood and behavioral symptoms (Kirshner, 2010). Identification of molecular targets to slow or hopefully to prevent the neurodegenerative process relies in a better understanding of both the biological functions of PGRN, and the role of protein haploinsufficiency in the development of dementia. For this purpose, familial forms of the disease with known pathogenic mutations provide an opportunity to get inside in FTLD-TDP pathogenesis and for fast-track development of new therapies for PGRN-deficient FTLD.

Cell cycle-related events are now considered as an important pathogenic mechanism for neurodegenerative disorders including Alzheimer disease (AD) (Mosch et al., 2007; Yang et al., 2001), Parkinson disease (PD) (Hoglinger et al., 2007), ALS and FTLD (Husseman et al., 2000). In these studies, it was suggested that cell cycle signaling might affect neuronal death pathway. The cell cycle is associated with the phase specific expression or modification of defined sets of regulatory genes that control proliferation, differentiation or entry into a quiescent state (Ross, 1996). However, re-entry of quiescent, post-mitotic neurons into the cell cycle may result in a mitotic catastrophe and cell death (Copani et al., 2001; Herrup et al., 2004; Zhu et al., 2004). Therefore, understanding the molecular pathways underlying this cell cycle-mediated neurodegeneration may be important to find new therapeutic targets to slow or prevent the onset and progression of FTLD.

Interestingly, it seems that dysfunction of the cell cycle in neurodegenerative disorders is a general phenomenon affecting cells other than neurons (Nagy et al., 2002; Stieler et al.,