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On the role of RNA binding proteins in polyglutamine diseases: from pathogenesis to therapeutics

André Conceição, Rebekah Koppenol, Clévio Nóbrega*

Polyglutamine (polyQ) diseases are a group of different neurodegenerative disorders characterized by an abnormal expansion of the trinucleotide cytosine-adenine-guanine (CAG) within coding regions of each disease-associated gene. The abnormal expansion translates into a protein bearing an abnormally long tract of glutamines. The expanded proteins are prone to aggregate, promote aberrant interaction with other proteins and mRNAs and contribute to cellular pathway disruption (Matos et al., 2019). To date, nine different polyQ diseases are described, including among others, Huntington's disease, and six different spinocerebellar ataxias (SCA). Patients affected by polyQ diseases, suffer a myriad of motor symptoms that include ataxia, dysphagia, tremors, dysarthria, and even dementia. Unfortunately, there is no cure nor treatment able to delay the disease and patients rely only on symptomatic and supportive treatments culminating in premature death (Takahashi et al., 2010).

SCA2 and SCA3 (also known as Machado-Joseph disease [MJD]) are two polyQ diseases characterized by selective neurodegeneration within specific brain regions that include the cerebellum and brain stem. SCA2 patients have an abnormal CAG expansion above 33 glutamines within the *ATXN2* gene, whereas SCA3/MJD patients harbor CAG expansions above 61 glutamines in the *ATXN3* gene (Klockgether et al., 2019; Matos et al., 2019).

The molecular mechanisms by which a protein bearing abnormally expanded polyQ tracts leads to neurodegeneration are still not understood. Nevertheless, several mechanisms and pathways are involved, including impaired autophagy, RNA toxicity, enhanced oxidative stress, aberrant mRNA processing, and disrupted cellular calcium release (Lieberman et al., 2019; Marcelo et al., 2021b). In recent years, several studies suggest that stress granules (SGs) might play an important role in neurodegenerative diseases and their disruption might contribute to the pathology (Wolozin and Ivanov, 2019; Marcelo et al., 2021a). SGs are dynamic structures formed in response to cellular stress, being mainly formed by messenger RNAs, RNA-binding proteins (RBPs), and protein translation machinery components (Marcelo et al., 2021a). Additionally, it is also widely accepted that disruption of RBPs and widespread RNA processing defects are critical determinants of neurodegenerative diseases (Nussbacher et al., 2019). In fact, we showed that the RBP ataxin-2 is downregulated in the context of SCA3/MJD, and restoring its levels ameliorates the disease phenotype, due to its interaction with poly(A)-binding protein, which is another RBP (Nóbrega et al., 2015). It is noteworthy to mention that both ataxin-2 and poly(A)-binding protein are proteins recruited to SGs in response to cellular stress and disruption of cellular stress responses might contribute to disease pathology (Nunes et al., 2019; Marcelo et al., 2021a).

In this line, we sought to understand how the SGs nucleator Ras GTPase-activating protein-binding protein 1 (G3BP1), which is an RBP could impact SCA2 and SCA3/MJD disease phenotype. In cellular models, we showed that G3BP1 expression was able to reduce the levels and the number of aggregates of expanded ataxin-2 and ataxin-3 proteins (Koppenol et al., 2022). However, this mechanism seems to be independent of SGs assembly. To elucidate the mechanism of action of G3BP1 we focused on two important domains, an RNA-recognition domain that bind RNA and a nuclear transport factor 2-like domain, responsible to import proteins through the nuclear pore (Winslow et al., 2013). Using truncated forms of G3BP1 lacking specifically each of these domains, we found that nuclear transport factor 2-like domain seems important to the action of G3BP1 in the reduction of polyQ proteins levels and aggregates. We further explored the role of serine 149 phosphorylation site, described to have an important functional role in G3BP1 functions (Tourrière et al., 2003). Using a phospho-dead and a phosphomimetic form of G3BP1 at this site, we found that it plays a key role in the modulation of polyQ proteins levels and aggregation.

To evaluate the functional relevance of G3BP1 in SCA2 and SCA3/MJD molecular pathogenesis, we accessed the levels of G3BP1 mRNA and protein in SCA2 and SCA3/MJD patients and models. Importantly, we found that G3BP1 levels are reduced in the patient's fibroblasts and in a SCA3/MJD transgenic mouse model. Additionally, we observed that reducing endogenous G3BP1 levels in lentiviral mouse models of SCA2 and SCA3/MJD increases the number of ataxin-2 and ataxin-3 aggregates, respectively (Koppenol et al., 2022). Altogether, these results suggest that G3BP1 levels are reduced in SCA2 and SCA3/MJD and this contributes to disease pathogenesis.

Next, we evaluated the impact of G3BP1 levels reestablishment in a lentiviral mouse model of SCA2 (Marcelo et al., 2021b) and SCA3/MJD (Alves et al., 2008). We found that lentiviral-mediated G3BP1 expression mitigates mutant ataxin-2 and ataxin-3 disease-associated neuropathological abnormalities, reducing the number of aggregates and preserving the loss of the neuronal marker DARPP-32. Furthermore, we observed that lentiviral-mediated G3BP1 expression did not produce neuronal loss or neuroinflammation.

Aiming to translate these results to a more clinically relevant model, we investigated the impact of G3BP1 levels reestablishment in a symptomatic transgenic mouse model of SCA3/MJD, which is characterized by severe motor deficits and marked Purkinje cell loss (Torashima et al., 2008). G3BP1 expression in the cerebellum mediated by lentiviral vectors led to a significant improvement in motor performance and to a significant reduction of aggregates and preservation of Purkinje cells, compared to control animals injected with green fluorescent protein.

PolyQ diseases enclose sets of disrupted cellular and molecular mechanisms caused by abnormal glutamine expansions in 9 different proteins, all leading to neuronal dead and selective degeneration within specific brain regions. Our latest study shows, using *in vitro* and *in vivo* disease models for SCA2 and SCA3/MJD that the expression of G3BP1, an RBP, can ameliorate neuropathologic and motor features (**Figure 1**). The shared pathologic features of polyQ diseases, based on the same type of genetic mutation allow one to envision common therapeutic strategies to counteract the progressive pathologic cascade. In this line, our study suggests that viral delivery of G3BP1 might constitute a viable therapeutic target for SCA2 and SCA3/MJD, and even for the other PolyQ diseases.

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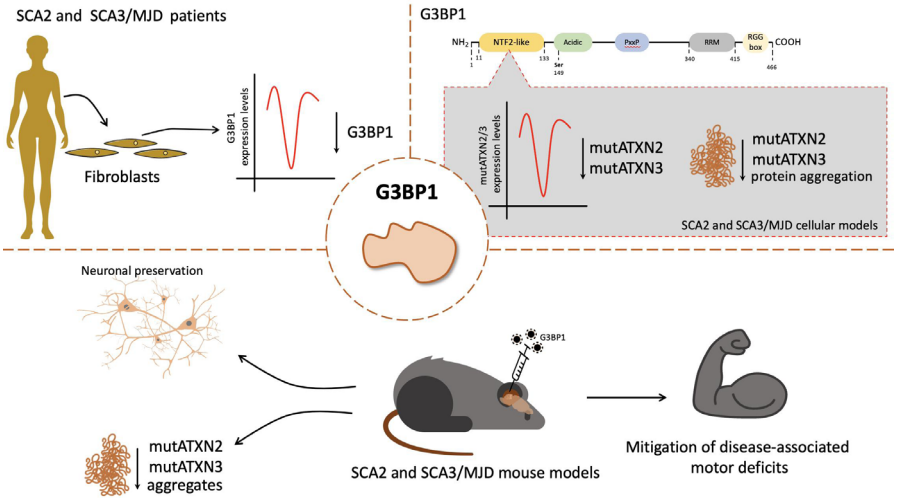

Figure 1 | ***G3BP1* gene expression mitigates SCA pathological phenotypes.**
G3BP1 levels are downregulated both in SCA2 and SCA3/MJD patients' fibroblasts. In cellular models of SCA2 and SCA3/MJD, the expression of G3BP1 was able to downregulate ataxin-2 and ataxin-3 mutant protein levels and reduce the number of aggregates. The mechanism of action of G3BP1 seems dependent on its NTF2-like motif. In a more relevant clinical context, lentiviral-mediated expression of G3BP1 into the brain of SCA2 and SCA3/MJD mouse models led to the preservation of neuronal tissue and was able to reduce the number of mutant ataxin-2 and mutant ataxin-3 aggregates, respectively. Importantly, lentiviral-mediated expression of G3BP1 was able to mitigate the pathological motor deficits in the severely affected SCA3 transgenic mouse model. G3BP1: Ras GTPase-activating protein-binding protein 1; MJD: Machado-Joseph disease; NTF2-Like: nuclear transport factor 2 like; SCA2: spinocerebellar ataxia type 2; SCA3: spinocerebellar ataxia type 3. Created with Affinity Designer and PowerPoint.

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