PQBP1, an intrinsically disordered/denatured protein at the crossroad of intellectual disability and neurodegenerative diseases

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ABSTRACT

PQBP1 (polyglutamine binding protein-1) is the earliest identified molecule among the group of disease-related intrinsically disordered/denatured proteins. PQBP1 interacts with splicing-related factors via the disordered/denatured domain and regulates post-transcriptional gene expression. The mutations cause intellectual disability due to decreased dendritic spines and abnormal expression of synapse molecules in neurons, and microcephaly due to elongated cell cycle time and abnormal expression of cell cycle proteins in neural stem progenitor cells. Meanwhile, PQBP1 interacts with polyglutamine tract sequences translated from CAG triplet disease genes via their disordered/denatured structures. The second hit on PQBP1 by such neurodegenerative disease proteins is supposed to similarly impair synapse functions in neuron and proliferation of stem cells. The alteration of gene expression profile and consequently induced phenotypes of neuron and stem cells via secondary impairment of the intrinsically disordered/denatured protein PQBP1, which are similar to developmental disorders by PQBP1 gene mutations, could be a part of the main pathologies shared by multiple neurodegenerative diseases.

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1. Introduction

Neurodegeneration has been believed to be a sequential process that is initiated by protein aggregation inside or outside of cells. The typical case is “amyloid theory” in which extracellular aggregation hypothesized located at the top of pathological cascades and supposed to trigger all the pathological events either directly or indirectly (Hardy and Selkoe, 2002). This “aggregation hypothesis” has been basically believed in the other types of neurodegenerative diseases such as Alzheimer’s disease (AD), frontotemporal lobar degeneration (FTLD)/amyotrophic lateral sclerosis (ALS),

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Parkinson's disease (PD)/Dementia with Lewy's body (DLB) and polyglutamine diseases (polyQ disease). However, the “aggregation hypothesis” is now challenged in most of neurodegenerative diseases, and new concepts to explain pre-aggregation pathology (or ultra-early phase pathology) are urgently demanded to overcome the difficulties of neurodegenerative disease researches.

PolyQ binding protein 1 (PQBP1) (Imafuku et al., 1998; Waragai et al., 1999; Okazawa et al., 2002) is a pioneering molecule whose physiological and pathological functions firstly challenged the “aggregation hypothesis". PQBP1 is the first disease-related molecule that was shown to compose intracellular foci, which could be a seed for protein aggregation (Okazawa et al., 2001, 2002; Okazawa, 2003), based on the self-assembly characteristics of intrinsically disordered/denatured proteins (Takahashi et al., 2009b; Rees et al., 2012), which is shared by recently reported examples of FUS or hnRNP1 from FTLD/ALS (Murakami et al., 2015; Molliex et al., 2015). PQBP1 is one of the first molecules that indicated involvement of disordered RNA metabolism in neurodegeneration pathway (Moore et al., 2010; Wang et al., 2013; Ito et al., 2015). PQBP1 is the first molecule that showed both higher and lower expression levels out of the appropriate range to be toxic in neurodegeneration and intellectual disability (Okuda et al., 2003; Ito et al., 2015; Tamura et al., 2013). PQBP1 is the first molecule that directly links neurodegenerative diseases accompanying dementia and brain atrophy (Waragai et al., 1999; Okazawa et al., 2002) and developmental symptoms such as intellectual disability (ID) and microcephaly (Kalscheuer et al., 2003; Lenski et al., 2004; Stevenson et al., 2005; Lubs et al., 2006).

These features of PQBP1 could be the essence of generating some hypotheses for “pre-aggregation pathology”. Therefore, in this review article, I summarize current knowledge about PQBP1, compare it with findings from the other neurodegenerative proteins, speculate two-hit theory for neurodegenerative diseases, and discuss about the molecular level concept that might be alternatives for “aggregation hypothesis”.

2. Discovery of PQBP1

PQBP1 was originally identified as a binding protein to polyQ repeat sequence by our group (Imafuku et al., 1998). We used a normal length of polyQ sequence (GAL-Q26-APP) derived from a brain-specific transcription factor Brn2 as the bait for two-hybrid screening of human embryonic brain cDNA library that was constructed in pG4-5 plasmid (Imafuku et al., 1998). The two-hybrid screening system and the cDNA library were generous gifts from Professor Roger Brent (Department of Genetics, Harvard University). We finally obtained six clones that showed strong interaction with polyQ sequences (Imafuku et al., 1998). They were TERA/VCP/p97 and five unknown sequences. We designated them as PQBP1, PQBP2, PQBP3, PQBP4 and PQBP5 (Imafuku et al., 1998).

We preferred to analyze unknown genes rather than the known gene TERA/VCP/p97, because we might open a new research field. Therefore we first obtained full-length cDNA of PQBP1 and analyzed the expression profile in multiple organs (Waragai et al., 1999). It is expressed in brain, but almost the similar levels of expression were observed in skeletal muscle, heart, pancreas, placenta, lung, kidney and so on (Waragai et al., 1999). In the brain, Northern blot showed similar levels of expression in various regions but in situ hybridization revealed higher expression in regions where neurons are compacted such as hippocampus and cerebellar granule layer (Waragai et al., 1999). The expression in neurons was also confirmed (Waragai et al., 1999). As expected from the original approach using polyQ sequence in Brn2, PQBP1 is dominantly located in the nucleus of cells, and overexpression of PQBP1 suppressed transcriptional activation of Brn2 (Waragai et al., 1999). However, we later noticed that both over- and under-expression of PQBP1 disturb various cellular functions including transcription and could be toxic consequently for animals (Okuda et al., 2003; Marubuchi et al., 2006; Yoshimura et al., 2006; Ito et al., 2009; Takahashi et al., 2009a; Tamura et al., 2010; Tamura et al., 2013; Ito et al., 2015).

Domain structure of PQBP1 was unique (Fig. 1). It has a WW domain, a protein interaction motif homologous to SH3, and a unique C-terminal domain as discussed following. Between the two domains, hepta- and di-amino acid repeat sequences are inserted (Waragai et al., 1999).

3. PQBP1 in transcription and RNA splicing

Next, we investigated transcriptional function of PQBP1 with a focus on WW domain, expecting that it would provide us some hints for unraveling functions of PQBP1. In collaboration with Professor Marius Sudol, we started to search for binding partners to the WW domain of PQBP1, but unexpectedly we found multiple candidates (our unpublished observation). This was natural if we think later, probably because WW domain recognizes short sequences rich in proline (Sudol, 1996; Einbond and Sudol, 1996; Macias et al., 2002), and such sequences exist in many molecules. However, among the list was RNA polymerase II (Pol II) that was obviously the most important molecule among the list.

Using filter-binding assay we searched for the sequence in Pol II to which PQBP1 specifically binds, and found it on the repetitive sequences in the C-terminal tail of Pol II. Especially the binding became strong when the second Serine was phosphorylated in the specific repeat sequence YSPGCPAYS PKQ (Fig. 2). This finding suggested that PQBP1 binds to Pol II actively transcribing and elongating (not initiating) pre-messenger RNA (Okazawa et al., 2002). Mutant Ataxin-1 (Atxn1), the disease protein of spinocerebellar ataxia type-1 (SCA1), interacts with PQBP1 and impairs transcription (Okazawa et al., 2002). Huntington (Htt), the causative protein of Huntington’s disease (HD), also bind to PQBP1 with a high affinity when polyQ sequence was elongated (Waragai et al., 1999), the similar mechanism to impair transcription is suspected in the HD pathology.

Impairment of general transcription is shown to cause atypical necrosis designated transcriptional repression-induced atypical cell death, TRIAD (Hoshino et al., 2006), Primary neurons undergo Type-III cell death with enormous enlargement of endoplasmic...
4. PQBP1 is an intrinsically disordered/denatured protein involved in RNA splicing

We noticed that C-terminal sequence of PQBP1 is conserved beyond species (Waragai et al., 2000; Okazawa et al., 2001). However, the sequence was unique to PQBP1 and no homologous sequence was found in other molecules (Okazawa et al., 2001). We named it as C-terminal domain (CTD) of PQBP1 and analyzed the function and structure. From screening of yeast two-hybrid screen by using the CTD as the bait, we identified US-15KD (Waragai et al., 2000), a component of U5 spliceosome (Fig. 1). It is of note that four of five finally isolated clones encoded US-15KD (Waragai et al., 2000), suggesting that the binding is rather specific. The similar results were also reported from another group (Zhang et al., 2000).

They searched for interacting molecule to Dim1p, the homologue of US-15KD, by using yeast two-hybrid screen, and identified PQBP1 as a binding protein (Zhang et al., 2000).

Our collaborators further investigated molecular structure of PQBP1-CTD. First, Professor Mizuguchi revealed that PQBP1-CTD is largely disordered (Takahashi et al., 2009b). According to the PONDR prediction of disordered protein, CTD could be disordered. Far-UV CD spectra from CD spectroscopy and 1H-15N HSQC spectra from NMR spectroscopy also revealed characteristics of unfolded conformation (Takahashi et al., 2009b). Furthermore, limited proteolysis supported that CTD is basically disordered to possess multiple cleavage sites (Takahashi et al., 2009b). The Mizuguchi group further narrowed the region for interacting with US-15KD to 23-residue segment in CTD (Takahashi et al., 2009b), and revealed that YxxPxxVL motif within the 23-residue segment is essential for the interaction with US-15KD (Fig. 3). The interaction motif is lost in all frame shift mutations of human intellectual disability (Mizuguchi et al., 2014) (Fig. 1). Next, the group of Professor Chen reconfirmed intrinsically disordered structure of PQBP-CTD by CD, NMR and EOM analyses (Rees et al., 2012), and proposed a disordered model with partially remained residual structures (Rees et al., 2012) (Fig. 3).

Consistently with characteristics of the other intrinsically disordered proteins like FUS and hnRNPA1 (Murakami et al., 2015; Mollieix et al., 2015), PQBP1 forms subcellular foci, nuclear body (Okazawa et al., 2002) and stress granules (Kunde et al., 2011) that contain mutant proteins of neurodegenerative diseases (Okazawa et al., 2002), ID protein like FMRP (Kunde et al., 2011) and RNA (Kunde et al., 2011). Mutant forms of PQBP1 linked to ID might also interact with FMRP in such cytoplasmic granule and might enhance degradation of FMRP (Zhang et al., 2017).

High-resolution imaging revealed colocalization of Atxn1 in such nuclear foci (or nuclear body) of PQBP1 (Okazawa et al., 2001, 2002). Interestingly, PQBP1 and Atxn1 form lamellar distribution specific to each molecule in a single nuclear body (Okazawa et al., 2002). The lamellar pattern might suggest that each molecule self-assembles but each could be a seed for assembly of another molecule (Fig. 2).
5. PQBP1 mutations cause intellectual disability and microcephaly

Unexpectedly, news came from genetic study of European Consortium of X-linked Mental Retardation (MR-ID) that PQBP1 mutations were associated with familial MR patients who had been genetically undiagnosed (Kalscheuer et al., 2003). The similar associations of MR-ID and PQBP1 were reported repeatedly from different groups (Lenski et al., 2004; Lubs et al., 2006), thus PQBP1 is now generally considered as one of the major causative genes for MR. The clinical diagnoses of these patients include Renpenning syndrome, Golabi-Ito-Hall syndrome, Sutherland-Haan syndrome and so on (Stevenson et al., 2005). All these syndromes share intellectual disability, microcephaly, low stature, lean body and hypogonadism (Stevenson et al., 2005).

In the Renpenning syndrome spectrum (Germainaud et al., 2011), pathogenic mutations are located in WW domain, polar amino acid-rich domain, and C-terminal domain (Fig. 1). In the first group, the mutation of tyrosine to cysteine in directly changes CTD for binding with U5-15kD (Mizuguchi et al., 2014). The second and third group mutations lead to loss of YxxPxxVL motif in CTD and causes frame shift of amino acid reading frame. The decrease or increase of AG-di-nucleotide-repeat causes frame shift of amino acid-rich domain, and C-terminal domain (Fig. 1). In the species back to complexes (Makarov et al., 2002). U5-15kD is conserved beyond recognition judging from its dynamics among various splicing complexes (Makarov et al., 2002). U5-15kD is supposed a regulatory protein of splicing site function (Ito et al., 2015). Thus considering the generally accepted concept of primary microcephaly, it is possible that PQBP1 dysfunction leads to abnormal cell cycle of neural stem cells (NSCs) during development and causes brain size reduction. In addition, the abnormal cell cycle in the other types of tissues might also explain the small stature and lean body that are frequently found in the patients (Kalscheuer et al., 2003; Lenski et al., 2004; Stevenson et al., 2005; Lubs et al., 2006).

We addressed the question by generating newly generated conditional knockout mice of PQBP1 in NSCs using nestin-Cre (nestin-KO mice) and by analyzing the cell cycle of NSCs in vitro and in vivo (Ito et al., 2015). Although we could not make general knock out mice of PQBP1 due to unspecified reasons, we successfully generated nestin-KO mice (Ito et al., 2015). Unexpectedly, the body weight of nestin-KO mice was lower than the background or floxed mice, even though PQBP1 was depleted only in neural cell lineage. The brain weight was decreased to about 70% of the background or floxed mice, as expected (Ito et al., 2015). Analyses of cerebral cortex did not reveal obvious changes in layer structure and the thickness ratio of cortical layers, which seemed consistent with MRI of human patients (Ito et al., 2015). Cell death evaluated by Tunel staining was not changed in ventricular zone or in cerebral cortex during development (Ito et al., 2015) even though PQBP1 had been implicated in alternative splicing of Bcl-x and in apoptosis (Moore et al., 2010).

Intensive analyses of cell cycle of NSCs including cumulative labeling in vivo elucidated cell cycle time elongation is not limited to a single phase, instead total cell cycle time was elongated rather proportionally among four cell cycle phases (Ito et al., 2015)(Fig. 4). The result was in reverse consistent with the concept that abnormal M phase affects asymmetrical cell division, increases neurogenesis from NSCs, and changes of thickness ratio among multiple cortical layers (Caviness et al., 2003; Cox et al., 2006; Dehay and Kennedy, 2007; Fietz and Huttner, 2011; Gotz and Huttner, 2005). In the case of PQBP1 mutations, such changes do not occur and instead the total number of cell division times during development is decreased in each NSC (Ito et al., 2015). In this case, however, we need to hypothesize that the timing of layer specific differentiation is regulated by embryonic days rather than cell division times of NSCs, and this hypothesis needs to be confirmed.

As underlying mechanism of cell cycle time elongation, we found that expression levels of numerous molecules related to different cell cycle phases were changed by transcription or splicing (Ito et al., 2015) (Fig. 5). Especially, APC4 seemed to be important because electroporation of APC4 partially rescues cortical expansion (Ito et al., 2015). However, expression changes at transcription and splicing levels were widely observed, the other molecules could also affect different phases of cell cycle (Ito et al., 2015).

Regarding the function of PQBP1 in splicing, different finding was also reported from the group of Professor Silver. They had previously reported that PQBP1 is a splicing factor whose deletion shifts the balance of alternative splicing isoforms from the anti-apoptotic isoform Bcl-xL toward the proapoptotic isoform Bcl-xS (Moore et al., 2010). They used UV-cross linking combined with immunoprecipitation to search binding partners of PQBP1 and found a component of U2 splicing complex, SF3B1. Knock-down of PQBP1 in embryonic neurons changes alternative splicing of a number of genes related to synaptic transmission, dendrite development, axogenesis and so on (Wang et al., 2013). PQBP1 knock-down changes alternative splicing of NCAM140 and suppresses dendrite growth, which was rescued by wild type PQBP1 but not disease mutants (∆AG and Y65C) (Wang et al., 2013).

As described, microcephaly is a critical symptom of PQBP1-linked intellectual disability. PQBP1-linked microcephaly belongs to primary microcephaly without architectural change of the brain (Ito et al., 2015). Thus considering the generally accepted concept of primary microcephaly, it is possible that PQBP1 dysfunction leads to abnormal cell cycle of neural stem cells (NSCs) during development and causes brain size reduction. In addition, the abnormal cell cycle in the other types of tissues might also explain the small stature and lean body that are frequently found in the patients (Kalscheuer et al., 2003; Lenski et al., 2004; Stevenson et al., 2005; Lubs et al., 2006).

6. PQBP1 causes symptoms via deregulation of gene expression

We next investigated the effect of PQBP1 on RNA splicing given that U5-15kD is supposed a regulatory protein of splicing site recognition judging from its dynamics among various splicing complexes (Makarov et al., 2002). U5-15kD is conserved beyond species back to fission yeast (Berry and Gould, 1997), and the mutation of the yeast homologue Dim1p affects cell cycle and leads to G2 arrest (Berry et al., 1999). Therefore, it is easy to imagine that effect of PQBP1 mutations would change cell cycle through dysfunction of U5-15kD.
The third case of the effect of PQBP1 knockdown was observed in neuronal cilia by the group of professor Bonni (Ikeuchi et al., 2013). They screened the effect on cilia of primary hippocampus neurons by shRNA-mediated knockdown of X-linked intellectual disability (XLID) disease proteins, and found that the PQBP1 reduction severely disrupts growth of neuronal cilia (Ikeuchi et al., 2013). The pathway is mediated by interaction between dynamin2 and PQBP1 via WW domain, thus Y65C mutation of PQBP1 might influence neuronal cilia (Ikeuchi et al., 2013).

Though our approaches did not detect SF3B1, it is possible that both of the two splicing factors (U5-15kD and SF3B1) bind to PQBP1 additively, collaboratively, alternatively or exclusively, and influence RNA splicing under physiological and pathological conditions. The possibility needs further investigation. Collectively, PQBP1 interacts with Pol II and some splicing related proteins, plays the role at the junction between transcription and RNA splicing of Pol II, and modulate gene expression patterns. Abnormal gene expression patterns under the functional deficiency of PQBP1 impair cell cycle of NSCs, dendrite elongation of neurons, neuronal cilia and so on. It is highly plausible that such cell functional phenotypes lead to the patient phenotypes like microcephaly, intellectual disability and other symptoms.

7. Other animal models of PQBP1

To investigate physiological and pathological functions of PQBP1, other types of animal models were reported. First, we generated human PQBP1-overexpression fly model, which showed impairment of long-term memory by Pavlovian olfactory conditioning (Yoshimura et al., 2006). In addition the hPQBP1-Tg flies showed abnormal courtship (Yoshimura et al., 2006). However, it should be noted again that Drosophila homologue of PQBP1 lacks CTD and expression of human full-length PQBP1 might be artificial. Therefore, we next investigated a hypomorph mutant Drosophila model (Tamura et al., 2010). In this mutant fly, a piggyBac transposon was inserted in the first exon of Drosophila PQBP1 homologue (CG11820), and dPQBP1 protein expression was severely decreased (Tamura et al., 2010). Olfactory conditioning revealed impairment of learning acquisition but not short-term, medium-term, long-term or anesthesia-resistant memory (Tamura et al., 2010). The underlying mechanism was shown to be decreased expression of NMDA receptor subunit 1 (NR1) in projection neurons (Tamura et al., 2010) (Fig. 6). Targeted knockdown of dPQBP1 in projection neurons but not in mushroom body showed similarly impaired learning acquisition, and the symptom of the mutant fly was recovered by selective expression of NR1 in projection neurons (Tamura et al., 2010). These findings indicated that NR1 is a core protein in the pathology of PQBP1-linked ID (Fig. 6). In fact, gene expression profiles of PQBP1-cKO mice also
revealed the similar decrease of NR1 (our unpublished observation).

Nematode (C. elegans) also possesses two homologues of PQBP1 (Takahashi et al., 2009a). TD21D12.3 is a complete homologue possessing WW domain and CTD, while T07A9.1 does not have WW domain but possesses a homologous sequence to CTD (Takahashi et al., 2009a). At L1 stage, PQBP1 orthologue TD21D12.3 is expressed in progenitor neurons in head ganglion. At L2-L4 stage, pharyngeal and intestinal cells that store lipid expressed PQBP1 at high levels (Takahashi et al., 2009a). The mutant of TD21D12.3 but not that of T07A9.1 revealed decrease of lipid storage in intestine cells and decrease of triglyceride contents in the nematode body (Takahashi et al., 2009a). These data collectively suggest involvement of PQBP1 in lipid metabolism.

8. Appropriate levels of PQBP1 are essential

Mouse PQBP1-transgenic mice overexpressing PQBP1 under the control of CMV enhancer and chicken beta-actin promoter were generated (Okuda et al., 2003). PQBP1-mRNA rather than PQBP1-protein is increased in the transgenic mice, and F0 mouse shows a late-onset motor neuron disease phenotype including loss of spinal motor neurons (Okuda et al., 2003). Therefore, though the phenotypes were different, both overexpression and underexpression of PQBP1 are shown to cause neurological deficits (Okuda et al., 2003; Ito et al., 2015).

Combined analyses of multiple Drosophila models also revealed that appropriate levels of PQBP1 are essential for keeping intelligence and lifespan (Tamura et al., 2013). Both over- and under-expressions of PQBP1 shorten the lifespan of Drosophila, while underexpression but not overexpression impairs short-term memory (Tamura et al., 2013). Finally, it is also of note that duplication of PQBP1 gene was found in human ID patients, which could lead to overexpression of PQBP1 in the brain (Honda et al., 2010).

9. PQBP1 is a sensor of cell stress?

Recently, surprising aspects of PQBP1 were uncovered. First, PQBP1 seems to be a cytoplasmic receptor of HIV-1 (human immunodeficiency virus-1). PQBP1 interacts with reversely transcribed cDNA of HIV-1-RNA and also cGAS to trigger IRF3-mediated transcription of immune response genes in innate immune cells like dendritic cells (Yoh et al., 2015). The cGAS-cGMP-STING pathway is generally used to sense cytoplasmic DNA that does not exist normally (Cai et al., 2014). The exogenous DNA from virus infection and endogenous DNA damage repair systems overlay (Trigg and Ferguson, 2015). Thus, especially in neurons, DNA damage and repair signals, which is one of the common pathology of neurodegeneration (Shiwaku and Okazawa, 2015), may merge with such exogenous DNA-triggered signals. In this process, PQBP1 may also be involved. Interestingly, C-terminal domain of PQBP1 interacts with HIV1-cDNA (Yoh et al., 2015) like the interaction with splicing factors (Waragai et al., 2000; Zhang et al., 2000; Mizuguchi et al., 2014), suggesting that the similar torsion of gene expression profiles may occur in multiple pathological conditions such as neurodegenerative diseases, intellectual disability and virus infections (Fig. 7).

As mentioned, PQBP1 forms nuclear and cytoplasmic foci, nuclear body (Okazawa et al., 2002) and stress granules (Kunde et al., 2011). Various cellular stresses could influence formation and distribution of both structures, and mutant proteins of neurodegenerative diseases could be understood as one of such cellular stresses (Okazawa et al., 2002; Okazawa, 2003).

Second, PQBP1 is suggested to be one of the major contributing molecules to coronary heart diseases (Talukdar et al., 2016). The analyses are based mainly on systems biology with mRNA expression data (Talukdar et al., 2016), thus further investigation with wet
Integration of knowledge of PQBP1 leads to “two hit hypothesis” of neurodegeneration. Step 1 is a scheme that has been accepted in the research of neurodegeneration, though the toxicity was believed triggered by aggregates. Instead, in this “two hit hypothesis”, the misfolded protein before aggregation interacts with PQBP1 or other intrinsically disordered denatured proteins based on the assembly mechanism of denatured proteins, and directly trigger cell dysfunction and cell death (Step 2). The Step 2 processes are actually similar to the developmental disorder causing microcephaly corresponding to brain atrophy (dendritic atrophy, dendritic spine loss, cell death) and intellectual disability corresponding to dementia (synapse dysfunction).

10. Two-hit theory that explains pre-aggregation neurodegeneration

As described, PQBP1 was identified as a protein binding to the polyQ tract sequence (Imafuku et al., 1998) that possesses a higher affinity to an expanded allele of two polyQ disease proteins, Atxn1 (Okazawa et al., 2002) and Htt (Waragai et al., 1999). On the other hand, human genetics of XLID revealed that PQBP1 is a causative gene for ID and microcephaly (Kalscheuer et al., 2003; Lenski et al., 2004; Stevenson et al., 2005; Lubs et al., 2006).

From these considerations, it is possible to assume “two-hit hypothesis” (Fig. 8). Functional disturbances of PQBP1 and other associating molecules may occur by such interaction with neurodegenerative disease proteins in the nuclear or cytoplasmic foci of PQBP1. Both PQBP1 and neurodegenerative disease protein possess the similar characteristics to be disordered/denatured. The accumulation of disease proteins in PQBP1 foci should impair the functions of PQBP1 in RNA transcription, RNA splicing, and protein translation. Meanwhile, in developmental disorders like ID, PQBP1 is functionally deficient and causes cellular dysfunction and possibly cell death. This scheme is probably applicable in general to other neurodegenerative diseases caused by dysfunctions of TDP43, FUS or hnRNPA1. In that case, functions of TDP43, FUS or hnRNPA1 in RNA splicing would be impaired by accumulation of such disease proteins.

Importantly, according to the “two-hit theory”, cellular dysfunctions occur before protein aggregation, and functional decline of neural networks in the brain, which causes dementia, paresis, ataxia and so on, can be explained without protein aggregation. Even brain atrophy might be partially explained by the “two-hit theory” because loss of PQBP1 remarkably reduces a single neuron volume due to loss of dendrites and shrinkage of cell body (Ito et al., 2015). Adult neurogenesis might also be affected by dysfunction of PQBP1 according to the “two-hit theory”, while experimental verification is awaited.

11. Conclusion and perspective

PQBP1 is located at the crossroad of neurodegenerative and developmental diseases. PQBP1 is a typical example of newly emerged key molecules for neurodegeneration that possess intrinsically disordered/denatured structure or low complexity domain. Judging from the morphological and molecular phenotypes of neurons in ID and microcephaly, it is highly plausible that PQBP1 is a second hit mediator of neurodegeneration pathway. The two-hit theory could be widely applicable to neurodegenerative diseases in general, and the new theory would be able to replace “aggregation theory” or at least to complement the weak points of “aggregation theory”.

Interestingly various isoforms of PQBP1 by alternative splicing exist (Iwamoto et al., 2000). Following the discussion in previous sections, such alternative splicing isoforms should be functional or non-functional, and the non-functional isoforms could be dominant-negative to functional isoforms. Therefore, the ratio among various isoforms might be related to intellectual ability and should be investigated.

In conclusion, future researches of PQBP1 would open new fields of neurodegenerative diseases, intelligence and developmental disorders, and would provide us critical hints for clinical applications.

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