Trends in Genetics

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Spotlight

Open Access: A Role for p53 in c9ALS/FTD?

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Poly(PR), a toxic dipeptide-repeat protein, translated from the pathogenic G_4C_2 repeat expansion in *C9orf72*, contributes to c9 amyotrophic lateral sclerosis/frontotemporal dementia (c9ALS/FTD). However, precisely how poly(PR) elicits neurodegeneration has remained unclear. Maor-Nof *et al.* now establish that poly(PR) remodels the neuronal epigenome to promote proapoptotic p53 activity involving PUMA, which drives neurodegeneration in several models.

ALS and FTD are two devastating fatal neurodegenerative diseases, which share many features, including the accumulation of cytoplasmic TDP-43 aggregates in degenerating neurons [1]. Treating ALS/FTD presents a serious unsolved challenge because the underlying mechanisms that drive disease are poorly defined. The expansion of a G_4C_2 hexanucleotide repeat in the *C9orf72* gene is a frequent genetic cause of ALS and FTD (these cases are termed 'c9ALS/FTD') [2]. However, it is still unclear how the G_4C_2 hexanucleotide repeat expansion elicits neurodegeneration [2]. There is evidence for three, non-mutually exclusive hypotheses that attempt to explain c9ALS/FTD etiology: one based on changes in *C9orf72* expression itself; one based on the accumulation of G_4C_2-repeat expansion-containing RNA (c9RNA); and one based on the production and aggregation of the translated products of c9RNA, dipeptide-repeat proteins (DPRs) [2]. Among the c9DPRs, poly(PR) and poly (GR) are particularly toxic [3]. Intriguingly, recent work suggests that the epigenome is also affected in ALS and FTD [4,5]. In a new study, Maor-Nof *et al.* investigated how the chromatin accessibility landscape of neurons changes in response to toxic poly(PR) or TDP-43 overexpression [6]. They discovered that poly(PR), but not TDP-43, specifically increases the accessibility of binding sites for the transcription factor (TF) p53, and that p53 is essential for poly(PR)-mediated neurodegeneration (Figure 1). Thus, Maor-Nof and colleagues describe a novel epigenetic mechanism linking the pathogenic G_4C_2 repeat expansion in *C9orf72* with neurodegeneration in ALS/FTD [6].

First, to probe changes to chromatin accessibility in a relevant model, Maor-Nof *et al.* adapted the assay for transposase-accessible chromatin using sequencing (ATAC-seq) for neurons, a feat that had not been achieved before [6]. Performing ATAC-seq on primary murine cortical neurons revealed a complex landscape of genomic regions with altered accessibility upon TDP-43 or poly(PR) overexpression [6]. Changes to chromatin accessibility also suggest changes to patterns of gene expression. Open chromatin is transcriptionally active, whereas closed chromatin is transcriptionally inactive. Thus, bioinformatics were deployed to search for TF-binding motifs that were differentially accessible in each condition. Strikingly, TDP-43-expressing neurons exhibited changes in accessibility for many TFs, whereas poly(PR) expression was associated with increased accessibility for a single TF group: the p53 family [6]. This finding was notable because p53 levels can be elevated in patients with ALS, and p53 has been linked to motor neuron degeneration [7].

To explore the relationship between poly (PR) expression and p53 activity, Maor-Nof *et al.* measured p53 levels and found that they were elevated in poly(PR)-expressing neurons [6]. This increase was due to the stabilization of p53 itself, because the high levels of p53 persisted after treatment with cycloheximide to prevent additional translation [6]. Astoundingly, in primary cortical neurons from *p53*-knockout (KO) mice, neither poly(PR) expression nor the expression of another c9DPR, poly(GR), caused cell death [6]. This neuroprotective effect was specific to poly(PR) and poly(GR), because TDP-43 expression was still toxic in *p53*-KO neurons [6]. Furthermore, in the *p53*-KO neurons, poly(PR) levels were not changed, indicating that poly(PR) is not immediately toxic to neurons (unlike yeast, which lack p53 [8]), but instead that poly (PR) toxicity is strictly mediated by p53 [6].

Remarkably, these findings were extended to a mouse model. In mice where poly(PR) was expressed throughout the brain via adeno-associated virus [5], *p53* KO significantly increased mouse lifespan relative to mice that were *p53* positive [6]. As expected, complete p53 ablation also accelerated the rate of tumorigenesis [6]. Nonetheless, even a modest knockdown (KD) of *p53* was neuroprotective, indicating that just lowering p53 activity could be a therapeutic strategy for c9ALS/FTD [6]. In wild-type mice, p53 is abundant and almost entirely colocalized with poly(PR) in neurons [6]. Consistent with the *in vitro* experiments, poly(PR) levels were also high in the p53-KO mice [6]. Thus, poly(PR) neurotoxicity is stringently dependent on p53 expression *in vivo*. Additionally, in agreement with the neuronal culture data, the protective effect of *p53* KO was specific to poly(PR), because *p53* KO or KD did not improve outcomes for mice overexpressing TDP-43 [6].

Importantly, p53 depletion also mitigated toxicity of the G_4C_2 hexanucleotide repeat and not just artificial expression of poly(PR) or poly(GR) [6]. In *Drosophila* expressing $(G_4C_2)_{30}$, and in induced pluripotent stem cells (iPSCs) and motor neurons (iMNs) from patients with c9ALS, reducing p53 levels protected against toxicity [6]. c9iMNs exhibit increased DNA damage, which promotes apoptosis [9]. Maor-Nof *et al.* showed that the DNA damage itself is

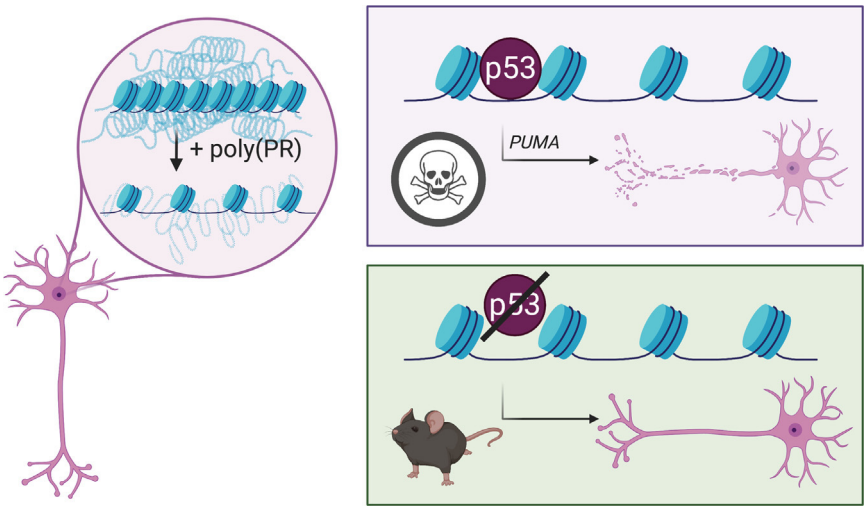

Figure 1. Poly(PR) Remodels Chromatin to Enable a p53 Transcriptional Program That Drives Neurodegeneration. Expression of poly(PR) causes changes to chromatin accessibility, specifically revealing binding motifs for the transcription factor, p53. Importantly, p53 enables a transcriptional program that drives neurodegeneration via PUMA, a proapoptotic member of the Bcl-2 protein family. Reducing p53 or PUMA levels confers robust neuroprotection against poly(PR). Figure created with BioRender.

related to *p53* expression, because decreasing p53 levels reduced the extent of DNA damage, thus preventing apoptosis [6].

Maor-Nof *et al.* next explored whether *Puma*, a p53-target gene and a pro-apoptotic member of the Bcl-2 family, which was upregulated in their ATAC-seq data, was a downstream effector of p53-associated cell death. PUMA is involved in axonal degeneration during development of the nervous system and, thus, might also be relevant in a neurodegenerative context. Excitingly, reducing PUMA levels in mouse cortical neurons prevented PR_{50} toxicity [6]. Thus, PUMA is among the targets of p53 that underlie its role in neurodegeneration. PUMA may represent a more viable therapeutic target than p53, because *Puma*-KO mice are not prone to spontaneous tumorigenesis. Thus, small-molecule inhibitors of PUMA or antisense oligonucleotides (ASOs) to reduce PUMA expression may provide a more actionable route to the clinic for c9ALS/FTD.

Through this pathbreaking work, Maor-Nof *et al.* establish that the expression of poly (PR) alters the epigenetic landscape of neurons, revealing p53-binding sites that enable expression of proapoptotic genes [6]. However, the mechanisms underlying the changes in chromatin accessibility are unclear, as is why p53 is stabilized and activated so specifically by poly(PR). These questions warrant further investigation. Despite potent effects in mitigating poly(PR) neurotoxicity, p53 KD was ineffective against TDP-43 neurotoxicity [6]. Since a large proportion of non-c9 ALS/FTD cases present with TDP-43 proteinopathy [1], targeting p53 or PUMA may only be useful in c9ALS/FTD. Moreover, patients with c9ALS/FTD also present with TDP-43 proteinopathy [2], which is not found in the poly(PR) mouse model used by Maor-Nof *et al.* [5,6]. Thus, it remains uncertain whether targeting p53 or PUMA would be beneficial in situations where both poly(PR) and TDP-43 simultaneously contribute to neurotoxicity, as is likely the case in c9ALS/FTD [2]. Further studies are required to address this issue. Reducing c9DPR toxicity and TDP-43 proteinopathy is possible, as has been demonstrated with ASOs targeting G_4C_2 repeats [10]. Finally, it will be of interest to further unravel how TDP-43 (and other proteins associated with neurodegenerative disease) alters the neuronal epigenetic landscape, which might suggest new disease mechanisms and treatments.

Declaration of Interests

C.M.F. has no interests to declare. J.S. is a consultant for Dewpoint Therapeutics and Maze Therapeutics.

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