Cell Research

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RESEARCH HIGHLIGHT

TDP-43 triggers immune response via mitochondrial DNA release

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Cell Research (2021) 31:379–380; https://doi.org/10.1038/s41422-020-00461-x

The molecular mechanisms of toxicity associated with cytoplasmic accumulation of TAR DNA binding protein-43 (TDP-43), a pathological feature of many neurodegenerative diseases, are not fully understood. A recent study by Yu et al. is proposing that noxious neuroinflammatory stimulation associated with TDP-43 pathology is caused by excess TDP-43 penetration into mitochondria followed by cytosolic release of mitochondrial DNA (mtDNA) and activation of the cGAS/STING immune sensor.

The loss of nuclear TDP-43 and its cytoplasmic accumulation in neurons are key pathologic hallmarks of many neurodegenerative diseases including amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), limbic-predominant age-related TDP-43 encephalopathy and Alzheimer's disease. It has remained a matter of debate whether disease pathogenesis occurs via the loss of TDP-43 nuclear function or through the adverse effects of cytoplasmic TDP-43 accumulations. There is evidence that the nuclear loss of TDP-43 can contribute to RNA splicing dysregulation[1] and to decondensation of chromatin structure.[2] On the other hand, cell culture studies revealed that the extent of cytoplasmic TDP-43 mislocalization was a good predictor of neuronal toxicity.[3] Cytoplasmic TDP-43 aggregates disrupt nuclear pore complexes and nucleocytoplasmic transport.[4] Moreover, TDP-43 deregulation can also provoke hyperactive innate immune responses in affected neurons.[5] For instance, in the spinal cord of ALS patients the nuclear factor κB (NF-κB) p65 was detected in the nucleus of neurons and glial cells, reflecting abnormal activation of the NF-κB pathway in the disease.[5] The involvement of neuronal NF-κB hyperactivation in ALS pathogenesis is further supported by the recent report that neuron-specific expression of a super-repressor form of the NF-κB inhibitor IκB ameliorated disease phenotypes in mouse models of ALS/FTD.[6] Swarup et al.[5] suggested a decade ago that hyperactivation of neuroinflammation in ALS was the result of excess levels of TDP-43 which can bind and co-activate NF-κB p65. Yet, this mechanism, which implied a direct interaction of TDP-43 with NF-κB in the nucleus, was difficult to reconcile with nuclear depletion of TDP-43 observed in affected neurons of ALS patients.

A novel and exciting mechanism is now proposed by Yu et al.[7] to explain an intrinsic neuronal immune response associated with TDP-43 proteinopathies. Their results indicate that immune stimulation in ALS motor neurons is caused by excess TDP-43 penetration into mitochondria followed by cytosolic release of mtDNA and activation of the cGAS/STING pathway (Fig. 1). In this paradigm, the gain-of-function toxicity is driven by the cGAS/STING pathway, an immune sensor of cytoplasmic DNA, which is activated after leakage of mtDNA due to excess TDP-43 entry into mitochondria. The researchers showed that CRISPR-mediated deletion of STING in cultured cells prevented induction of inflammation, as measured by levels of tumor necrosis factor and interferon (IFN) type I, in response to overexpression of TDP-43 wild-type (WT) or mutant species. Moreover, pharmacological inhibition of cGAS or STING pathway by drugs, RU.521 and H-151, respectively, blocked neuroinflammation in induced pluripotent stem cell (iPSC)-derived motor neurons from ALS patients bearing TDP-43 mutations. Immunoprecipitation of cGAS followed by qPCR analysis confirmed that the source of DNA activating cGAS in context of TDP-43 pathology was of mitochondrial origin. High-resolution microscopy of cells overexpressing TDP-43 allowed visualization of mtDNA leakage into the cytosol that was associated with TDP-43 translocation into mitochondria.

How is TDP-43 translocated into mitochondria? Evidence based on knockdown experiments suggested that TDP-43 penetrates the mitochondria matrix via the mitochondrial import inner membrane translocase TIM22.[8] The mitochondrial TDP-43 is soluble and uncleaved. TDP-43 is not a transmembrane protein but it contains some stretches of hydrophobic amino acids that were found to be critical for the mitochondrial import via TIM22 translocase.[8] The study by Yu et al.[7] showed that competitive peptides spanning these hydrophobic motifs, which blocked mitochondrial import of TDP-43,[8] also prevented leakage of mtDNA into the cytosol of ALS patient iPSC-derived motor neurons. The presence of TDP-43 in mitochondria was found to cause loss of membrane potential and upregulation of reactive oxygen species generating mtDNA damages. Such mitochondrial destabilization can result in opening of the mitochondrial permeability transition pore (mPTP) with subsequent mtDNA release. In support of this mechanism, pharmacological inhibition of mPTP with cyclosporine A stopped the TDP-43-mediated leakage of mtDNA into the cytosol and downstream inflammation. The voltage-dependent anion channel 1 (VDAC1) is another component that may contribute to mtDNA release since an inhibitor of VDAC1 (VBIT-4) also reduced cytosolic mtDNA and inflammation in cultured ALS patient iPSC-derived motor neurons.

To validate in vivo the involvement of the cGAS/STING pathway in the TDP-43-mediated pathogenesis, Yu et al.[7] generated transgenic mice overexpressing human TDP-43^{A315T} which are Sting deficient. It should be noted that in agreement with the proposed disease mechanism, there was activation of the cGAS/STING and presence of cytosolic mtDNA in the cortex and spinal cord of TDP-43^{A315T} mice. Remarkably, the absence of Sting in TDP-43^{A315T} mice slowed down disease progression by 58% and

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Published online: 18 February 2021

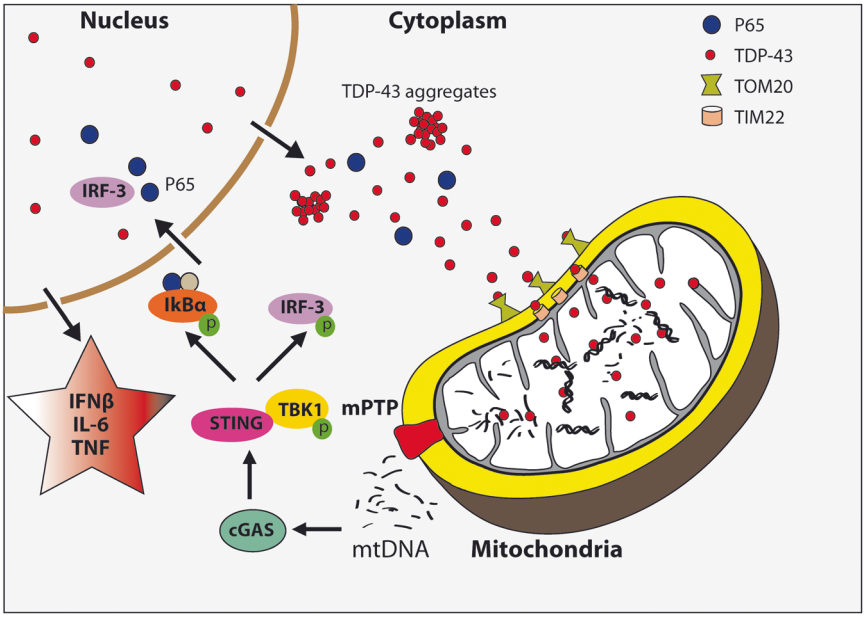

Fig. 1 Excess levels of cytoplasmic TDP-43 lead to mitochondrial penetration of TDP-43 which may trigger mtDNA release with ensuing activation of the cGAS/STING immune sensor. Activation of this pathway induces pathogenic NF-κB and IFN signaling.

extended the life span by 40%. Moreover, the deletion of Sting in TDP-43^{A315T} mice ameliorated motor performance, downregulated levels of type I IFN and NF-κB, and reduced the loss of cortical neurons. Similarly, pharmacological treatment of TDP-43^{A315T} mice with a STING inhibitor (H-151) after disease onset decreased the levels of neuroinflammatory markers, mitigated neuronal loss in the cortex and ameliorated motor performance in the rotarod test. This new model of TDP-43-mediated toxicity was depicted by Yu et al.[7] as an intrinsic neuronal pathway. However, such adverse effects of TDP-43 overexpression or mutations may not be limited to neurons. A previous study showed that overexpression of WT or mutant TDP-43 in glial cells can make them hyperactive to immune stimulation resulting in enhanced toxicity toward neighboring neuronal cells.[5]

Many potential therapeutic targets emerge from this new TDP-43-mediated pathogenic pathway. One promising target is cytoplasmic TDP-43. Therapeutic approaches aiming to reduce the levels of cytoplasmic TDP-43 have recently yielded promising results with different model systems. Thus, neuronal delivery of antibodies targeting TDP-43 were found to reduce the cytoplasmic levels of TDP-43 via proteasome degradation and to ameliorate disease phenotypes in mouse models of ALS/FTD.[9] Clearance of excess TDP-43 pharmacologically by inducing autophagy also conferred beneficial effects in mouse models of ALS/FTD.[10] This study by Yu et al.[7] should stimulate ALS clinical studies with drugs, alone or in combination, aiming to block mitochondrial import of TDP-43, to mitigate mtDNA damage or to inhibit cGAS/STING with ensuing downstream NF-κB and IFN signaling. The remarkable protection of STING inhibitor in a mouse model of ALS is consistent with previous reports that inhibition of NF-κB signaling reduced pathology and ameliorated motor and cognitive performance in transgenic mice expressing TDP-43 mutants.[5,6] It is also of interest that suppression of neuronal NF-κB signaling can concurrently enhance autophagy activity thereby mitigating the cytoplasmic accumulation of TDP-43,[6] the primary driver of this novel neuronal inflammation pathway.

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