identifying new oncogenic pathways and mutated genes, some of which were virtually unknown in cancer. These findings also have clinical relevance, providing elements for refined diagnosis and new therapeutic strategies.

The most surprising finding was the frequent somatic mutations in the genes encoding the transcription factor *TCF3* and its negative inhibitor *ID3*, affecting up to 70% of sporadic and immunodeficiency-associated BL and 40% of endemic tumors. *ID3* mutations (38–68%) were more common than *TCF3* mutations (11%), and most of the former were inactivating and biallelic or were associated with deletion of the other allele, suggestive of a tumor-suppressor function. In contrast, *TCF3* mutations were monoallelic and occurred at conserved residues, suggesting that they could be activating. Functional studies showed that action of the TCF3 protein and mutated ID3 were critical for the survival and proliferation of BL cells[7]. Schmidt *et al.* studied the transcriptional program of TCF3 in BL cells and found that it modulates essential genes for germinal center function (**Fig. 1a**) and also upregulates ID3, creating an inhibitory loop that in normal cells would attenuate TCF3 action[7]. Interestingly, the *ID3* locus is a direct target of MYC and may also contribute to downregulating the TCF3 pathway in normal cells[8]. Therefore, it is likely that the inactivating mutations of *ID3* in BL release TCF3 from its inhibitory function (**Fig. 1b**). TCF3 also promoted the survival of BL cells by intensifying B-cell receptor (BCR) signaling through the phosphoinositide-3-kinase (PI3K) pathway, and it promoted their proliferation by modulating cell cycle–related genes such as *CCND3* (ref. 7) (**Fig. 1a**). Interestingly, activating mutations in *CCND3* were also found in 38% of sporadic BL but only in a minority of endemic tumors[5,7]. These results are remarkable because a recent mouse model in which PI3K signaling cooperates with MYC develops a lymphoma that recapitulates the characteristics of human BL, including frequent *CCND3* mutations[9]. These observations strongly support the cooperation of *MYC* translocation with the mutations of *ID3*, *TCF3* and *CCND3* in the pathogenesis of BL (**Fig. 1b**).

Richter *et al.*[5] concluded that *ID3* mutations have the imprint of the mutational machinery of the germinal center, a mechanism that acts physiologically on the immunoglobulin genes to increase their affinity for the respective antigen[5]. This finding is intriguing because diffuse large B-cell lymphomas (DLBCL), a different type of aggressive lymphoma that also originates in cells that have experienced this mutational microenvironment, do not have *ID3* mutations[5–7]. The reason for this apparent discordance is not clear, but it suggests that BL and DLBCL may originate in cells of the germinal center with different dependence of the ID3-TCF3 pathway. The lymphoid germinal center has two distinct topographic and functional areas, recognized as the dark and light zones (DZ and LZ) (**Fig. 1a**). The DZ is highly proliferative, whereas cells in the LZ are beginning to differentiate towards effector cells. TCF3 is more abundant in DZ than in LZ cells, and the expression profile of BL is more similar to that of DZ cells whereas the profile of DLBCL is closer to that of LZ cells, supporting the idea that these two types of lymphomas may arise from different subpopulations of the germinal center[10].

Clinical implications and next steps

The finding of *ID3* mutations exclusively in BL may help in the diagnosis of aggressive lymphomas[11]. Richter *et al.* found that tumors with a gene expression profile intermediate between those of BL and DLBCL but carrying *ID3* mutations had clinical and biological features closer to those of conventional BL[5]. Current therapeutic protocols have improved the outcome in children but are less successful in adults[12], although the toxicity of these treatments is high and they are difficult to apply in low-income countries[3,12]. The identification of this new pathogenic pathway essential for BL cells may provide new potential therapeutic targets.

The mutations recognized in these studies do not seem to be present in all patients, and their different distribution in sporadic and endemic BL is intriguing[7]. It will be important to clarify whether the remaining patients have mutations in genes of the same or other pathways. The relationship between these genetic alterations and EBV infection also needs further study, particularly in relation to the different distribution of the virus and *ID3* or *CCND3* mutations in the epidemiological subtypes of the disease.

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TDP-43 toxicity and the usefulness of junk

Shuying Sun & Don W Cleveland

A new study shows that loss of the lariat debranching enzyme Dbr1 suppresses TDP-43 toxicity. The accumulated intronic lariat RNAs, which are normally degraded after splicing, likely act as decoys to sequester TDP-43 away from binding to and disrupting functions of other RNAs.

A paradigm shift in understanding the mechanisms of several neurodegenerative diseases is underway, with an increasing focus on errors of RNA metabolism and processing. The catalyst for this shift was the discovery that 43-kDa TAR DNA-binding protein (TDP-43) is a major component of ubiquitinated protein aggregates found in sporadic amyotrophic lateral sclerosis (ALS) and the most common form of frontotemporal dementia (disease with ubiquitinated inclusions, or FTLD-U)[1,2]. Subsequently, mutations in the genes encoding TDP-43 and a related RNA binding protein, FUS/TLS, were found to cause both diseases. Moreover, TDP-43 immunoreactive inclusions have been observed not just in inherited and sporadic ALS and FTLD-U, but also in Alzheimer's, Parkinson's and Huntington's diseases[3]. Typical pathology in affected individuals includes reduced accumulation of TDP-43 in nuclei and the presence of cytoplasmic inclusions[4]. While the disease mechanisms

Shuying Sun and Don W. Cleveland are at the Ludwig Institute for Cancer Research and Department of Cellular and Molecular Medicine, University of California at San Diego, La Jolla, California, USA.
e-mail: dcleveland@ucsd.edu

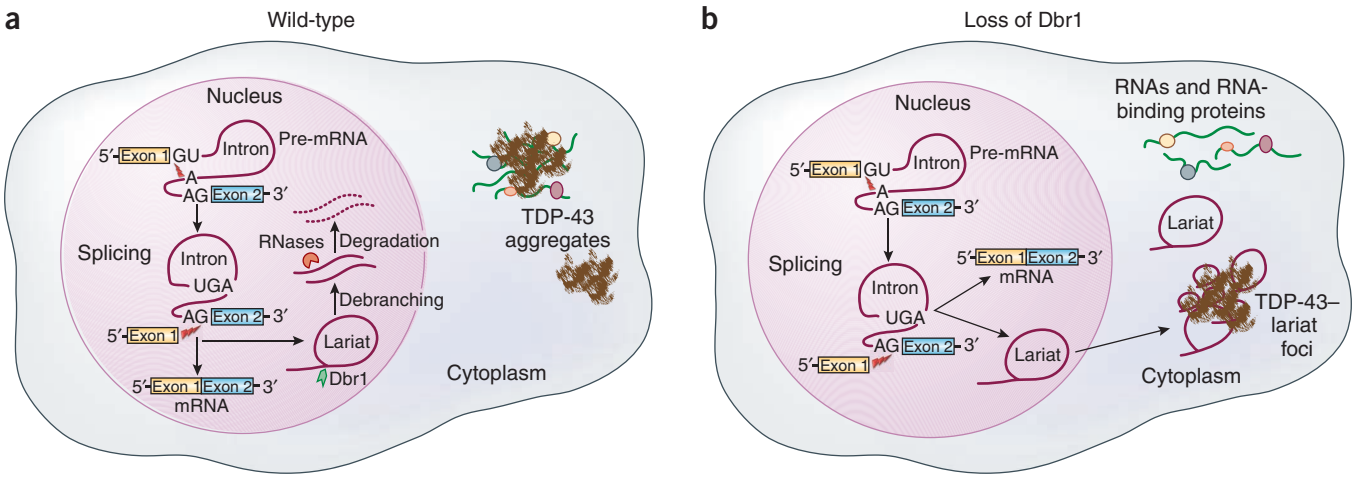

Figure 1 Model for suppression of TDP-43 toxicity following loss of Dbr1. (**a**) Dbr1 normally converts intronic lariats into linear RNAs that are subsequently degraded by exonucleases. Cytoplasmic TDP-43 aggregates sequester normal RNAs and RNA-binding proteins, thereby reducing their function. (**b**) Without Dbr1, lariats accumulate cytoplasmically, sequestering TDP-43 and diminishing its interference with normal RNAs.

remain unclear, they probably involve both loss of normal nuclear TDP-43 function and gain of toxic properties from cytosolic aggregates. There is intense interest in elucidating the mechanisms underlying TDP-43-induced pathogenesis, as this could provide therapeutic opportunities for a range of diseases. In this issue, Aaron Gitler and colleagues[5] apply genome-wide loss-of-function screens in yeast to discover an unexpected, potentially druggable modifier of TDP-43 cytotoxicity.

Yeast modifier screens

Although an overexpression screen to identify modifiers of TDP-43 aggregation and toxicity in yeast cells has been reported previously[6], Armakola *et al.*[5] have now performed two genome-wide loss-of-function screens to identify new candidate genes that modify toxicity from high expression of TDP-43 in yeast. They identified six enhancers and eight suppressors in the first screen and an amazing number of potential enhancers and suppressors (2,581 and 2,056, respectively) in the second screen. They then focused on one of the most effective suppressors of TDP-43 toxicity, which emerged from both screens: loss of the lariat debranching enzyme Dbr1, which cleaves the 2′-5′ phosphodiester linkage at the branch point of circular lariats formed upon the excision of introns from precursor mRNAs (pre-mRNAs). Dbr1 converts lariats into linear RNAs that are subsequently degraded[7,8], and this function is conserved from yeast to humans. After the initial screen, further analyses of point mutants defective in debranching activity showed that inhibition of Dbr1 enzymatic function is required to suppress TDP-43 toxicity.

Next, Armakola *et al.*[5] tested whether reducing Dbr1 activity could mitigate TDP-43-dependent toxicity in two mammalian cell contexts. Initially, they used siRNA to transiently lower Dbr1 in a mitotically cycling, undifferentiated human neuroblastoma cell line in which doxycycline treatment induced accumulation of the ALS-causing mutant TDP-43^{Q331K} to a level comparable to that which would be expected for a dominantly inherited mutation. By the end of the assay, mutant TDP-43 produced toxicity in a minority (20%) of cells (the effects of wild-type TDP-43 were not reported), and this toxicity was mostly alleviated by siRNA-mediated reduction of Dbr1. The authors then performed a more relevant test using primary cortical neurons transiently transfected with a construct encoding TDP-43-EGFP, along with siRNA against Dbr1 or a control siRNA. Expression of wild-type TDP-43 provoked toxicity over an 8-day period (the amount of accumulated TDP-43 was not determined), and suppression of Dbr1 provided what the authors argue is a degree of protection, albeit one that, it must be acknowledged, many readers will probably find too modest (42% versus 50% survivors in the presence or absence of Dbr1, respectively) to be fully persuasive.

Useful RNA junk

How might accumulation of intron-derived lariats protect against TDP-43 toxicity? High-throughput sequencing and splicing-sensitive microarrays have shown TDP-43 depletion to have broad effects on mRNA levels and alternative splicing[9,10]. Many of the target genes encode proteins that are related to neuronal functions and/or implicated in neurological diseases. Genome-wide sequencing of RNAs bound by TDP-43 has also been performed in several systems[9–12], and a consensus GU-rich binding motif has been identified[9–13], with most TDP-43 binding sites lying deep in introns[9–12]. This makes it reasonable to hypothesize that lariat intron—the 'junk' RNA that is normally degraded in wild-type cells but accumulates in *dbr1Δ* cells—serves as a decoy to sequester TDP-43 away from binding to and inhibiting the functions of normal RNAs that would otherwise be trapped in TDP-43 aggregates (**Fig. 1**).

Armakola *et al.*[5] also used a combination of immunoprecipitation and electrophoretic mobility shift assays to show that TDP-43 directly binds to lariat RNAs. To determine how and where the benefit of lariat accumulation occurs, they visualized lariats in living yeast with a tethering technique using an RNA-binding MS2 coat protein, combined with immunocytochemistry. Lariat RNAs were found to colocalize with TDP-43 in bright foci in the cytoplasm of *dbr1Δ* yeast cells. This surprising result provokes many questions. How does the lariat RNA get from the nucleus into the cytoplasm? Does the same phenomenon occur in mammalian cells, especially in non-cycling, differentiated neurons? Why might the relocalization of TDP-43 from one cytosolic location (aggregates) to another (lariat foci) reduce its toxicity? Is the lariat structure itself or the sequence typically bound by TDP-43 in the intron (a GU-rich domain) essential for binding?

Furthermore, as none of three other yeast mutations that produce defects in nonsense-mediated decay (NMD), and thereby accumulate a different class of non-functional RNAs that are normally targeted for rapid degradation, affected TDP-43 toxicity, Armakola *et al.*[5] propose that rescue of TDP-43 toxicity is specific to intronic lariat RNA accumulation. However, it is also possible that the amount of RNA accumulating in these NMD mutant strains is not as high as after loss of Dbr1 and that toxicity is therefore not alleviated because TDP-43 is not successfully competed away from normal cellular RNAs.

Clarifying the sequestration mechanism in mammalian cells is essential for developing therapeutic strategies based on reducing Dbr1 activity. First, it will be important to establish whether any other RNA-binding proteins are sequestered onto lariat introns and away from their normal interaction partners, as this could obviously be harmful to cells. This concern is especially important because splicing is rare for pre-mRNAs in budding yeast, whereas it is prominent in most mammalian pre-mRNAs. It must also be determined whether TDP-43 sequestration to lariat decoys in the cytoplasm still promotes loss of its crucial nuclear function in the nervous system[9–12].

Whatever the case, this study[5] sheds new light on how dysfunction of RNA metabolism acts in ALS and other neurodegenerative diseases. Future work to clarify the underlying pathogenic mechanisms will include characterization of candidate genes beyond *Dbr1* that were identified in the genetic screens and whose loss of function either alleviates or exacerbates TDP-43 toxicity.

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Digenic inheritance and Mendelian disease

James R Lupski

Powerful genomic technologies, such as exome sequencing, are providing new insights into the genetics underlying Mendelian traits. A new study identifies a role for digenic inheritance and an epigenetic modifier in facioscapulohumeral muscular dystrophy type 2.

Traditional wisdom states that phenotypes that seem to segregate in a Mendelian fashion are caused by alterations in a single gene or a unique locus. Recently, exceptions to this notion have been identified. In this issue, Silvère van der Maarel, Stephen Tapscott and colleagues show that facioscapulohumeral muscular dystrophy type 2 (FSHD2, also known as FSHD1B; MIM 158901) results from digenic inheritance of an allele of the D4Z4 microsatellite array on chromosome 4, which is permissive for the expression of the embedded *DUX4* gene, and single-nucleotide variation (SNV) at the *SMCHD1* locus[1]. Their work shows that a point mutation in *SMCHD1* (encoding structural maintenance of chromosomes flexible hinge domain containing 1) acts as an epigenetic, epistatic modifier of the D4Z4 allele and thus seems to be a genetic determinant underlying the FSHD2 disease trait. It is possible that *SMCHD1* variants may also be involved in other human diseases and phenomena, such as X-chromosome inactivation, that are subject to epigenetic dysregulation.

James R. Lupski is in the Department of Molecular and Human Genetics and the Department of Pediatrics at the Baylor College of Medicine, Houston, Texas, USA, and at the Texas Children's Hospital, Houston, Texas, USA.
e-mail: jlupski@bcm.edu

FSHD2 and digenic inheritance

FSHD (MIM 158900) is a form of muscular dystrophy characterized clinically by facial and upper extremity muscle weakness that can be asymmetric and progress to involve both upper and lower extremities. The clinical phenotypes of FSHD1 and FSHD2 are indistinguishable, and both are associated molecularly with DNA hypomethylation and decreased levels of repressive heterochromatin (referred to as chromatin relaxation) at the D4Z4 array on chromosome 4. Each D4Z4 repeat contains a copy of *DUX4*, and the permissive D4Z4 allele encodes a polyadenylation signal that stabilizes *DUX4* mRNA. Chromatin relaxation results in inefficient epigenetic repression of *DUX4*, yielding a variegated pattern of DUX4 protein expression in subsets of skeletal muscle nuclei. Such ectopic expression of this homeobox developmental gene causes derepression and overexpression of stem cell and germline genes that can eventually result in apoptotic cell death[2].

Lemmers *et al.*[1] developed a method to quantify methylation patterns at D4Z4 arrays on chromosomes 4 and 10 and noted that, in some kindreds with FSHD2, the D4Z4 hypomethylation trait segregated in a pattern consistent with autosomal dominant inheritance but without linkage to either the chromosome 4 or chromosome 10 D4Z4 array haplotype. These data suggested that variation at a separately segregating locus might determine the ultimate penetrance of the FSHD2 clinical trait. Whole-exome sequencing was used to identify heterozygous *SMCHD1* variants that cosegregated with the D4Z4 hypomethylation trait in selected families with FSHD2 or occurred *de novo* in individuals with the sporadic hypomethylation trait and FSHD2. Notably, *Smchd1* was first identified in a mouse mutagenesis screen for modifiers of the variegated expression of a multicopy transgene[3].

Other examples of digenic inheritance

Early evidence for digenic inheritance in humans was found in retinitis pigmentosa, with variants at two unlinked genes with photoreceptor-specific expression, *ROM1* and *PRPH2* (RDS/peripherin)[4]. Subsequent molecular characterization showed that the encoded proteins interact and that such interactions are critical for stable photoreceptor disc formation[5]. A more complex digenic model with triallelic inheritance was identified in studies of Bardet-Biedl syndrome[6]. In this disease, mutations at two loci are often required for penetrance of the clinical phenotype; however, at one locus, two mutant alleles seem to be necessary, whereas, at the other locus, just one allelic mutation is required to cause disease[6]. Subsequent work has provided strong evidence that epistatic interactions contribute to both penetrance and variable phenotypic expression across the ciliopathy spectrum[7], further establishing the concept of mutational load in human genetic disease.