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EDITORIAL

Making sense of the antisense transcripts in C9FTD/ALS

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In medicine, Occam's razor is employed to enforce diagnostic parsimony upon complicated cases. Stated simply, Occam's razor says that the single disease that best accounts for all of a patient's symptoms is likely to be the primary cause of all of them. In biomedical research, a similar logic is often applied, triggering searches for a single central pathogenic mechanism as the primary (or sole) pathway that causes a given disease. The rationale for this approach is clear: only when we know the one true cause of a disease can we know where to focus our efforts for therapeutic development. In this issue of Acta Neuropathologica, five new papers suggest that successful application of Occam's razor to the recently described C9-associated frontotemporal dementia and amyotrophic lateral sclerosis (C9FTD/ALS) is going to be a challenge.

C9FTD/ALS is a dominantly inherited intronic GGGGCC hexanucleotide repeat expansion in C9orf72, a neuronally expressed gene of unknown function [7, 20]. It accounts for a significant fraction of all inherited cases and ~4 % of sporadic cases of amyotrophic lateral sclerosis and frontotemporal dementia in Caucasian populations [7, 9]. Pathologically, patients exhibit multiple types of proteinaceous neuronal inclusions, including TDP-43 positive cytoplasmic aggregates as well as P62 and ubiquilin positive but TDP-43 negative cytoplasmic inclusions [6, 7, 10, 16, 22, 23]. Additionally, neurons in patient tissues exhibit nuclear RNA foci containing GGGGCC repeat transcripts [7].

Nucleotide repeat expansion disorders are thought to elicit toxicity via three non-exclusive mechanisms [19]. As DNA, repeats can alter local chromatin structure and impact RNA transcription in cis, leading to suppressed RNA and protein expression from the gene in which they reside [5]. Alternatively, transcribed repeats as RNA can bind to and sequester RNA-binding proteins and prevent them from performing their normal functions [17]. Lastly, translated repeats can alter the normal functions of the proteins in which they reside while also directly eliciting toxicity via alterations in proteostasis [24]. Two additional factors add complexity to the determination of which mechanism is at play in any given disorder. First, many repeats are bi-directionally transcribed, leading to two potentially toxic RNAs from any given repeat [15]. Second, many nucleotide repeats appear capable of triggering protein translation in the absence of an initiator AUG codon through a process known as "RAN Translation" [4].

In C9FTD/ALS, there is evidence for each of these potential pathogenic mechanisms. The GGGGCC repeat expansion is associated with a decrease in detectable amounts of multiple C9orf72 mRNA isoforms [7, 9]. In addition, multiple groups observe the appearance of GGGGCC RNA foci and a growing list of potential RNA-binding proteins that could be sequestered by this RNA await further characterization [13, 18, 25]. Earlier this year, two different groups demonstrated that RAN translation leads to production of three different dipeptide repeat containing proteins (Gly-Arg, Gly-Pro, and Gly-Ala) that form P62 positive aggregates observed in patients [1, 14].

The new papers published here provide further support for each of these possible disease mechanisms, while adding a new player to consider: toxicity elicited by production

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of an antisense transcript through the repeat. This CCCCGG repeat containing transcript triggers formation of neuronal and (less frequently) glial RNA foci in C9FTD/ALS patient tissues [8, 21]. These foci are seen predominantly in the nucleus, but they can occur in the cytoplasm, suggesting that these transcripts could also be targets for RAN translation. Indeed, both Gendron et al. [8] and Mori et al. [12] demonstrate the presence of antisense-derived RAN translation products in all three reading frames, producing Arg-Pro, Gly-Pro, and Ala-Pro dipeptide repeat proteins. These antisense RAN products accumulate in P62 positive aggregates, just like the RAN products derived from GGGGCC sense transcripts. Interestingly, the presence of RNA foci and the RAN aggregates appears to be distinct (and potentially competing) events, such that the majority of neurons with sense or antisense RNA foci do not exhibit RAN-mediated inclusions and vice versa [8, 21]. Consistent with this observation, Mackenzie and colleagues find that the distribution of one particular RAN product within the CNS of C9FTD/ALS patients is similar across clinical phenotypes and is anti-correlated with both cytoplasmic TDP-43 aggregate formation and neurodegeneration [11]. As they wisely point out, this could indicate either that these aggregates are in fact protective or that production of C9RAN proteins is not a factor in pathogenesis.

Lastly, Belzil et al. [2] demonstrate that the local chromatin structure around the repeat is altered in both patient tissue samples and in blood from patients, with increased histone H3 and H4 tri-methylation in two neighboring CpG islands. These chromatin marks are typically associated with decreased transcription and they observe less mRNA in C9orf72 isoforms in patient samples and cells by RT-PCR. Interestingly, they were able to reactivate transcription of the gene in patient-derived cells with demethylating agents.

So what should we make of all of this? First, the pathology is telling us that all of these processes do occur in the majority of patients with the clinical disease. What remains unclear is (1) which event (if any) is proximal in triggering neurodegeneration; (2) what components are necessary and sufficient to elicit toxicity and (3) what additive or synergistic interactions these components exhibit in disease pathogenesis. Early data suggest that both RNA alone and C9orf72 loss of function alone are capable of eliciting relevant phenotypes in simple model systems [3, 25]. However, the relevance of these findings in mammalian systems at expression levels seen in patients is less clear. In addition, teasing out the relative contributions of each transcript and each RAN product will be difficult, given our current inability to dissociate the production of these two components. Lastly, although one might anticipate that antisense transcripts and RAN translation products will be less abundant than their sense transcript counterparts, one must be cautious about dismissing even low level accumulation of potentially highly toxic molecules.

The corollary in clinical medicine to Occam's razor is known as Hickam's dictum, which states that "Patients can have as many diseases as they damn well please". Future studies will undoubtedly extend our understanding of how each of these different processes contributes to C9FTD/ALS disease pathogenesis individually. However, we may in the end need to accept that effects of the repeat on local chromatin structure as DNA, as a sink for GGGGCC and CCCCGG repeat binding proteins as RNA, and as different RAN protein products have additive (and perhaps synergistic) influences on disease biology. Such a messy reality will make development of effective therapeutics more difficult, but ignoring such a reality carries equal risk.

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