Targeting RNA G-quadruplexes as new treatment strategy for C9orf72 ALS/FTD

Martin H Schludi1,2 & Dieter Edbauer1,2

The recent discovery of a pathogenic expansion of a (GGGGCC)n repeat in the C9orf72 gene in amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) led to a burst of mechanistic discoveries. In this issue, Simone et al (2018) describe novel compounds targeting the G-quadruplex (G-Q) structure of the (GGGGCC)n repeat RNA that alleviate the hallmarks of C9orf72 disease in patient-derived neurons and increase survival in a Drosophila model. Lack of overt off-target effects and toxicity suggest that these small molecules are promising lead compounds to the development of a therapy.

See also: R Simone et al (January 2018)

Since the discovery of the (GGGGCC)n repeat expansion upstream of the coding region of C9orf72 as the most common genetic cause of ALS and FTD, tremendous progress toward understanding disease mechanisms and developing therapies has been made (Edbauer & Haass, 2016). The repeat RNA forms small foci within the nucleus and is thought to sequester several RNA-binding proteins and thereby alter gene expression and splicing. Surprisingly, both sense and antisense transcripts are translated in all reading frames by an unconventional mechanism into five co-aggregating dipeptide repeat (DPR) proteins: poly-GA, poly-GP, poly-GR, poly-PA, and poly-PR. The DPRs also bind key cellular proteins (including RNA-binding proteins), and their relative role in pathogenesis is under intense investigation. The so-called repeat-associated non-ATG (RAN) translation has been first discovered for (CAG)n repeat expansion disorders (Zu et al, 2011) and was later reported for several other repeat expansions diseases (Cleary & Ranum, 2017). The mechanism remains elusive, but seems to depend on the secondary structure of the repeat RNA. Therefore, several groups have analyzed the RNA structure of (GGGGCC)n repeats in vitro and in vivo and found that the repeat RNA can form both so-called G-quadruplexes (G-Qs) and hairpins. G-Qs are four-stranded structures containing stacks of four guanines that associate through Hoogsteen hydrogen bonding within one plane (Fig 1). This structure can form from a single or up to four separate RNA or DNA strands. In contrast to G-Qs formed from DNA, RNA-based G-Qs are more stable and compact as a consequence of more intramolecular interactions. Moreover, RNA G-Qs preferentially assemble in a parallel conformation. Thus, DNA and RNA G-Qs can be selectively targeted. Endogenous RNA G-Qs within untranslated regions and introns regulate transcription, alternative splicing, and protein binding. Hairpins composed of a base-paired stem and a loop are the most common RNA structures and can also affect transcription and alternative splicing. Thus, targeting the secondary structure of the disease-associated (GGGGCC)n RNA is a potential therapeutic strategy.

In 2014, Su et al repurposed a compound originally identified as an interactor of (CGG)n in fragile X-associated tremor ataxia syndrome for the C9orf72 repeat RNA (Su et al, 2014). This study describes in total three compounds (1a, 2, 3), which interfere mainly with the hairpin structure of the (GGGGCC)n RNA resulting in translational inhibition. In patient-derived neurons, compound 1a significantly reduced RNA foci and DPR proteins and showed no overt toxicity, but their more potent compound 2, an aromatic diamidine, was too toxic to validate in patient-derived neurons. None of the compounds were tested in animals.

In contrast, Simone et al (2018) specifically targeted the G-Q structure and screened a library of 138 small molecules with known or suspected binding to G-Q structures (Fig 1). They used a FRET-based melting assay for the initial screen and selected three compounds that had a much larger effect on the G-Q formation of (GGGGCC)n RNA than of (GGGGCC)n DNA. The three best compounds have a nearly identical atomic structure and are, like the most potent compound in Su et al, aromatic diamidines. Circular dichroism spectroscopy confirms direct binding to repeat RNA with 200–400 nM affinity. At 1 μM concentration, two of the compounds reduced RNA foci in iPSC-derived spinal motor neurons and cortical neurons by about 50%. At higher concentration (16 μM) and later time points, both compounds also reduced poly-GP expression by up to 50%, which is likely due to the long half-life time of poly-GP. In contrast to the aromatic diamidine compound from Su et al, no toxicity was observed at the effective concentration. In addition to these biochemical and cellular assays, Simone et al (2018) fed their best compound (DB1273) to flies modeling (GGGGCC)n repeat toxicity (Mizielinska et al, 2014). Adult flies expressing (GGGGCC)36 showed a pronounced reduction in poly-GP. Moreover, feeding larvae with the compound led
Another way to boost the efficiency of the nanospheres or nanocapsules (Lu et al, 2014) also developed a new poly-GR immunoassay to directly show the effect on the main toxic species. Indeed, (GGGGCC)n modified compounds to (GGGGCC)n G-Qs and azides could promote cross-linking of the RNA structure, because 1,3-dipolar cycloaddition of alkynes to a modest increase in survival. Since poly-GR is the main driver of toxicity in this model, Simone et al (2018) also developed a new poly-GR immunoassay to directly show effect on the main toxic species. Indeed, stabilizing the G-Q structure of (GGGGCC)36 using DB1273 also reduced poly-GR levels by 33%, which is consistent with the moderate survival benefit. Since brain penetrance of DB1273 is still low, medicinal chemists may be able to improve the in vivo effects significantly in the future.

The Isaacs laboratory is covering a lot of ground already by validating their novel compounds specifically inhibit RAN translation or would also inhibit ATG-initiated translation of the structured repetitive RNA. RAN translation seems to highly depend on the RNA structure, because only (CAG)n, but not (CAA)n repeats are translated into poly-Q in the absence of an ATG-start codon (Zu et al, 2011), suggesting that targeting the secondary structure of RAN-translated repeat RNAs is a potential strategy to slow or stop disease progression. A compound affecting RAN translation of different repeat RNAs might be beneficial for many diseases (Cleary & Ranum, 2017).

In conclusion, this paper is an encouraging and timely study, because our understanding of C9orf72 pathogenesis is growing rapidly and the first treatment options, such as antisense oligonucleotides are already on the horizon (Jiang et al, 2016). Patients can only benefit from the intense interest in the C9orf72 mutation as academia and industry race for a treatment.

Acknowledgements
We apologize for not citing all relevant original literature due to space constrains. D.E. received funding from the European Community’s Health Seventh Framework Programme under grant agreement 617198 [DPR-MODELS].

References
Bernat V, Disney MD (2015) RNA structures as mediators of neurological diseases and as drug targets. Neuron 87: 28–46
Cleary JD, Ranum LP (2017) New developments in RAN translation: insights from multiple diseases. Curr Opin Genet Dev 44: 125–134
Di Antonio M, Biffi G, Mariani A, Raiber EA, Rodriguez R, Balasubramanian S (2012) Selective RNA versus DNA G-quadruplex targeting by in situ click chemistry. Angew Chem Int Ed Engl 51: 11073–11078
Edbauer D, Haass C (2016) An amyloid-like cascade hypothesis for C9orf72 ALS/FTD. Curr Opin Neurobiol 36: 99–106
Jiang J, Zhu Q, Gendron TF, Saberi S, McAlonis-Dowmes M, Seelam A, Stauffer JE, Jafar-Nejad P, Drenner K, Schulte D et al (2016) Gain of toxicity from ALS/FTD-linked repeat expansions in C9ORF72 is alleviated by antisense oligonucleotides targeting GGGGCC-containing RNAs. Neuron 90: 535–550
Lu CT, Zhao YZ, Wong HL, Cai J, Peng L, Tian XQ (2014) Current approaches to enhance CNS delivery of drugs across the brain barriers. Int J Nanomedicine 9: 2241 – 2257

Figure 1. Small molecules stabilize the GGGGCC RNA G-quadruplex structure.
Small aromatic compounds with two amidine residues preferentially interact with the parallel G-Q of the (GGGGCC)n repeat RNA and stabilize this structure. Stabilization of the G-quadruplex structure reduces RNA foci formation and inhibits repeat translation. Note that other G-Q confirmations are possible.
pathology in vitro and in vivo. *EMBO Mol Med* 10: 22–31

Su Z, Zhang Y, Gendron TF, Bauer PO, Chew J, Yang WY, Fostvedt E, Jansen-West K, Belzil VV, Desaro P et al (2014) Discovery of a biomarker and lead small molecules to target r(GGGGCC)-associated defects in c9FTD/ALS. *Neuron* 83: 1043–1050

Zu T, Gibbens B, Doty NS, Gomes-Pereira M, Huguet A, Stone MD, Margolis J, Peterson M, Markowski TW, Ingram MA et al (2011) Non-ATG-initiated translation directed by microsatellite expansions. *Proc Natl Acad Sci USA* 108: 260–265