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siRNA-knockdown screening for potential RAN translation modulators in cellular models of *C9orf72* FTLD/ALS

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A hexanucleotide expansion in the intron of *C9orf72* is the most frequent cause of genetic form of FTLD/ALS. The repetitive DNA repeat suffers transcription and produces repeat RNA. The repeat RNA further undergoes repeat associated non-AUG (RAN) translation. In the expanded *C9orf72* repeat, RAN translation produces five distinct dipeptide repeat proteins (DPR). Of the DPRs, poly-(Gly-Ala), poly-(Gly-Arg) and poly-(Pro-Arg) are thought to be highly neurotoxic, at least in disease models, and may be so in human patients. Therefore, selective inhibition of RAN translation could have therapeutic potential. Although several factors have been proposed as potential RAN translation modulators, complete picture of RAN translation remains obscure.
Here we have utilized nano-luciferase based RAN translation reporters that can monitor either poly-GA or poly-GR expressions. Conventional translation was monitored with firefly luciferase reporter activity expressed at AUG initiation codon following the optimal Kozak sequence. With 22 different siRNA-mediated knockdowns of translation-related factors, we have identified several factors that significantly inhibit/stimulate relative signals of RAN translation reporter over conventional AUG dependent reporter. Multiple siRNA targeting different region of the top hit molecules on the screening confirmed similar effect, further validating the involvement of these factors on RAN translation. These results implicate that *C9orf72* RAN translation could be modulated via multiple endogenous factors. Further experimental work will clarify how these factors contribute to RAN translation, thereby elucidating one important aspect of the pathogenesis of *C9orf72* FTLD/ALS.

Conflicts of interest

None to disclose.