Alleviation of RNA toxicity by small molecules targeting repeat RNAs responsible for neurological diseases

Department of Regulatory Bioorganic Chemistry, The Institute of Scientific and Industrial Research (SANKEN)

Assistant Professor Tomonori Shibata Researchmap https://researchmap.jp/7000010597

Professor Kazuhiko Nakatani Researchmap https://researchmap.jp/read0042668

Abstract

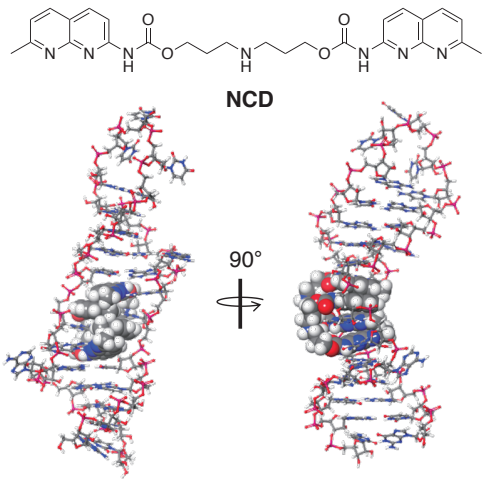
Aberrant expansion of repeat sequences causes hereditary neuromuscular diseases called repeat expansion diseases. Transcription of the expanded repeats produces repeat RNAs termed as toxic RNAs, which is thought to be associated with a pathogenetic mechanism of repeat expansion diseases. Small molecules modulating structure and function of the toxic repeat RNAs have therapeutic potential for repeat expansion diseases. We have discovered a small molecule that binds to the UGGAA repeat responsible for spinocerebellar ataxia type 31 (SCA31) by screening using in-house chemical library containing nucleic acid-targeting small molecules. The UGGAA repeat-binding small molecule showed inhibitory effect on RNA-protein interaction and the formation of RNA aggregates consisting of UGGAA repeats in the nucleus, alleviating the RNA toxicity caused by UGGAA repeats in the *Drosophila* model of SCA31.

Background & Results

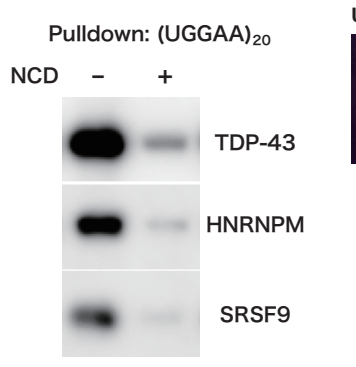
Spinocerebellar ataxia type 31 (SCA31) is an inherited autosomal dominant spinocerebellar degeneration caused by the insertion of TGGAA repeats into the intron region shared by the *BEAN1* and *TK2* genes in chromosome 16. The UGGAA repeats transcribed from the inserted TGGAA repeats are thought to exhibit RNA toxicity by sequestration of RNA-binding proteins followed by the formation of RNA aggregates in the nucleus. Previous studies have reported that TDP-43, which has been identified as a UGGAA repeat-binding protein, alleviates the RNA toxicity of SCA31. Based on these reports, we performed the exploratory study on UGGAA repeat-binding small molecules to alleviate the RNA toxicity of SCA31 by small molecules. We found naphthyridine carbamate dimer (NCD) as UGGAA repeat-binding small molecules by screening using in-house chemical library containing nucleic acid-binding small molecules. The structural analysis of the NCD-RNA complex by NMR clarified that two molecules of NCD bound to the 5'-GGA-3'/3'-AGG-5' internal loop in UGGAA/UGGAA pentad via hydrogen bonding with guanine. In vitro pulldown assay revealed that NCD interfered with the binding of RNA-binding proteins to UGGAA repeats. The inhibitory effect of NCD on the formation of RNA aggregates containing UGGAA repeats in the nucleus was examined by RNA fluorescence in situ hybridization, demonstrating that NCD disrupts RNA aggregates. Feeding NCD to larvae of the *Drosophila* model of SCA31 suppressed the degeneration of compound eyes in the adults. These results demonstrate the alleviation of the RNA toxicity in SCA31 by a small molecule binding to UGGAA repeats.

Significance of the research and Future perspective

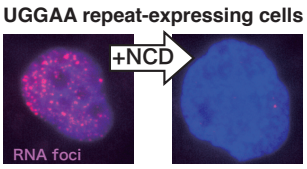
Although RNA has been thought to be undruggable, it has recently been attracting attention as a drug target. Small molecules that target disease-causing RNAs have the potential to open up new therapeutic strategies for intractable diseases that have not been targeted by conventional protein-targeted drug discovery. The discovery of small molecules targeting repeat RNAs and the elucidation of their mechanisms of action are expected to develop therapies of incurable repeat expansion diseases.



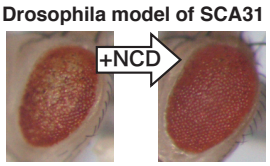
Structure of NCD–RNA complex



Inhibition of RNA–protein interaction



Disruption of RNA foci



Alleviation of compound eye degeneration

Patent Japanese Patent Application No. 2019-182767

Treatise Shibata, T et al. Small molecule targeting r(UGGAA)$_n$ disrupts RNA foci and alleviates disease phenotype in *Drosophila* model. Nat. Commun. 2021; (12): 236. doi: 10.1038/s41467-020-20487-4.

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Keyword RNA, repeat expansion diseases, RNA-targeting small molecules, spinocerebellar degeneration, drug discovery