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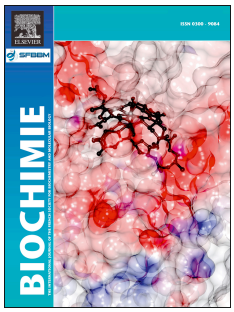

Comprehensive identification of proteins binding to RNA G-quadruplex motifs in the 5′ UTR of tumor-associated mRNAs

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Abstract

G-quadruplex structures in the 5' UTR of mRNAs are widely considered to suppress translation without affecting transcription. The current study describes the comprehensive analysis of proteins binding to four different G-quadruplex motifs located in mRNAs of the cancer-related genes *Bcl-2, NRAS, MMP16,* and *ARPC2*. Following metabolic labeling (Stable Isotope Labeling with Amino acids in Cell culture, SILAC) of proteins in the human cell line HEK293, G-quadruplex binding proteins were enriched by pull-down assays and identified by LC-orbitrap mass spectrometry. We found different patterns of interactions for the G-quadruplex motifs under investigation. While the G-quadruplexes in the mRNAs of *NRAS* and *MMP16* specifically interacted with a small number of proteins, the *Bcl-2* and *ARPC2* G-quadruplexes exhibited a broad range of proteinaceous interaction partners with 99 and 82 candidate proteins identified in at least two replicates, respectively. The use of a control composed of samples from all G-quadruplex-forming sequences and their mutated controls ensured that the identified proteins are specific for RNA G-quadruplex structures and are not general RNA-binding proteins. Independent validation experiments based on pull-down assays and Western blotting confirmed the MS data. Among the interaction partners were many proteins known to bind to RNA, including multiple heterogenous nuclear ribonucleoproteins (hnRNPs). Several of the candidate proteins are likely to reflect stalling of the ribosome by RNA G-quadruplex structures. Interestingly, additional proteins were identified that have not previously been described to interact with RNA. Gene ontology analysis of the candidate proteins revealed that many interaction partners are known to be tumor related. The majority of the identified RNA G-quadruplex interacting proteins are thought to be involved in post-transcriptional processes, particularly in splicing. These findings indicate that protein-G-quadruplex interactions are not only important for the fine-tuning of translation but are also relevant to the regulation of mRNA maturation and may play an important role in tumor biology. Proteomic data are available via ProteomeXchange with identifier PXD005761.