ABSTRACT

Influenza A virus (IAV), a cause of seasonal epidemics and occasional pandemics, is a significant research subject. Despite the high variability of its genome, viral RNA (vRNA) secondary structures remain conserved across IAV strains. Moreover, the role of vRNA structural motifs in the viral replication cycle has been previously described, making them interesting targets in anti-influenza strategies.

Among the particularly interesting nucleic acid secondary structures are G-quadruplexes (G4s). The G4 motifs are noncanonical DNA or RNA structures formed within guanine-rich regions. As a result, these motifs have significant biological functions, taking part in many cellular processes. In recent years, G-quadruplexes have been identified in viral genomes, where they play an important role in the viral replication cycle. Therefore, G-quadruplexes represent a promising target in antiviral therapies.

The first stage of my studies aimed to determine whether potential quadruplex-forming sequences (PQSs) are present within the IAV genome (A/California/04/2009(H1N1) strain). To this end, bioinformatic analyses were conducted, enabling the identification of 12 highly conserved PQSs within the IAV vRNA. In the next step of the research, we confirmed the RNA G-quadruplex formation using several spectroscopic techniques and polyacrylamide gel electrophoresis assay. Based on the obtained results, we revealed that three PQSs form stable RNA G-quadruplexes *in vitro*. Interestingly, two of these motifs were found within sequences encoding polymerase subunits and one within the hemagglutinin-coding region, suggesting their role in the viral replication cycle.

In the second part of the research, we investigated whether G4-specific ligands interact with RNA G-quadruplexes from the IAV genome. The aim of our study was to observe differences in binding modes of G4-specific ligands (*in vitro*). For this purpose, two small-molecule compounds, TMPyP4 and BRACO-19, were selected. We studied the G4/ligand interactions using reverse transcription stop assay, isothermal titration calorimetry, circular dichroism, fluorescence spectroscopy, and UV melting analysis. Importantly, differences were observed in the interaction properties between specific RNA G-quadruplexes and their ligands, with distinct binding modes identified for TMPyP4 and BRACO-19. Furthermore, we conducted biological studies in cell culture to evaluate the influence of TMPyP4 and its analogue, TMPyP2, on IAV minireplicon activity. The experiment results revealed a significant inhibition of viral replication upon the ligand addition, suggesting that selected G4s may be potential anti-influenza drug targets. To complement our studies, we employed molecular

modeling to simulate the folding of one selected RNA G-quadruplex. This method allowed us to gain additional insights into G4 topology and structure stability.

Overall, we concluded that RNA G-quadruplex structures are present within the A/California/04/2009(H1N1) genome and are highly conserved across strains. Notably, these motifs can be targeted by G4-specific small-molecule ligands. What is more, viral replication is effectively inhibited via G4 interaction with TMPyP4 and TMPyP2 compounds. All our findings suggest that selected PQS motifs from the IAV genome can serve as potential novel antiviral therapeutic targets.