Investigations on structural and physicochemical features potentially correlated with G-quadruplexes antiproliferative activity

Abstract

Despite substantial advancements in treatment of many malignancies in recent years, cancer is still the second in terms of mortality rates around the world. This emphasizes the necessity of developing the diagnostic and anticancer approaches, as well as strengthening the research on the origins of cancer formation.

G-quadruplexes are DNA or RNA structures that are formed by guanosine-rich sequences. Although guanosine-based compounds capacity to form tetrameric structures has been known since 1962, the possibility that G-quadruplex structures might be used as drugs for medicinal purposes has only received significant consideration in the last two decades. These structures are promising molecular tools that can be used to target a variety of biologically significant ligands. Moreover, G-quadruplexes have relatively small size, can be easily chemically modified and can have high stability. Additionally, they can be produced economically in large quantities and are easily internalized into cells with great resistance to nucleases, what makes them useful, targeted delivery agents.

The main goal of my research was to investigate the structural and physicochemical features of diverse G-quadruplex structures and to correlate them with their biological potential as anticancer agents.

In order to comprehend the most recent advancements in G-quadruplex-based tools, we performed a reviewed study focused on G-quadruplex based aptamers and their potential in therapeutic and diagnostic applications.
We realized that G-quadruplex structures formation is essential for the efficient inhibition of cancer cell proliferation, but little is known about the correlation between their structural elements and potential anticancer properties. Having that into account, we performed experimental studies aimed at analysis of potential correlation between G-quadruplex physicochemical properties, their structural elements and therapeutic

potential. We started by selecting five G-quadruplex-forming, sequence-related DNA molecules and studied their thermodynamic and structural properties, biostability and cellular uptake. Afterwards, we selected three sequences that demonstrated the best antiproliferative potential and we performed further studies. In the second research we investigated the influence of modified nucleic acid residues (locked nucleic acid (LNA), unlock nucleic acid (UNA), and 2′-O-methyl-RNA (2′-O-Me-RNA)) on G-quadruplex structural, physicochemical and biological properties. For this purpose, a single-position substitution in the loops or G-tetrads were introduced and the influence of modified nucleoside residues was analyzed for a total of twenty-seven modified G-quadruplex variants. Moreover, to better understand the correlation between the G-quadruplex structure, its chemical character and properties, a third type of research was performed. In this studies we examined a set of sequence-related RNA G-rich sequences and compared them with the properties of their DNA counterparts to find a possible correlation between the structure of G-quadruplexes, their thermal stability, and biological activity. All analysed G-quadruplexes differed slightly in loop length, number of G-tetrads and homogeneity of the core to facilitate finding of a structure-function relationship that could be helpful during the development of potential anticancer therapeutic agents.

The results presented in the dissertation suggest that G-quadruplex structural elements are intrinsic to their biological activity and that slight variations in sequence can initiate changes in G-quadruplex properties. More precisely, the G-quadruplexes with shorter G-tetrad cores and longer loops were more effective in inhibiting cancer cell growth and demonstrated improved ability to bind the NCL protein. In contrast, the nuclease resistance and the effectiveness of internalization of the studied oligonucleotides are strictly correlated with their thermodynamic characteristics, favoring structuralized and expanded G-tetrad core with shorter loops. We also indicated that UNA modifications are efficient modulators of the G-quadruplex thermodynamic stability, however they are poor tools to improve the anticancer properties. In contrast, LNA and 2’-O-Me-RNA modified G-quadruplexes revealed some antiproliferative potential. Moreover, DNA G-quadruplexes are better candidates as inhibitors of cancer cells proliferation compared to RNA G-quadruplexes.

Understanding the G-quadruplex structural features and their role in the biological activity of G-rich molecules might simplify the development of new and more potent G-quadruplex-based therapeutics with exceptional anticancer properties.