Molecular Approaches for Intervening Biological Functions of DNA Secondary Structures

Abstract

Nucleic acids exist as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Apart from the well-known canonical double helix, nucleic acids can also fold into various non-canonical secondary structures. The non-canonical DNA secondary structures include G-quadruplex, i-motif, Z DNA, hairpin, cruciform, triplex, etc. G-quadruplex structures are widely abundant in the eukaryotic genomes at regions of biological significance, like the telomeres (DNA and RNA G-quadruplexes), promoters of oncogenes (*c-MYC*, *BCL-2*, *c-KIT*, *k-RAS*, etc), untranslated regions of genes (*n-RAS*, *VEGF*, etc), as well as hotspots of recombination. These G-quadruplex secondary structures can act as molecular switches having roles in gene regulation, DNA damage and repair, genomic instability and are involved in diseases like cancer, diabetes, and neurological disorders.

In recent times, G-quadruplexes have evolved from mere structural curiosity to highly integral therapeutic targets evidenced from their functions in regulation of essential cellular processes like transcription, replication, genome integrity and epigenetic expression. Thus, therapeutic intervention of these G-quadruplex structures using small molecules are considered as alternative strategies for anticancer discovery.

Chapter 1 provides a general introduction of the G-quadruplex secondary structure. It also details the function and occurrence of both DNA and RNA G-quadruplexes in the geneome. Moreover, the therapeutic strategy to target G-quadruplexes have been discussed. The role of computational and biological tools to search and explore novel G-quadruplex targets have also been emphasized.

Chapter 2 demonstrates the inhibition of the most explored G-quadruplex found in the telomeric region of eukaryotic chromosomes. Stabilization of telomeric G-quadruplexes has been reported as an effective method of inhibiting telomerase mediated telomere lengthening. Indoloquinoxaline containing small molecules were screened and it was found that **IQ2** with carboxamide side chain exhibited selective binding interaction with the human telomeric G-quadruplex DNA and inhibited telomerase activity. The ligand also

triggered DNA damage, induced cell cycle arrest in S and G2/M phases and activated caspases-3/7 that ultimately led to cancer cell death through apoptotic pathway.

Chapter 3 illustrates the downregulation of an oncogenic promoter gene by finding a selective G-quadruplex ligand for the *c-KIT* G-quadruplex which is implicated in different cancer types including gastrointestinal stromal tumors, melanomas, mastocytosis, and acute myeloid leukemia. Different biophysical and biological assays were used to study the interaction of a library of small molecules with different side chains and different heteroaromatic scaffolds to ascertain the most effective G4 binder for *c-KIT* G4. A carbazole derivative, **MC-4** was found to have the highest capability for selective recognition and stabilization of the parallel *c-KIT 1* G4. Detailed investigations revealed that the **MC-4** facilitates the visualization of stabilized cellular G4s. In addition, the ligand induced *c-KIT* gene downregulation, cell cycle arrest and apoptosis in leukemia cells.

Chapter 4 emphasized the need to find novel G4 structures in the DNA and RNA sequences of a critically important gene, mammalian target of rapamycin (*mTOR*) which is implicated in various cancers. Bioinformatics tools were employed to explore the presence of potential G-quadruplex forming sequences in the mTOR gene. It was found that a G-rich sequence was capable of forming the G-quadruplex structure both in the DNA (P2) as well as the 5'UTR of the mRNA (rP2) of *mTOR* gene. The presence of the novel *mTOR* G-quadruplex was confirmed using various techniques including circular dichroism, DMS footprinting, nuclear magnetic resonance (NMR) and its biological implications were affirmed using gene expression studies. This is the first report of a novel G-quadruplex in the mRNA of the *mTOR* gene with a role in the gene regulatory mechanism of *mTOR*.

Dash 6/10/2023

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