Abstract

A number of studies have established that DNA targeting is a successful strategy in anticancer therapy. In recent years, however, the focus has shifted from double-stranded DNA to alternative DNA motifs such as G-quadruplex and i-motif structures that are often found in the telomeric and transcriptional regulatory regions of genes in almost all eukaryotic organisms. The mutually exclusive formation of G-quadruplex and i-motif structures by G/C rich sequences, which in turn impact a variety of DNA transactions, suggest that stabilization of such structures by small molecules offer alternative options for the treatment of genetic and life-style diseases. Indeed, a large number of G-quadruplex stabilizing ligands have been extensively studied for their anti-cancer activity both *in vitro* and *in vivo*.

Acetyl-CoA carboxylase catalyses the ATP-dependent carboxylation of cytosolic acetyl-CoA to malonyl-CoA. The synthesis of malonyl-CoA is the first committed step for de novo fatty acid biosynthesis pathway. Numerous lines of evidence suggest that the expression and specific activity of acetyl-CoA carboxylase is highly regulated at transcriptional, translational, and post-translational levels. The expression of human acetyl-CoA carboxylase 1 gene (ACC1) is regulated by three alternative promoters **(**PI, PII, and PIII), but all three promoters produce the same protein coding sequence. Notably, promoter 1 and 2, but not promoter 3, harbour G/C-rich *cis*-elements whose secondary structure and function remain unknown. In the present study, using multiple complementary methods such as CD spectroscopy, FRET, electrophoretic mobility shift assay, chemical foot printing and computational methods we show that G-rich *cis*-elements of PI and PII promoters fold into thermodynamically stable G-quadruplex structures, and then establish unambiguously the topologies of these structures. Furthermore, we found that PI promoter folds into two distinct G-quadruplex structures with 1:1:1 loop arrangement, while PII promoter folds into 1:4:1 loop arrangement. Human nucleolin, a conserved major nuclear protein is known to regulate gene expression through interaction with G/C-rich sequences in the genome. We show that nucleolin binds to G-quadruplex DNA formed by ACC1 promoters with high specificity and in a dose-dependent manner. Most importantly, G-quadruplex formation in ACC1 gene promoter region blocks DNA replication, suppresses transcription, and this effect was further augmented by G-quadruplex stabilizing ligands. Taken together, these results not only demonstrate the existence of G-quadruplex structures in ACC1 promoters, but also attest to their functional significance.

The formation of a G-quadruplex structure within a genomic duplex DNA region requires the separation of the G-rich strand from its complementary C-rich strand. As our study revealed that the G-rich sequences in ACC1 promoters, PI and PII, fold into G-quadruplex DNA, then the question arises whether the C-rich strand folds into i-motif structures. Using multiple complementary methods such as CD spectroscopy, FRET and electrophoretic mobility shift assay, we show that the C-rich sequences of PI and PII promoters fold into intramolecular imotif structures. The results of chemical foot printing assays indicated that whereas PI promoter folds into two distinct i-motif structures with 2:2:2 loop arrangements, PII promoter folds into 2:3:2 loop arrangement. Consistent with the significance of I-motif structures in the cellular context, we found that molecular crowding agents abet the formation of I-motif structures. These findings were ascertained by luciferase reporter activity assay. The data revealed that the C-rich sequence

complementary to the G-rich sequence in ACC1 promoters markedly attenuated luciferase expression. The attenuated activity of the reporter could be unleashed by mutations in the C-rich sequence of ACC1 promoters. Several classes of small molecules that selectively stabilize G-quadruplex structures over duplex DNA are known. G-quadruplexes adopt a wide range of conformations and thus the ligands that can stabilize one structure may not stabilize others. Previously, we have shown that benzimidazole-carbazole conjugates selectively bind and stabilize the telomeric G quadruplex structures over B-form DNA and inhibit telomerase activity and proliferation of human cancer cells. In addition, our previous studies have shown that these ligands induce topological conversion from non-parallel to parallel forms in the human telomeric G quadruplex structure. In the present study, we have investigated the interaction of bezimidazole-carbazole ligands with *c-MYC, c-KIT1, c-KIT2, VEGF* and *BCL2* promoter G quadruplex structures. CD measurements suggested that ligands induce topological changes from hybrid to stable parallel G-quadruplex DNA. Our CD melting and FID assays revealed that these ligands show higher affinity and confers stability to promoter G-quadruplexes. Further, to investigate the probable modes of binding of the ligands to various G4 DNAs at the promoter and telomeric regions, we have performed the docking studies, and which shows ligands interacts with promoter G-quadruplex. Overall, this study suggests that ligands that are earlier shown to interact with telomeric G-quadruplex DNA also stabilize promoter G-quadruplexes.

The development of ideal anti-cancer therapies that are highly efficient and exhibit minimal systemic toxicity is an important research area. The pro-drug approach has generated significant interest for selective targeting of cancerous cells. Photodynamic therapy (PDT) is one of the novel pro-drug approaches that involve activation of the pro-drug by a light stimulus. PDT involves light and a photosensitizer (PS) that in conjunction with molecular oxygen elicits cell death. A large number of ruthenium complexes have been examined for their DNA binding properties and photo-reactivities. In the present study, we have synthesized and characterized four new ruthenium azo-8-hydroxyquinoline complexes, their DNA binding properties and anticancer activities. Our studies revealed that these complexes can be stimulated by visible light to induce ROS mediated DNA photocleavage activity in a cellular environment. Interestingly, these complexes display potent cytotoxic activity in cancer cells, which is further augmented by exposure to visible light. Thus, we propose that the new Ru-complexes have the potential to be used in photodynamic therapy and as anticancer agents. Thus, the current work not only provides new insights into the regulation of ACC1 gene expression, but also identified a promising set of ruthenium metal complexes.