Journal of Physical Chemistry & Biophysics

Sabin, J Phys Chem Biophys 2013, 3:5
DOI: 10.4172/2161-0398.1000e117

Editorial Open Access

Current and Future Views in G-Quadruplex Secondary Structure as an Anti-Cancer Target

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The double stranded helical structure of B-DNA, which was proposed by Watson and Crick [1], is not the only conformation the DNA exists. Depending upon the nucleotide sequences and the physiological conditions the DNA can form different secondary structures [2]. G-quadruplex is one of the non-canonical secondary structures of the DNA, which is formed in the guanine rich regions. G-quadruplex structures arise from the stacking of G-tetrads, which is a square planar arrangement of four guanine residues attached to each other through Hoogsteen hydrogen bonding [3]. The stability of these structures depend upon the presence of alkali cations like Na^+ and K^+, which form an electrostatic interaction between the negatively charged G-tetrad center due to the presence of the carbonyl oxygen of the guanine residues. The sequence motif required for the quadruplex formation is at least four runs of guanine repeats separated by any nucleotides that are involved in the loop formation, which connects the tetrads [3].

The formation of quadruplex structures *in vitro* using guanine repeat oligonucleotides was confirmed by many biophysical methods like NMR, Circular Dichroism etc. But, it was complicated to provide a direct evidence for the formation of these structures insidea cell. Whole genome sequencing revealed that the human genome consists of many guanine rich regions in the telomere region, promoter regions of many oncogenes like RET, VEGF, c-Myc, Bcl-2 etc. and in the immunoglobulin switch regions [4]. The evidence for the formation of quadruplex structures from these guanine rich sequences was convincible when the telomere binding proteins and the DNA binding proteins were found to interact with the quadruplex structures *in vitro* [5].

Telomeres are non-coding regions present at the ends of linear chromosomes and are rich in guanine repeat sequences [6]. The telomere region has a short 3' overhang, whose integrity is critical and has to be protected from the end-end fusion and from the DNA double stranded repair machinery proteins which result in chromosomal instability.The single stranded 3' overhang region folds into a quadruplex structure and maintains its integrity [7]. The length of the telomere region is also involved in the regulation of the cell cycle [8]. During each round of DNA replication the telomere regions are shortened in length and when they reach a particular length, the cells activate p53 and undergo senescence. The cancer cells, which are immortal, evade the p53 induced senescence mechanism. This is accomplished by maintaining the telomere length by an enzyme called telomerase. Normal cells do not express telomerase but it is overexpressed in most of the cancers. The formation of G quadruplex in the telomere region inhibits the interaction of telomerase and suppresses its function [8].

The formation of the non B-DNA structures like G-qaudruplex is less favorable when the DNA is double stranded. Their formation is favorable only when the duplex DNA unwinds and becomes a single stranded DNA [9]. Unwinding of DNA occurs mainly during the DNA replication and transcription processes. Since, the promoter region of several oncogenes has guanine rich sequences; the formation of quadruplex structures was believed to regulate the transcription mechanism. The formation of quadruplex inhibits the binding of RNA polymerase and other transcriptional factors, which results in the down regulation of several oncogenes transcription [10].

Based on the biological function of the quadruplex structures in inhibiting the telomerase function and down regulating the transcription of the oncogene, these structures are considered as a potential anti-cancer target. To identify and characterize the small molecules that bind and stabilize quadruplex, it is necessary to understand the mode of interaction of a compound with quadruplex. Most of the quadruplex interacting compounds are planar and similar in size to the G-tetrad. This results in the stacking of these compounds over the G-tetrad through π-π interaction. Interaction in between the G-tetrads is thermodynamically unfavorable because the G-tetrads are highly stable and the energy required to distort them to allow the binding of the compound is too high. There are several methods developed to determine the interaction of the compounds with quadruplex [11].

Fluorescence Resonance Energy Transfer (FRET) is an important technique used to study the formation of quadruplex structures. The energy transfer between a donor flurophore and an acceptor flurophore is inversely proportional to the distance between them. Guanine rich oligonucleotides are labelled with a donor and acceptor flurophores at both the ends. The formation of quadruplex structure folds the oligos and brings both the ends together, which results in the increase in energy transfer. The energy transfer between the flurophores is measured at increasing temperature in the presence and in the absence of the compound. The temperature at which the quadruplex structure unfolds is called the melting temperature (TM), which is increased when the compounds stabilize the structure [12].

The biological effects of the compounds through the stabilization of quadruplex are determined using cell based assays. As discussed above, one of the biological relevance of quadruplex is the down regulation of oncogene transcription. The transcriptional inhibition of a compound through the specific binding to the quadruplex structure present in the

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Received December 03, 2013; **Accepted** December 06, 2013; **Published** December 11, 2013

Citation: Kumarasamy VM, Sun D (2013) Current and Future Views in G-Quadruplex Secondary Structure as an Anti-Cancer Target. J Phys Chem Biophys 3: e117. doi:10.4172/2161-0398.1000e117

promoter region is determined using luciferase reporter gene assay. A vector containing the luciferase reporter gene with an upstream promoter sequence similar to any oncogene promoter sequence, which has quadruplex forming sequences, is transfected into the cells. The decrease in the luciferase expression level corresponds to the transcriptional inhibitory effect of the compound.

Apart from the telomere region and the oncogene promoter region, the guanine rich sequences are also found in the other regions of the genome. As mentioned earlier, the formation of quadruplex structures are favorable only when the DNA is single stranded and since, DNA replication also involves the unwinding of the duplex DNA, the formation of quadruplex plays an important role in DNA replication. During DNA replication the lagging strand synthesis is a discontinuous process in which the daughter strand synthesis occurs in short fragments in the 5' to 3' direction called okazaki fragments. DNA helicase unwinds the double stranded helix for a certain length before the okazaki fragment synthesis is initiated. This single stranded region favors the formation of quadruple structure. When the DNA polymerase encounters the quadruplex, it stops the replication and results in the strand breakage and chromosomal instability [9].

The major challenge involved in targeting the quadruplex structures in anti-cancer therapy is the specificity of the small molecule in quadruplex interaction. As guanine rich regions are present in the promoter region, telomere region and also in the other regions of the genome (as described above), the most predominant region, which allows the quadruplex formation is still unclear. The compounds that interact with quadruplex formed in the promoter region also have affinity for the quadruplex structures formed in the other regions of the genome. Further research in distinguishing the quadruplex structures by using small molecules that induce their formation in specific regionis required to overcome these problems in the future.

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