

Quantitative Demonstration of DNA Quadruplex in Human Cells - A Step Forward to Cancer Biology

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BACKGROUND

Guanine-rich DNA sequences capable of adapting non-Watson-Crick model of DNA structures in vitro are prevalent in human genome. But decades have passed to ascertain that they exist in vivo in mammalian cells. G-quadruplexes (G4) are structures which seriously challenge the age-old dogma that the double helical structure of DNA is the only model of DNA capable of existing and influencing cellular functions considerably in human cells. G4 are square structures formed only of guanine residues linked together by Hoogsteen hydrogen bond and additional stabilizing energy provided by a monovalent cation coordinated with oxygen (O6) lone pairs of each guanine residue [1]. Thermodynamically highly stable, these quadruplexes are involved in gene expression, cell division, maintenance of chromosome stability and apoptosis [2, 3, 4, 5, 6]. They actually interrupt the 'normal' double helical structure of DNA forming knots and tangles.

WHY WAS THE STUDY CONDUCTED?

Several years ago, a group of researchers showed that four-stranded DNA can exist in the telomeres of macronuclei of ciliates [7]. They were also produced in test tubes [8]. However, the presence of G-quadruplex structure in mammalian cell nuclei was not observed. The study aimed to engineer a structure-specific antibody highly selective for DNA G-quadruplex structures such that quadruplex structures might be detected in human genome.

HOW WAS THE STUDY DONE?

Giulia Biffi, one of the members of this study group, produced an antibody called BG4 which specifically bound with quadruplex DNA and it

did not tag with RNA hairpin, ssDNA and dsDNA. BG4 was isolated from a library of single chain antibody clones by the method of phage display. With the help of ELISA, it was assured that BG4 did not bind with any other nuclear materials except G-quadruplex DNA. Further experiments also showed that BG4 did not possess any differential affinity for any particular G-quadruplex conformation.

A secondary antibody and then a tertiary fluorochrome-labelled antibody were tagged with the primary antibody i.e., BG4 and was applied into the fixed incubated cells to visualize the DNA G-quadruplex. To confirm the specificity of BG4, the following series of experiments were carried out: a. Pre-incubation of the antibody with excess pre-folded G-quadruplex oligonucleotides, b. Treatment with DNase I, c. Treatment with RNase, d. G4 transfection. Changes in the number of BG4 foci were noted after each intervention.

To detect the distribution of G-quadruplexes at the level of individual chromosome, they were incubated with colcemid and then BG4 was applied.

To prove the previous hypothesis of replication-dependent formation of DNA quadruplex, the amount of DNA quadruplex formed in relation to the cell cycle progression was measured. To ascertain the finding, aphidicolin was applied to inhibit DNA polymerase activity and the amount of BG4 foci was further investigated.

WHAT DID THE STUDY FIND?

The BG4 antibody was shown to specifically attach with the regions of DNA rich with G-quadruplex. Following observations further strengthen the conclusion:

- Pre-incubation of the antibody with excess pre-folded G4 oligonucleotides - Loss of BG4 foci
- DNase I treatment - Loss of BG4 foci
- RNase treatment - No change
- G4 transfection - Increase in BG4 foci

On individual chromosome observation, discrete BG4 foci were observed both within the non-

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telomeric regions and at the telomeres. In some cases, symmetrical staining of sister chromatids were observed. This finding suggests the formation of G4 within the same genetic loci of a newly replicated DNA. Greater amount of antigen-antibody complex were formed when a cell expresses some dysfunctional state and also when there was increased cell division. Lowest BG4 staining was observed during G₀/G₁ checkpoint and highest during S phase. On administration of aphidicolin during S phase, number of BG4 formation was found to decrease. The study also found that when agents like pyridostatin, shown to cause cell damage in vitro by operating on DNA G-quadruplexes [9], were administered in mammalian cells, marked increase in nuclear staining was observed. This finding confirms that agents like pyridostatin actually work primarily through the stabilization of G4.

LEARNING POINTS FROM THE STUDY

Production of genetically engineered highly specific BG4 antibody is a major breakthrough because it is thought to be an ideal tool to detect the genome wide distribution of G4 DNA structures in human in future. The antibody when applied to the mammalian cell not only helped to visualize the G4 foci in chromosomes but also it fostered the quantitative demonstration of G4 and its replication dependent modulation in formation. Therefore, the study has confirmed the physical and functional presence of G4 quadruplex DNA and opened up a huge research area to design drugs particularly small molecular agents which stabilize these G4 leading to cellular damage and subsequent apoptosis.

LIMITATIONS

The study did not actually visualize the G-quadruplex DNA as such but it concluded its finding from indirect evidence of antigen-antibody reaction. Skepticism is going on the accuracy of these antibodies working with big structures like the G-quadruplex and their ability to co-localize with cell division is rife. Although the finding is remarkable, further repeated investigations are needed to verify the results. Indirect evidences from other sources and also direct evidence is sought for.

WHAT IS THE BOTTOMLINE?

Increased formation of G4 DNA during S phase

of cell cycle points to the possibility of increased production of these structures in neoplasia. Researchers have shown that the production of G4 DNA suppresses the telomere elongation [10] and the proliferation of tumor cells [11] by inhibiting telomerase [12]. Thus, these quadruple DNA are thought to be potential target for pharmacotherapeutic agents for newer forms of cancer therapy in the future. Presence of these structures in human cells and the fact that their formation is being modulated with the course of various phases of cell cycle prompts us to think whether this might lead us to an era of personalized cancer chemotherapy.

REFERENCES

1. Sen D, Gilbert W. Formation of parallel four-stranded complexes by guanine-rich motifs in DNA and its implications for meiosis. *Nature* 1988; 334:364-6.
2. Cahoon LA, Seifert HS. An alternative DNA structure is necessary for pilin antigenic variation in *Neisseria gonorrhoeae*. *Science* 2009; 325:764-7.
3. Eddy J, Maizels N. Gene function correlates with potential for G4 DNA formation in the human genome. *Nucleic Acids Res* 2006; 34:3887-96.
4. Gomez D, Wenner T, Brassart B, Douarre C, O'Donohue MF, El Khoury V, Shin-Ya K, Morjani H, Trentesaux C, Riou JF. Telomestatin-induced telomere uncapping is modulated by POT1 through G-overhang extension in HT1080 human tumor cells. *J Biol Chem* 2006; 281:38721-9.
5. Rodriguez R, Müller S, Yeoman JA, Trentesaux C, Riou JF, Balasubramanian S. A novel small molecule that alters shelterin integrity and triggers a DNA-damage response at telomeres. *J Am Chem Soc* 2008; 130:15758-9.
6. Schaffitzel C, Berger I, Postberg J, Hanes J, Lipps HJ, Plückthun A. In vitro generated antibodies specific for telomeric guanine-quadruplex DNA react with *Stylonychia lemnae* macronuclei. *Proc Natl Acad Sci* 2001; 98:8572-7.
7. Lipps HJ, Rhodes D. G-quadruplex structures: in vivo evidence and function. *Trends Cell Biol* 2009; 19:414-22.
8. Fahlman RP, Sen D. Cation-regulated self-association of "synapsable" DNA duplexes. *J Mol Biol* 1998; 280:237-44.
9. Rodriguez R, Miller KM, Forment JV, Bradshaw CR, Nikan M, Britton S, et al. Small-molecule-induced DNA damage identifies alternative DNA structures in human genes. *Nat Chem Biol* 2012; 8:301-10.
10. Izbiccka E, Nishioka D, Marcell V, Raymond E, Davidson KK, Lawrence RA, et al. Telomere-interactive agents affect proliferation rates and induce chromosomal destabilization in sea urchin embryos. *Anticancer Drug Des* 1999; 14:355-65.
11. Zimmermann S, Martens UM. Telomeres and telomerase as targets for cancer therapy. *Cell Mol Life Sci* 2007; 64:906-21.
12. Zahler AM, Williamson JR, Cech TR, Prescott DM. Inhibition of telomerase by G-quartet DNA structures. *Nature* 1991; 350:718-20.