Expert Review of Clinical Pharmacology

ISSN: 1751-2433 (Print) 1751-2441 (Online) Journal homepage: informahealthcare.com/journals/ierj20

G-quadruplexes: selective DNA targeting for cancer therapeutics?

Kyle M Miller & Raphaël Rodriguez

To cite this article: Kyle M Miller & Raphaël Rodriguez (2011) G-quadruplexes: selective DNA targeting for cancer therapeutics?, Expert Review of Clinical Pharmacology, 4:2, 139-142, DOI: 10.1586/ecp.11.4

To link to this article: https://doi.org/10.1586/ecp.11.4

Published online: 10 Jan 2014.

G-quadruplexes: selective DNA targeting for cancer therapeutics?

Expert Rev. Clin. Pharmacol. 4(2), 139–142 (2011)

Kyle M Miller

The Gurdon Institute, The University of Cambridge, Tennis Court Road, Cambridge, CB2 1QN, UK

Raphaël Rodriguez

Author for correspondence The University of Cambridge, Department of Chemistry, Lensfield Road, Cambridge, CB2 1EW, UK rr324@cam.ac.uk

"...G-quadruplex DNA motifs ... occur throughout the human genome and are involved in a myriad of biological processes, including telomere maintenance, replication and transcription."

The identification of novel cancer therapeutics is an intensive area of research that spans interdisciplinary fields that includes biologists, chemists and clinicians. These fields have recently turned to drug discovery for new anticancer therapies designed against specific molecular targets. One such target are G-quadruplex DNA motifs, which occur throughout the human genome and are involved in a myriad of biological processes, including telomere maintenance, replication and transcription. These targets offer potential opportunities for selective DNA targeting using small molecules that have been designed to bind these structures with high specificity. We will discuss the biology of G-quadruplexes and the potential use and challenges for these drugs in cancer therapy.

"...a more accurate knowledge of DNA damage and repair machineries has enabled the emergence of novel strategies based on enzyme inhibitors of DNA damage proteins, including kinases and poly ADP-ribose polymerase."

Therapeutic intervention for the treatment of cancers often involves combinations of radio- and chemotherapies including ionizing radiation and cisplatin, as well as replication and topoisomeras inhibitors. This stems from the fact that many cancer cells have impaired DNA damage detection and repair pathways [1]. Chemical agents have proven to be efficacious in the treatment of certain types of cancers [2]. Typically, small-molecule drugs, including ecteinascidin 743 and actinomycin D, promote a discontinuity in the integrity of DNA and its immediate environment, resulting in growth arrest and cell death. In this respect, the association of drugs with a propensity to act synergistically in cancer cells versus normal tissues holds great potential. However, despite tremendous efforts deployed by scientists and clinicians, promising new cancer therapeutics are in short supply. The complexity and diversity of cellular phenotypes associated with various cancers have made this task a considerable challenge for cancer biologists [3]. A poor understanding of the exact mode of action of DNA-interacting drugs has prevented the elaboration of more selective drugs and the development of selective chemotherapies. Rather, a more accurate knowledge of DNA damage and repair machineries has enabled the emergence of novel strategies based on enzyme inhibitors of DNA damage proteins, including kinases and poly ADP-ribose polymerase (PARP) [4].

G-quadruplex nucleic acids have recently emerged as potentially relevant clinical targets. These elements are nonclassical four-stranded secondary structures arising from the folding of a single DNA strand that comprises stretches of tandem guanines [5]. These types of sequences have

Keywords: cancer • DNA damage • drug • G-quadruplex • HDAC inhibitors • pyridostatin • quarfloxin • RHPS4 • telomestatin

  ISSN 1751-2433

been computationally identified in particular regions of the human genome, including promoters and gene bodies, with a higher propensity occurring in oncogenes [6–9,101]. These motifs are also clustered at telomeres and are highly conserved throughout evolution, suggesting functional importance for these DNA sequences. Despite a number of studies suggesting the biological relevance of G-quadruplex DNA, their existence *in vivo* has remained a matter of controversy. Two questions may be formulated independently: do G-quadruplex DNA exist in cells and if so, what is their biological significance? And can these motifs be selectively targeted by G-quadruplex-binding small molecules to modulate their function(s)?

> "...the potent antiproliferative properties of this molecule at noncytotoxic doses have provided additional evidence of the clinical relevance of drugs that target G-quadruplex DNA..."

Biological evidence of functional G-quadruplex DNA

A few significant studies have recently shed light on this subject in many biological systems, including humans. G-quadruplex structures are present in telomeres and are inhibitory to telomerase activity *in vitro,* although *in vivo* evidence has been difficult to obtain [10]. However, in ciliates, the formation of telomeric G-quadruplexes have been demonstrated *in vivo* using a G-quadruplex structure-specific antibody [11]. Cahoon and Seifert demonstrated that the human pathogen, *Neiseria gonorrhoeae*, utilizes a 16-nucleotide G-rich sequence that can form a G-quadruplex for the initiation of recombination to promote antigenic variation of surface structures [12]. The Sale group found that in chicken DT40 cells, G-quadruplex DNA was poorly replicated in REV1-deficient cells, a protein important for DNA translesion synthesis during replication. Interestingly, silent loci in REV1 mutants were derepressed and epigenetic information was lost in G-quadruplex DNA regions owing to the inability of these cells to properly replicate and regulate histone supply at these G-rich sequences [13]. Finally, ATR-X, which is mutated in the human syndrome ATR-X characterized by mental retardation, binds to G-quadruplex DNA and regulates gene expression of key genes. The use of genome-wide deep sequencing allowed for the determination of the binding sites of ATR-X, which correlated with long G-repeat DNA that can form G-quadruplexes[14]. Taken together, these studies highlight the vast potential for the biological functions of G-quadruplexes *in vivo*.

Clinical relevance of targeting G-quadruplex DNA

Riou *et al.* have shown that the natural product telomestatin uncaps telomere-binding proteins in human cancer cells, preventing the elongation of telomeres and resulting in premature chromosome shortening, a process reminiscent of replicative aging. These authors proposed a model whereby telomestatin stabilizes G-quadruplex structures at telomeres, which in turn prevents the recognition, protection and elongation of telomeric sequences by telomere-binding proteins responsible for the phenotype observed [15]. Biroccio *et al.* showed that the pentacyclic acridinium derivative RHPS4 (3,11-difluoro-6,8,13-trimethyl-8H-quino[4,3,2-kl]acridinium methosulfate) interferes with the replication of telomeres, promoting the activation of a DNA-damage response and aberrant chromosome segregation due to dysfunctional telomeres [16]. Similarly, the authors have proposed that RHPS4 selectively targets telomeric G-quadruplexs during the S-phase to impede replication fork progression through telomeres in human cancer cells. The recruitment and activation of the single-strand break protein PARP1 by uncapped telomeres implicates PARP1 in the repair of targeted telomeres [17]. The association of RHPS4 and a PARP1 inhibitor, the latter currently in clinical trials, was shown to act *de concert* in the treatment of human colorectal adenocarcinoma mice xenografts, demonstrating the clinical relevance of a telomeric G-quadruplex-based therapy. Recently, the drug quarfloxin entered Phase II clinical trials for the treatment of carcinoid/neuroendocrine tumors [18]. In this study, the authors suggested that quarfloxin accumulates in the nucleolus and selectively binds to rDNA-containing G-quadruplexes. As a result, a redistribution of the G-quadruplex-binding protein nucleolin was observed that correlated with a decrease in RNA Pol I transcriptional activity, which is required for rRNA synthesis and ribosome biogenesis. Ribosomal RNA synthesis is critical for tumor growth and these results suggest that these defects explain the anti-tumor activity of the drug that was observed in a murine xenograft cancer model. In other work, Hurley and Brooks have proposed an alternative mechanism by which the redistribution of nucleolin in the nucleus would facilitate the folding of another G-quadruplex element in the promoter region of the *MYC* oncogene. These authors reported a repression of MYC by 85% in tissues obtained from a HCT-116 colorectal mouse xenograft, which was in agreement with the proposed model [19]. Thus, a G-quadruplex element could act as a transcriptional switch that upon binding of a protein could trigger the off position, conferring tumor-suppressing properties to the drug, as seen in the case of quarfloxin and MYC. Finally, we have observed that the highly selective G-quadruplex-binding compound pyridostatin induces DNA damage at telomeric and nontelomeric regions of the genome in a panel of human cancer cells [20,21; Rodriguez R, Miller KM, Forment J *et al.,* Unpublished Data]. The exact binding loci of the drug and the precise mode of action *per se* have not yet been established as these studies are still in progress. Nevertheless, the potent antiproliferative properties of this molecule at noncytotoxic doses have provided additional evidence of the clinical relevance of drugs that target G-quadruplex DNA structures.

Conclusion & perspectives

In the last decade, a great deal of studies have provided invaluable knowledge on the structure and dynamics of G-quadruplex nucleic acids and their targeting with small molecules. The development of potent drugs has suffered from the absence of known biologically relevant G-quadruplex targets required for a suitable design. As a result, nonvalidated G-quadruplex targets have been used to identify potent binders. This reverse-genetics approach seems to have revealed a number of targets, including telomeres

and gene promoters. However, we believe that there are two critical questions that need addressing: what are the *in vivo* targets of G-quadruplex-binding small molecules? And what regulates their accessibility? Although telomeres, rDNA and promoter regions have been identified as potential targets, there are over 350,000 predicted sequences in the genome that can fold into G-quadruplex structures, raising the daunting task of determining selective targets for these drugs [8]. Whether these molecules are able to bind and target one specific or clusters of G-quadruplex structures is still an open question. Our recent unpublished observations, as well as the advancement of using small-molecule probes as selective G-quadruplex-DNA antibodies, suggests that the implementation of systematic genome-wide analysis, including chromatin immunoprecipitation followed by deep sequencing and RNA-Seq, will be instrumental in providing validated *in vivo* targets. As to the question of accessibility, these drugs must be able to interact with DNA, which is normally bound by chromatin proteins, including histones. Histones will negatively regulate the accessibility of DNA-interacting molecules, including G-quadruplex binders, and it is surprising that the role of chromatin in G-quadruplex biology has not been addressed. Histone deacetylases are a class of enzymes that regulate chromatin dynamics and constitute a major class of targets for anticancer therapies. As inhibition of histone deacetylases impairs DNA repair, and acts synergistically with radio- and chemotherapies, the dual use of these anticancer drugs along with G-quadruplex small-molecule binders could have therapeutic potential [22,23]. Answers to these questions, as well as the development of site-specific interacting probes to G-quadruplexes, are certain to unravel additional biological functions, as well as unanticipated therapeutic targets, for the treatment of human diseases. There is no doubt that G-quadruplex DNA will continue to emerge as relevant druggable structures.

Acknowledgements

The authors thank Sébastien Britton and Blerta Xhemalce for critical reading of the manuscript.

Financial & competing interests disclosure

Kyle M Miller thanks the Wellcome Trust for funding. Raphaël Rodriguez is a Herchel Smith Research Fellow. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

References

Papers of special note have been highlighted as:
• of interest
•• of considerable interest

1 Jackson SP, Bartek J. The DNA-damage response in human biology and disease. *Nature* 461(7267), 1071–1078 (2009).

2 Espinosa E, Zamora P, Feliu J, González Barón M. Classification of anticancer drugs – a new system based on therapeutic targets. *Cancer Treat. Rev.* 29(6), 515–523 (2003).

3 Weinberg RA. *The Biology of Cancer.* Garland Science, NY, USA, (2007).

4 Fong PC, Boss DS, Yap TA *et al.* Inhibition of poly(ADP-ribose) polymerase in tumors from *BRCA* mutation carriers. *N. Engl. J. Med.* 361(2), 123–134 (2009).

5 Patel DJ, Phan AT, Kuryavyi V. Human telomere, oncogenic promoter and 5′-UTR G-quadruplexes: diverse higher order DNA and RNA targets for cancer therapeutics. *Nucleic Acids Res.* 35(22), 7429–7455 (2007).

6 Eddy J, Maizels N. Gene function correlates with potential for G4 DNA formation in the human genome. *Nucleic Acids Res.* 34(14), 3887–3896 (2006).

7 Eddy J, Maizels N. Conserved elements with potential to form polymorphic G-quadruplex structures in the first intron of human genes. *Nucleic Acids Res.* 36(4), 1321–1333 (2008).

8 Huppert JL, Balasubramanian S. Prevalence of quadruplexes in the human genome. *Nucleic Acids Res.* 33(9), 2908–2916 (2005).

•• **Comprehensive bioinformatic analysis of G-quadruplexes in the human genome.**

9 Huppert JL, Balasubramanian S. G-quadruplexes in promoters throughout the human genome. *Nucleic Acids Res.* 35(2), 406–413 (2007).

10 De Cian A, Cristofari G, Reichenbach P *et al.* Reevaluation of telomerase inhibition by quadruplex ligands and their mechanisms of action. *Proc. Natl Acad. Sci. USA* 104(44), 17347–17352 (2007).

11 Paeschke K, Simonsson T, Postberg J, Rhodes D, Lipps HJ. Telomere end-binding proteins control the formation of G-quadruplex DNA structures *in vivo*. *Nat. Struct. Mol. Biol.* 12(10), 847–854 (2005).

• **First evidence of functional G-quadruplex motifs in cells.**

12 Cahoon LA, Seifert HS. An alternative DNA structure is necessary for pilin antigenic variation in *Neisseria gonorrhoeae*. *Science* 325(5941), 764–767 (2009).

• ***In vivo* evidence for a biological function of G-quadruplexes in a human pathogen.**

13 Sarkies P, Reams C, Simpson LJ, Sale JE. Epigenetic instability due to defective replication of structured DNA. *Mol. Cell* 40(5), 703–713 (2010).

14 Law MJ, Lower KM, Voon HP *et al.* ATR-X syndrome protein targets tandem repeats and influences allele-specific expression in a size-dependent manner. *Cell* 143(3), 367–378 (2010).

•• **Identification of a G-quadruplex-binding protein that regulates gene expression and is mutated in a human genetic disease.**

15 Gomez D, Wenner T, Brassart B *et al.* Telomestatin-induced telomere uncapping is modulated by POT1 through G-overhang extension in HT1080 human tumor cells. *J. Biol. Chem.* 281(50), 38721–38729 (2006).

16 Rizzo A, Salvati E, Porru M *et al.* Stabilization of quadruplex DNA perturbs telomere replication leading to the activation of an ATR-dependent ATM signaling pathway. *Nucleic Acids Res.* 37(16), 5353–5364 (2009).

17 Salvati E, Scarsella M, Porru M *et al.* PARP1 is activated at telomeres upon G4 stabilization: possible target for telomere-based therapy. *Oncogene* 29(47), 6280–6293 (2010).

• **Synergystic interaction between a G-quadruplex-binding molecule and a DNA repair inhibitor *in vivo*.**

18 Drygin D, Siddiqui-Jain A, O'Brien S *et al.* Anticancer activity of CX-3543: a direct inhibitor of rRNA biogenesis. *Cancer Res.* 69(19), 7653–7661 (2009).

•• **First G-quadruplex-binding drug in clinical trials.**

19 Brooks TA, Hurley LH. Targeting MYC expression through G-quadruplexes. *Genes Cancer* 1(6), 641–649 (2010).

20 Müller S, Kumari S, Rodriguez R, Balasubramanian S. Small-molecule-mediated G-quadruplex isolation from human cells. *Nat. Chem.* 2(12), 1095–1098 (2010).

• **A novel, highly selective G-quadruplex-binding small molecule.**

21 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.* 130(47), 15758–15759 (2008).

22 Camphausen K, Tofilon PJ. Inhibition of histone deacetylation: a strategy for tumor radiosensitization. *J. Clin. Oncol.* 25(26), 4051–4056 (2007).

23 Miller KM, Tjeertes JV, Coates J *et al.* Human HDAC1 and HDAC2 function in the DNA-damage response to promote DNA nonhomologous end-joining. *Nat. Struct. Mol. Biol.* 17(9), 1144–1151 (2010).

Website

101 Quadparser: a program for finding putative quadruplexes in DNA sequences. www.quadruplex.org/?view=quadparser