Quadruplex Ligands in Cancer Therapy

Subjects: Pathology

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Nucleic acids can adopt alternative secondary conformations including four-stranded structures known as quadruplexes. To date, quadruplexes have been demonstrated to exist both in human chromatin DNA and RNA. Quadruplexes are associated with key biological processes ranging from transcription and translation of several oncogenes and tumor suppressors to telomeres maintenance and genome instability. In this context, quadruplexes have prompted investigations on their possible role in cancer biology and the evaluation of small-molecule ligands as potential therapeutic agents.

Keywords: cancer ; G-quadruplexes ; i-Motifs

1. Introduction

Nucleic acids have considerable potential to fold into three-dimensional secondary structures based on particular sequence motifs. Single-stranded guanine-rich DNA sequences can fold into stable intramolecular and intermolecular four-stranded G-quadruplexes (G4s). G4s arise from Hoogsteen hydrogen bonding of four guanines arranged within a planar quartet, which is further stabilized by interactions between the O-6 lone- pair electrons of each guanine and monovalent or divalent cations. Self-stacking of two or more G-quartets generates a G4 structure. Further studies established that many RNA sequences featuring G-tracts can also fold into G4 structures, sometimes demonstrating increased thermodynamic stability and reduced steric hindrance. Therefore, G4s are found both in DNA and RNA. In addition, single-stranded cytosine-rich sequences can form hemiprotonated cytosine–cytosine base pairs (C–C+), adopting a structure called an i-Motif. Although quadruplexes are related to each other in terms of primary sequence, they in fact comprise a diverse family of structures that can fold into different topologies including parallel, antiparallel, and hybrid structures for G4s and R-forms or S-forms for i-Motifs.

In general, quadruplex sequences are non-randomly distributed but are mainly clustered in pivotal genomic regions, such as DNA replication origins, telomeres, gene promoters, and untranslated regions (UTRs). In this regard, quadruplexes display key cancer-related functions. Quadruplexes are linked to the control of the expression of several oncogenes and tumor suppressors, both at the transcriptional and translational levels. In addition, quadruplexes participate in lengthening telomeres and induce genome instability, processes which are frequently altered in cancer in order to sustain limitless replication. Therefore, quadruplex ligands have emerged as potential strategies for anticancer drug discovery.

This study aimed to concisely review the most recent advances in quadruplex targeting in antitumoral therapy. Given that the field of quadruplexes is continuously developing, we cover the current state of the art of quadruplex ligands in cancer research. Moreover, previous reviews separately focus on DNA G4, RNA G4 and i-Motif ligands in cancer. To the best of our knowledge, the present review is the first to bring together these quadruplex ligands, all of which are relevant for cancer therapeutics.

2. Quadruplex Ligands

Enormous efforts are being made to target quadruplexes as a therapeutic approach given their profound implication in carcinogenesis. Ligands are chemical compounds that specifically bind to and stabilize the structure of quadruplexes. Without this mechanism, quadruplexes would unfold immediately after their formation in the cell as a result of helicases. Quadruplexes provide recognition sites for ligands since different quadruplex structures adopt specific conformations. Binders generally have an aromatic surface for π–π stacking interactions with quadruplexes, a positive charge or basic groups to selectively bind to the loops or grooves of the quadruplex, and a steric bulk to prevent intercalation with double-stranded DNA. To date, an arsenal of around 1000 small molecules that target quadruplexes has been reported. In the section below, quadruplex ligands with an anticancer effect are summarized. The majority of ligands have emerged in recent years.

- DNA G4 ligands

Herein we review the plethora of existing small molecules targeting DNA G4s, from classical ligands to the most recently discovered and selective binders, through the main chemo-families.

- RNA G4 ligands

As described in the Background Section, increasing evidence suggests that UTRs, coding sequences, and splicing sites of cancer-relevant genes contain putative RNA G4s, which can be targeted. In addition, RNA G4s play important roles associated with telomeric function.

- I-motif ligands

While there are hundreds of ligands that interact with DNA and RNA G4s, there are very few compounds that target i-Motifs. Several ligands act as dual i-Motif/G4-interactive compounds. Thus, we would like to highlight the importance of evaluating their behavior towards the i-Motif counterpart, when studying G4-targeting compounds. In this section, DNA i-Motif ligands that were reported to exert antitumoral activity are grouped based on telomeric or extratelomeric targeting.

3. Discussion

Cancer is a major disease that poses a serious threat to human life and health. As a result of its complex and heterogeneous pathogenesis, there are still many challenges in cancer therapy. Finding novel anti-tumor drugs with high selectivity and few side effects is still the main focus of cancer research. As demonstrated in the present review, an imbalance in quadruplex dynamics contributes to carcinogenesis, and its manipulation by quadruplex ligands provides a novel opportunity to defeat cancer. Initial efforts were mainly focused on targeting telomeric quadruplexes in order to inhibit telomere extension in cancer cells using telomerase, whereas later studies attempted to transcriptionally modulate individual cancer genes by targeting their quadruplexes. Although there is a long way to go in the development of potent drugs, various promising lead compounds have been obtained; however, the results have thus far been limited. Firstly, at this stage, the variety of binding sites for these ligands and the differences in their effects on the quadruplex structures make it difficult to unravel how quadruplexes influence biological function, i.e., whether the stabilization or destabilization of quadruplexes promotes or inhibits gene expression. Secondly, the correlation between stabilization in vitro and cell activity is not straightforward. In particular, a G4 target characterized in vitro may not be the sole G4 targeted in cells. Furthermore, there is also inherent cell variability, which has an impact on the relationship between in vitro and in vivo results. A further point to be addressed for the majority of ligands described thus far is that they are generally characterized by high-molecular weights and protonated side chains, which may affect their cellular uptake. However, the major limitation for the clinical application of quadruplex ligands seems to be directly related to selectivity. In fact, the selectivity pattern of several quadruplex ligands is dose-dependent. Although global or multiple G4 targeting approaches may be effective, targets need to be clearly defined in advance. Other conceivable obstacles are the potential side effects of the ligands on normal tissues. Moreover, the predictive response biomarkers need to be identified if a personalized anticancer management is to be achieved. Nevertheless, given the rapid accumulation of data on quadruplex structures and the related biological functions, and the rapid development of ligands, we are confident that these limitations can be overcome. In this regard, a wealth of new derivatives with lower cytotoxicity and superior selectivity will emerge in the near future.

4. Conclusions

In this review, we give an overview of a range of compounds that target quadruplexes, including DNA G4s, RNA G4s, and i-Motifs, and discuss their limitations. The quadruplex-mediated antitumoral effects reported herein may pave the way for cutting-edge therapeutic approaches in the future treatment of human cancer.
