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REVIEW ARTICLE

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The influencing factors and functions of DNA G-quadruplexes

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G-quadruplexes form folded structures because of tandem repeats of guanine sequences in DNA or RNA. They adopt a variety of conformations, depending on many factors, including the type of loops and cations, the nucleotide strand number, and the main strand polarity of the G-quadruplex. Meanwhile, the different conformations of G-quadruplexes have certain influences on their biological functions, such as the inhibition of transcription, translation, and DNA replication. In addition, G-quadruplex binding proteins also affect the structure and function of G-quadruplexes. Some chemically synthesized G-quadruplex sequences have been shown to have biological activities. For example, bimolecular G-quadruplexes of AS1411 act as targets of exogenous drugs that inhibit the proliferation of malignant tumours. G-quadruplexes are also used as vehicles to deliver nanoparticles. Thus, it is important to identify the factors that influence G-quadruplex structures and maintain the stability of G-quadruplexes. Herein, we mainly discuss the factors influencing G-quadruplexes and the synthetic G-quadruplex, AS1411.

Significance of the study: This review summarizes the factors that influence G-quadruplexes and the functions of the synthetic G-quadruplex, AS1411. It also discusses the use of G-quadruplexes for drug delivery in tumour therapy.

KEYWORDS

AS1411, G-quadruplex, G-tetrad, nanoparticles

1 | INTRODUCTION

The G-quadruplex, which is formed by the folding of tandem repeats of guanine sequences,[1] is a special secondary structure of DNA and RNA. As a square plane, the G-tetrad, which is the structural unit of G-quadruplexes, is formed by connecting four guanines through eight hydrogen bonds. In the G-tetrad, two hydrogen bonds pairing adjacent guanines are involved in N1, N7, O6, and N2 of each guanine nucleotide (Figure 1A).[2-5] These G-tetrads are connected by four G-tracts, which denote four separate runs of three guanines. In

addition to the quadrimolecular G-quadruplex, the intervening sequences connecting two adjacent G-tracts are pushed out to form a single-stranded loop (Figure 1B).[3] Two or more G-tetrads that are parallel to each other are stacked to form a G-quadruplex. Because of the 2′-hydroxyl group of the ribose in the pentose phosphate skeleton, the G-quadruplexes of RNA are more thermodynamically stable in different environments than G-quadruplexes of DNA with the same sequences. RNA G-quadruplexes almost exclusively adopt a parallel quadrimolecular conformation.[3,6-9] However, the conformation of DNA G-quadruplexes is influenced by many factors. The three main factors are the number of nucleotide strands, the polarity of the main strands of the G-quadruplexes, and the type of loops.[10,11] Furthermore, cations and G-quadruplex binding proteins also have important effects on the conformation of G-quadruplexes.[12-14]

The human genome harbours >376 000 potential G-quadruplex sequences,[15,16] which are widely distributed in many important gene regions, such as telomeres, gene promoter regions, and replication origins.[12,13,17,18] The G-quadruplexes in these regions have important biological functions, mainly involving the inhibition of telomerase activity and the regulation of transcription, translation, and DNA replication.[9,19,20] The G-quadruplex is regarded as an important drug for cancer treatment.[21] Some chemically synthesized G-quadruplex sequences have been shown to have biological activity. For example, the synthetic G-quadruplex, AS1411 is used as an exogenous drug to inhibit the proliferation of malignant tumours, with no effect on normal cells.[22,23] In recent years, the number of studies of AS1411 has gradually increased, mainly focusing on its use as a nanoparticle (NP) delivery vehicle or for targeted cancer treatment.[24] G-quadruplexes have good development prospects for the treatment of diseases. Since different conformations of G-quadruplexes influence their stability and function, it is important to know what factors influence G-quadruplex structures.

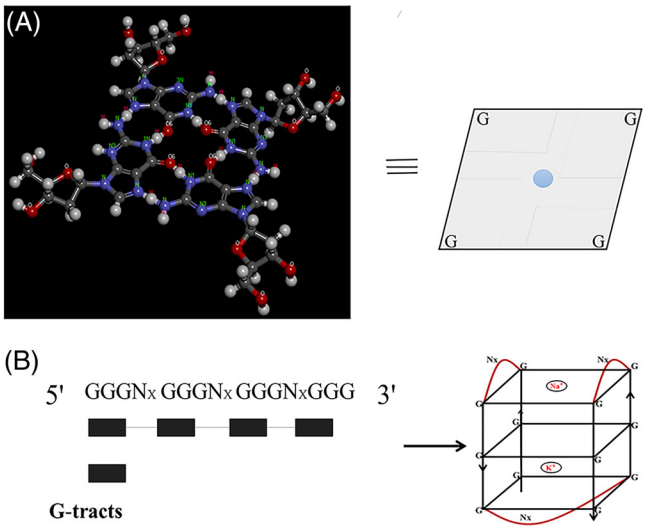

FIGURE 1 The structures of G-quadruplexes. A, Structure of G-tetrads. The G-tetrad, the structural unit of the G-quadruplex, is a square plane formed by connecting four guanines through eight hydrogen bonds. The monovalent cations are represented as blue. B, G-tracts denote four separate runs of three guanines. The intervening sequences between two G-tracts in single-stranded DNA or RNA are pushed out to form single-stranded loops (Nx: N denotes any of A, G, C, T, or U; 1 < x < 7). The loops are represented as red. The G-quadruplex is formed by the folding of the four G-tracts of three tandem repeats of guanine sequences separated by loop regions

2 | FACTORS INFLUENCING G-QUADRUPLEX STRUCTURE

2.1 | The number of nucleotide strands forming the G-quadruplex

Based on the number of nucleotide strands forming G-quadruplexes, there are three main types of G-quadruplex structures: quadrimolecular, bimolecular, and unimolecular. The quadrimolecular G-quadruplex consists of four independent strands; the bimolecular G-quadruplex consists of two strands; and the unimolecular G-quadruplex consists of one strand, which belongs to the intramolecular G-quadruplex. The quadrimolecular and bimolecular G-quadruplexes belong to the intermolecular type of G-quadruplexes (Figure 2).[14,25-27] Moreover, the long single strand that forms the unimolecular G-quadruplex can also form bimolecular and quadrimolecular G-quadruplexes. Intramolecular G-quadruplexes are predicted to contain at least four G-tracts in which each G-tract contains at least three guanine nucleotides (G ≥ 3–Nx – G ≥ 3–Nx–G ≥ 3–Nx–G ≥ 3; N denotes any of A, G, C, T, or U; 1 < x < 7). Otherwise, the stability may be low if each G-tract only contains only two guanine nucleotides.[3] However, for intermolecular G-quadruplexes, it is possible for each G-tract to only contain two guanine nucleotides. For example, some studies have shown that the synthetic G-quadruplex of AS1411 is stable in the presence of serum-containing medium.

2.2 | The polarity of nucleotide strands

Under different environmental conditions, three typical configurations of G-quadruplexes are formed, namely, parallel, antiparallel, and mixed configurations.[6,21] Four guanines, connected by hydrogen bonds to form a G-tetrad, adopt anti or syn arrangements about glycosidic bonds, depending on the glycoside angle of the guanines. The H8 and sugar H1′protons of anti guanine glycoside angles have a longer distance than those of the syn arrangement (Figure 3A and 3B).[25] Moreover, the polarities of the relative nucleotide strands forming the G-quadruplexes are also related to the glycosidic angles of guanines in the same G-tetrad. The guanine arrangement of each G-tract can be influenced by monovalent cations.[4] For quadrimolecular DNA G-quadruplexes, if four G-tracts of one G-quadruplex have the same polarity, a parallel G-quadruplex is formed. With K^+ or Na^+ as the coordinated monovalent cation, the glycosidic angles of the four

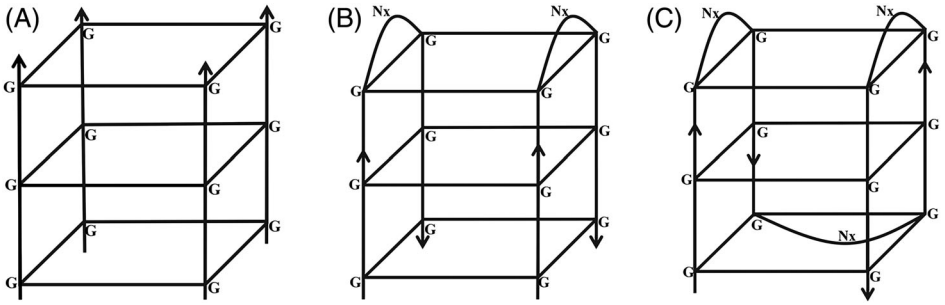

FIGURE 2 The number of nucleotide strands forming the G-quadruplex. A, Quadrimolecular G-quadruplexes consist of four independent strands and are intermolecular G-quadruplexes. B, Bimolecular G-quadruplexes consist of two strands, and they are intermolecular G-quadruplexes. C, Unimolecular G-quadruplexes consist of one strand, and they are intramolecular G-quadruplexes

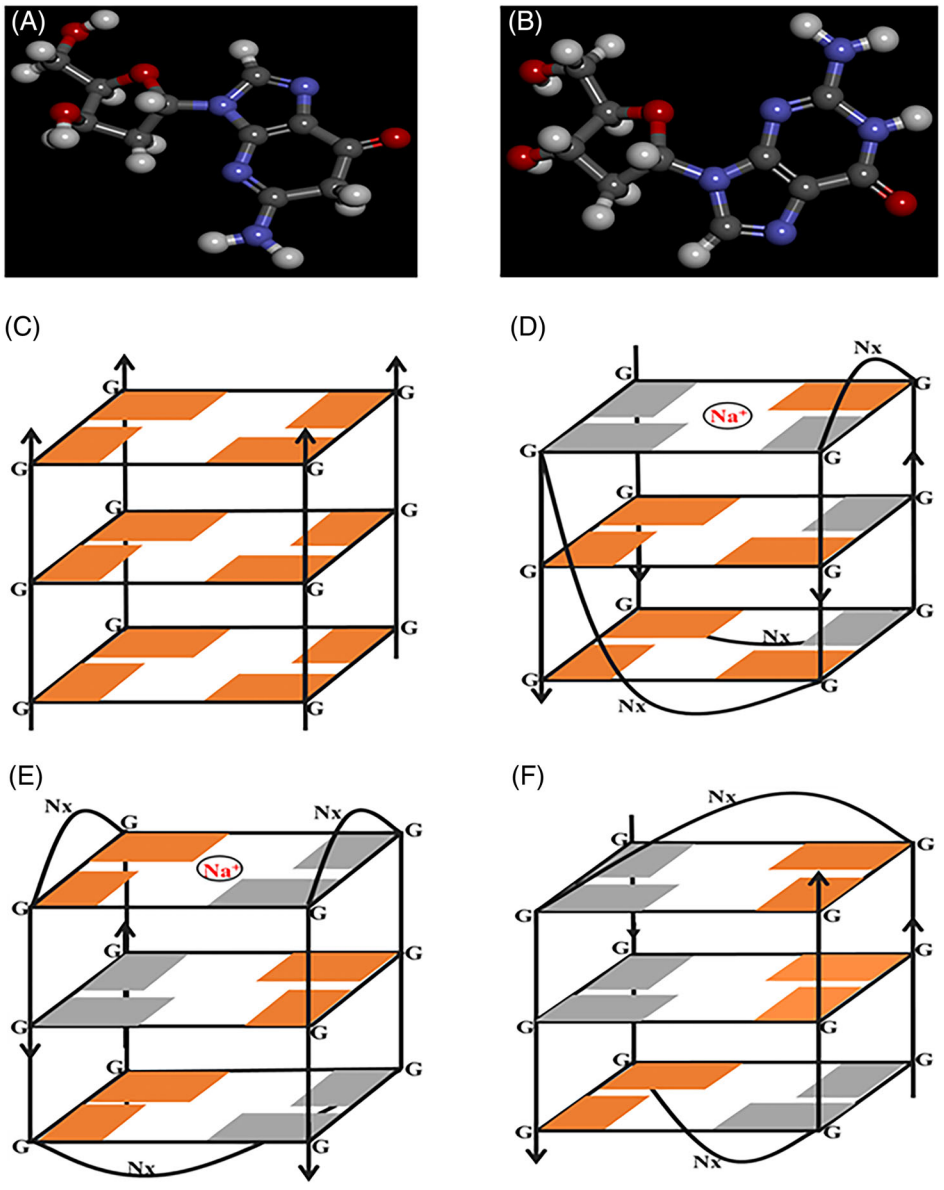

FIGURE 3 The polarity of nucleotide strands forming the G-quadruplex. A, The anti arrangements of guanine glycoside angles. B, The syn arrangements of guanine glycoside angles. C, The structure of parallel G-quadruplexes. The four G-tracts of the parallel quadrimolecular DNA G-quadruplex have the same polarity. D, The structure of mixed G-quadruplexes. Three of the four G-tracts of the unimolecular G-quadruplex have the same polarity, and the fourth has the opposite polarity. E, The structure of antiparallel G-quadruplexes. Two of the four G-tracts of the unimolecular G-quadruplex have the same polarity. F, The structure of antiparallel G-quadruplexes. Two of the four G-tracts of the bimolecular G-quadruplex have the same polarity. The anti guanines are represented as orange, and the syn guanines are represented as grey

guanines in each G-tetrad mainly adopt anti-anti-anti-anti arrangements, and the guanines of each G-tract adopt anti-anti-anti arrangements (Figure 3C).[4,28] For unimolecular G-quadruplexes, if three of the four G-tracts have the same polarity and the fourth has the opposite polarity, mixed G-quadruplexes are formed. With Na^+ as the coordinated monovalent cation, the glycosidic angles of the four guanines

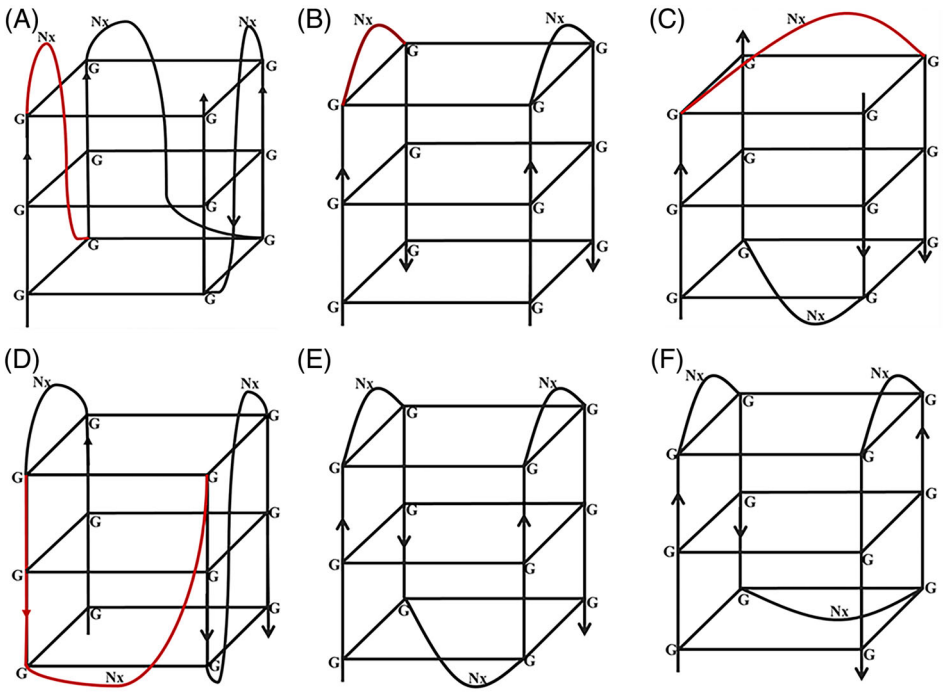

FIGURE 4 The loop of G-quadruplexes. A, The chain reversal loop. B, The lateral loop. C, The diagonal loop. D, The v-shaped loop. The loops are represented as red. E, Basket configurations of the antiparallel unimolecular G-quadruplex. F, Chair configurations of the antiparallel unimolecular G-quadruplex

in each G-tetrad adopt syn-anti-anti-anti or anti-syn-syn-syn arrangements, and the guanines of each G-tract adopt syn-anti-anti or syn-syn-anti arrangements (Figure 3D).[4,29] For unimolecular and bimolecular G-quadruplexes, if two of the four G-tracts of each G-quadruplex have the same polarity, an antiparallel G-quadruplex is formed; therefore, each G-tract has adjacent parallel or antiparallel neighbours. The glycosidic angles of the four guanines in each G-tetrad adopt syn–syn–anti–anti[30-32] or syn–anti–syn–anti arrangements.[33-36] However, the guanine arrangement of each G-tract is different between unimolecular and bimolecular G-quadruplexes. For unimolecular G-quadruplexes, the guanines of each G-tract adopt anti–syn–anti or syn–anti–syn arrangements, with Na^+ as the coordinated monovalent cation (Figure 3E). For bimolecular G-quadruplexes, the guanines of each G-tract adopt syn–anti–anti or syn–syn–anti arrangements, with K^+ or Na^+ as the coordinated monovalent cation (Figure 3F).[4] Because of the 2′-hydroxyl group of the ribose in the pentose phosphate skeleton, RNA G-quadruplexes prefer to form parallel G-quadruplexes, which are unaffected by the surrounding conditions.[2,37-41]

2.3 | The loop of G-quadruplexes

The intervening sequences between two G-tracts in single-stranded DNA or RNA are pushed out to form single-stranded loops.[1,3] The size of the loop affects the stability of the G-quadruplex structure. One loop generally includes one to seven nucleotides. A smaller loop results in greater stability of RNA G-quadruplexes, whereas DNA G-quadruplexes have greater stability with a longer loop.[38,42] The loop is a linker that connects G-tracts, and it mainly occurs in bimolecular and unimolecular G-quadruplexes. There are three main types of loops in G-quadruplexes, namely, chain reversal, lateral, and diagonal loops.[43] The chain reversal loop is located on the side of the G-quadruplex. It is a linker that connects two adjacent parallel strands and two guanine nucleotides that are located on the uppermost and lowermost G-tetrads (Figure 4A).[29,44,45] The lateral loop connects two adjacent antiparallel strands and two adjacent guanine nucleotides located on the same G-tetrad. These two adjacent guanine nucleotides are connected by two hydrogen bonds to form one edge of the G-tetrad (Figure 4B).[31,46] The diagonal loop connects two opposing antiparallel strands and two opposing guanine nucleotides located on the same G-tetrad (Figure 4C).[30,32] In addition, the mixed unimolecular G-quadruplex forms a special v-shaped loop between two adjacent guanine nucleotides located on the uppermost G-tetrad (Figure 4D).[47] Because of the different types of loops, the antiparallel unimolecular G-quadruplex presents two different configurations, namely, chair and basket configurations (Figure 4E and 4F).

2.4 | The effect of cations on G-quadruplexes

The formation and stability of G-quadruplexes are affected by monovalent cations, including K^+, Na^+, Rb^+, Cs^+, $NH4^+$, and Tl^+, and divalent cations. K^+ and Na^+ are the most extensively characterized monovalent cations. However, compared with Na^+, G-quadruplexes prefer K^+, because of its radius and free energy.[48] K^+ enters the middle of two adjacent G-tetrads and binds to eight carbonyl oxygen atoms. It can neutralize the negative electrostatic potential energy generated by the oxygen atoms in eight guanine nucleotides.[32,49] The radius of Na^+ is smaller than that of K^+, and therefore, Na^+ enters directly into a G-tetrad. Under these conditions, it only neutralizes the negative electrostatic potential energy produced by the oxygen atoms in four guanine nucleotides. Na^+ also increases the stability of G-quadruplexes,

although to a lesser extent than K^+.[14,49,50] Therefore, monovalent cations improve the stability of G-quadruplexes, seemingly due to their radius. A free energy cycle, which includes the free energy of hydration and the relative free energy of ion replacement, influences the ion selectivity of G-quadruplexes. Hud et al[48] reported that Na^+ actually binds to the G-quadruplex coordination site with a higher relative free energy of ion replacement than K^+, whereas the negative hydration free energy of Na^+ is greater. Considering the free energy cycle as a whole, K^+ can replace the position of Na^+. Divalent cations also promote the formation of G-quadruplexes and stabilize their structure but with a more complex mechanism than that of monovalent cations. Divalent cations stabilize G-quadruplexes in the following order: $Sr^{2+} > Ba^{2+} > Ca^{2+} > Mg^{2+}$.[51,52] When other cations are absent, Ca^{2+} and Mg^{2+} do not have the ability to promoting the formation of G-quadruplexes. In addition, the concentration of divalent cations also affects the stability of G-quadruplexes. Lower concentrations of divalent cations lead to more stable G-quadruplex structures.[49] The ion radius of Sr^{2+} is close to that of K^+, and for some unimolecular G-quadruplexes, Sr^{2+} plays a more important stabilizing role than K^+.[53-55]

3 | G-QUADRUPLEXES AND G-QUADRUPLEX BINDING PROTEINS

Helicases are molecular motors that use ATP-driven motor force.[56-59] On one hand, helicases unwind the double helices of DNA or complementary RNA to promote the formation of single-stranded nucleic acids. On the other hand, some helicases also have the ability to rewind or re-anneal two complementary single-stranded DNA or RNA molecules.[60] Helicases play a role in almost every aspect of DNA and RNA metabolism, including replication, repair, recombination, transcription, chromosome segregation, and telomere maintenance.[61-64] During DNA replication, replication helicases unwind the DNA double helices.[60] The leading strand is the template for DNA replication, and its synthesis is continuous. The synthesis of the lagging strand is discontinuous, and therefore, the leading strand transiently remains single-stranded, which provides a potential opportunity for guanine-rich strands to fold into stable G-quadruplexes, especially when DNA replication is slow.[3,5] The G-quadruplex has beneficial roles in cancer therapy, because of its inhibitory effect on DNA replication. However, it is necessary to unwind G-quadruplexes in normal cells. Some studies have shown that many helicases bind to and unwind G-quadruplex structures. The best characterized DNA G-quadruplex helicases are WRN, BLM, Pif1, and FANCJ. The mutation of DNA G-quadruplex helicases leads to severe hereditary diseases. These diseases are associated with the loss of G-quadruplex unwinding, which causes genomic instability. The absence of WRN is associated with premature ageing, and the deletion of BLM, Pif1, or FANCJ is associated with an increased risk of cancer.[5,65-69] The presence of a G-quadruplex on the template strand inhibits transcription; however, when it occurs on the complementary strand, the transcription level of some genes increases. In addition, G-quadruplex binding proteins affect transcription.[18] The mammalian oncogene, *MYC*, is one of the best models to study the effects of G-quadruplexes on transcription. In 80% of human cancer cells, the expression level of MYC is increased, and this promotes tumourigenesis.[70-72] Guanine-rich sequences in the promoter region of the *C-MYC* gene can be folded to form G-quadruplexes, which inhibit its expression.[73] Nucleolin, a 100 kDa nucleolar phosphoprotein, is abundant in eukaryotic cells. It binds to G-quadruplexes in the promoter region of *C-MYC* and promotes the formation of G-quadruplexes, which inhibit gene expression.[74,75] During translation, the guanine-rich sequences of mRNAs have the ability to form G-quadruplexes, and the main role of these G-quadruplexes is to inhibit translation.[76] Some proteins can bind to RNA G-quadruplexes and affect translation. DHX36 is an RNA G-quadruplex helicase, which mainly unwinds RNA G-quadruplexes.[77,78] PTX1 is a transcription factor associated with cancer. Booy et al showed that the expression of PTX1 increased when the *DHX36* gene was knocked out. This may be related to the effects of DHX36 on the G-quadruplex structure in the 3′-UTR of *PTX1* mRNA.[79] Above all, G-quadruplexes have important biological functions in DNA replication, transcription, and translation. G-quadruplex binding proteins affect the function of G-quadruplexes by promoting their formation and stabilizing or unfolding the G-quadruplex structures.

3.1 | The synthetic G-quadruplex, AS1411

3.1.1 | AS1411 acts as an exogenous drug

AS1411, which consists of 26 nucleotides, is a synthetic G-rich oligodeoxynucleotide with the sequence, 5′-GGT GGT TGT GGT GG-3′. It can form bimolecular G-quadruplexes and acts as an exogenous drug.[22,80] The structure of AS1411 is highly polymorphic in solution, with at least eight different G-quadruplex structures detected by chromatography and NMR.[81] AS1411 is stable in the presence of serum-containing medium, and fluorescence anisotropy analysis has shown that AS1411 is resistant to nuclease degradation.[22,82-84] Nucleolin, one of the molecular targets of AS1411,[22,85] acts as a receptor on the cell surface and plays an important role in the transport of substances between the nucleus and cytoplasm.[86-88] The overexpression of nucleolins on the surface of cancer cells is associated with malignant proliferation.[89] Moreover, nucleolins are also present in the cytoplasm and nucleus of cancer cells but only in the nucleus of normal cells.[90] AS1411 binds to nucleolin to suppress proliferation and induce the death of cancer cells in vitro. This may be due to the inhibition of DNA replication by AS1411, but it may also be linked to the stabilization of *BCL-2* mRNA, which is inhibited by nucleolin.[22,82,83,90-93] A phase I clinical trial demonstrated that AS1411 has no serious toxicity in humans, and it is currently being assessed as an anticancer agent in phase II clinical trials.[22] All in all, AS1411 appears to have extensive therapeutic potential, and thus, further studies of G-quadruplexes are warranted.

3.1.2 | AS1411 is used as a vehicle to deliver NPs

During the treatment of various diseases, it is important to effectively deliver drugs to the target sites or cells, to maximize the local concentration of the drug and reduce the potential side effects to other normal sites or cells.[94] NPs improve drug effects by increasing drug stability during blood transport and promoting drug absorption into cells.[95,96] Paclitaxel (PTX, also known as taxol), which is isolated from the bark of the Pacific yew, is considered one of the best natural antitumour drugs.[97,98] Because of its low solubility in aqueous environments (<0.03 mg/mL) and chemoresistance, only a small amount of PTX enters tumour cells, and therefore, its clinical application is limited.[98,99] Human serum albumin (HSA) is a plasma protein that is used as a delivery vehicle. It has the advantages of reducing the clearance and degradation of the drug, resulting in higher intratumour concentrations.[100-103] After the structure is destroyed, the hydrophobic domain of HSA is exposed, and the denatured molecules surrounding PTX cause self-assembly into nanoparticles (NPs-PTX), through hydrophobic interactions.[104] Recently, it was reported that a drug delivery system, Apt-NPs-PTX, was formed by modifying NPs-PTX with AS1411, via cross-linking with EDC and NHS. This drug delivery system was shown to be very stable. Apt-NPs-PTX is transported to the nucleus in combination with nucleolin. It increases the intake rate and inhibitive ability of PTX. Thus, Apt-NPs-PTX overcomes the clinical application limitations of PTX, with little effect on normal cells.[101] As a promising drug delivery system, the Apt-NPs-drug system reduces the side effects of traditional drug delivery systems and simultaneously improves tumour targeting and drug efficacy.

CONCLUSIONS

In recent years, G-quadruplexes have become a popular research topic globally. Experiments investigating DNA replication and transcription have provided the most convincing evidence to date of the existence of G-quadruplex structures in vivo.[105-107] Some studies have reported that the formation of DNA G-quadruplexes may be pathological and may only occur occasionally. This is related to discontinuous lagging strand replication, which provides a potential opportunity for guanine-rich strands to fold into G-quadruplex structures, especially when DNA replication is slow. However, the specific processes and mechanisms of G-quadruplex formation are not clear, and further research is needed. G-quadruplexes have important biological functions; however, the configuration of the G-quadruplex depends on many factors. It is important to investigate the influence of such factors and identify a generic modification strategy to maintain the stability of G-quadruplex structures. Recently, the chemical modification strategy has become a hot topic for future research. Some studies have reported that 2′-deoxyinosine can be used as a chemical modification strategy, to maintain the chemical stability and promote the biological effects of G-quadruplexes.

AS1411 appears to have extensive therapeutic potential, and a phase I clinical trial has shown that AS1411 has no serious toxicity in cancer patients. It would be of interest to identify the mechanism whereby AS1411 preferentially affects cancer cells and only has minimal side effects on normal cells. AS1411 is a type of nucleolin-nucleotide aptamer, and aptamer technology has been developed in the last few decades as a novel therapeutic approach. Furthermore, aptamers can be used as delivery vectors by targeting cell surface markers to deliver drugs, proteins, radionuclides, and NPs into cancer cells.[108,109] Some noncoding RNAs, such as siRNAs and microRNAs, also have therapeutic potential by selectively downregulating gene expression in diseased cells.[110-112] Some studies have shown that AS1411-microRNA conjugates can be constructed by chemically coupling microRNAs to AS1411 via a linker, and these constructs can be delivered into cancer cells by combining with nucleolins.[113] AS1411 provides a new direction for gene therapy. A recent study has shown that the biological activity of an antisense oligonucleotide is dependent on the mechanism of uptake, rather than uptake efficiency.[114] AS1411 can combine with nucleolins and enter cancer cells by a process known as macropinocytosis.[23] However, the specific site at which AS1411 combines with nucleolins on cancer cells and the reason why cancer cells uptake AS1411 by micropinocytosis are not clear. Thus, further research is needed to explore these mechanisms. From this review, it is clear G-quadruplexes have many important roles, but there are some specific details of their function that require further study.

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CONFLICT OF INTEREST

There are no other conflicts of interest to disclose.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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