Targeting signal transducer and activator of transcription 3 with G-quartet oligonucleotides: a potential novel therapy for head and neck cancer

Naijie Jing,1,2 Qiqing Zhu,1 Ping Yuan,4 Yidong Li,1 Li Mao,3 and David J. Tweardy1

Department of 1Medicine and 2Cancer Center, Baylor College of Medicine; 3Department of Thoracic/Head and Neck Medical Oncology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas; and 4Department of Pathology, Ruijin Hospital, Shanghai Jiaotong University, Shanghai, China

Abstract
Signal transducer and activator of transcription 3 (Stat3) is a critical mediator of oncogenic signaling activated frequently in many types of human cancer where it contributes to tumor cell growth and resistance to apoptosis. Stat3 has been proposed as a promising target for anticancer drug discovery. Recently, we developed a series of G-quartet oligodeoxynucleotides (GQ-ODN) as novel and potent Stat3 inhibitors, which significantly suppressed the growth of prostate and breast tumors in nude mice. In the present study, we showed that GQ-ODN specifically inhibited DNA-binding activity of Stat3 as opposed to Stat1. Computer-based docking analysis revealed that GQ-ODN predominantly interacts with the SH2 domains of Stat3 homodimers to destabilize dimer formation and disrupt DNA-binding activity. We employed five regimens in the treatment of nude mice with tumors of head and neck squamous cell carcinoma (HNSCC): placebo, paclitaxel, GQ-ODN T40214, GQ-ODN T40231, and T40214 plus paclitaxel. The mean size of HNSCC tumors over 21 days only increased by 1.7-fold in T40214-treated mice and actually decreased by 35% in T40214 plus paclitaxel–treated mice whereas the mean size of HNSCC tumors increased 9.4-fold in placebo-treated mice in the same period. These findings show that GQ-ODN has potent activity against HNSCC tumor xenografts alone and in combination with paclitaxel. [Mol Cancer Ther 2006;5(2):279–86]

Introduction
Signal transducer and activator of transcription 3 (Stat3), a critical mediator of oncogenic signaling, is constitutively activated in many human cancers (1–4) including 82% of prostate cancers (5), 70% of breast cancers (6), >90% of head and neck cancers (7), and over half of lung cancers (8). Stat3 plays a critical role in oncogenic signaling through the up-regulation of genes encoding apoptosis inhibitors (Bcl-xL, Mcl-1, and survivin), cell cycle regulators (cyclin D1 and c-myc), and inducers of angiogenesis (VEGF; ref. 3). Therefore, Stat3 is considered to be an important molecular target for human cancer therapy (4).

Head and neck squamous cell carcinoma (HNSCC) is believed to arise via multistep carcinogenesis (9–11). Common molecular events that contribute to the emergence of the tumor cell clone and its survival may be considered as potential therapeutic targets. Increasing evidence has emerged from studies examining tumor cells in culture and clinical samples that show Stat3 is a critical mediator of oncogenic signaling. In a recent report, 74 tumor specimens from 90 HNSCC patients were found to have constitutive levels of Stat3 activation (12). Within these patient samples, 74% and 47% showed high levels of Stat3 activity in the early and late classification of carcinogenesis, respectively. Previous studies using antisense treatment and transfection of dominant-negative Stat3 constructs have shown that Stat3 plays a crucial role in HNSCC cell growth in vitro (13, 14). Cumulatively, these results indicate that activation of Stat3 signaling contributes to the development of HNSCC and provide a strong rationale for targeting Stat3 in the treatment of head and neck cancer (15).

G-rich DNA sequences have been identified, cloned, and characterized in the telomeres of many organisms, such as fungi, ciliates, vertebrates, and insects (16). G-quartets arise from the association of four G-bases into a cyclic Hooft-Steenum H-bonding arrangement. G-quartets stack on top of each other to give rise to tetrad-containing helical structures. G-rich DNA can form different G-quartet structures, including intramolecular G-quartets, hairpin dimers, and parallel-stranded tetramers (17–19). Based on the G-quartet structure and its physical properties, GQ-ODNs act as non-antisense agents that directly interact with a target protein to interfere with its function. G-quartet forming oligodeoxynucleotides (GQ-ODN) have been developed to modulate several biological processes such as telomerase activity (20), human thymbin activity (21), HIV infection (22, 23), and HIV-1 integrase activity (24–27). GQ-ODNs were also developed as anticancer agents to inhibit DNA replication and induce S-phase cell cycle arrest (28, 29) and inhibit topoisomerase I (30). Topoisomerase I plays a crucial role in DNA replication, RNA transcription, and other cellular functions (31).
Recently, we developed GQ-ODNs, T40214, and T40231, which serve as potent inhibitors of Stat3 DNA-binding activity thereby suppressing expression of Stat3-regulated, antiapoptotic genes such as Bcl-2 and Mcl-1 in cancer cells (32). Computational methods predicted that GQ-ODNs insert between the two SH2 domains of the Stat3 homodimer, resulting in dimer destabilization. When administered i.v. through a novel drug delivery system, T40214 and T40231 dramatically inhibited the growth of prostate and breast tumor xenografts in nude mice. A biochemical examination of tumors from GQ-ODN–treated mice showed a significant decrease in the expression of antiapoptotic proteins, Bcl-2 and Bcl-xL, and a marked increase in apoptosis of tumor cells (33).

In the present study, we showed that GQ-ODN selectively inhibits the DNA-binding activity of Stat3, but not Stat1, derived from HNSCC cells and that it significantly increases apoptosis in these cells. Our in vivo results showed that the growth of HNSCC tumors in nude mice xenografts was strongly suppressed by treatment with GQ-ODN alone or combined with paclitaxel.

Materials and Methods

Materials

Oligonucleotides were synthesized by Midland Certified Reagent Co. (Midland, TX) and used without further chemical modification. The HNSCC cell lines MDA-1986, Tu167, and B488 were provided by Dr. Li Mao (Thoracic/Head and Neck Medical Oncology, M.D. Anderson Cancer Center, Houston, TX). The cells were grown in DMEM medium containing 10% fetal bovine serum with penicillin and streptomycin. Polyethyleneimine (25 K) was purchased from Aldrich Chemical Co. (Milwaukee, WI). Antibodies to Stat1 and Stat3 were purchased from R&D Systems, Inc. (Minneapolis, MN). Antibodies to Stat1 and Stat3 were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

Electrophoretic Mobility Shift Assay

Electrophoretic mobility shift assay was done as previously described (32). Briefly, IL-6 (25 ng/mL) or IFN-γ (25 ng/mL) was added into wells containing 5 × 10^5 to 7 × 10^5 HNSCC cells. Cells were washed and extracted using high-salt buffer. The protein concentrations of the extracts were determined using a Bradford assay (Bio-Rad Laboratories, Hercules, CA). The 32P-labeled duplex DNA probe (hSIE, 5'-AGCTTCATTTCCCGTAAATCCTA) was purified using G-25 columns (GE Healthcare/Amersham Biosciences, Piscataway, NJ). Labeled hSIE probe was mixed with 5 µg of cell protein in 1× binding buffer and 2 µg of polyelectroinonic-deoxyctydylidic acid) and incubated at room temperature for 15 minutes with or without GQ-ODN. Samples were loaded onto 5% polyacrylamide gel containing 0.25× Tris-borate EDTA and 2.5% glycerol. The gel was run at 160 to 200 V for 2 to 3 hours at room temperature, dried, and autoradiographed.

Computational Analysis

GRAMM and HEX docking programs were employed to predict the interaction between GQ-ODN T40214 and Stat3 dimer. GRAMM uses a geometry-based algorithm to generate quantitative data that will predict the structure of a protein-protein complex and forecast the quality of the contact between molecules of known structures (34). The GRAMM docking program constructed the complexes of GQ-ODN/Stat3 dimer, without any prior restriction for the binding site, and generated a low-energy conformation as an initial orientation for HEX. An advanced docking technology tool, HEX can rapidly search a high-resolution structure in six dimensions for rigid ligand docking based on its spherical polar Fourier correlations (35). We systematically rotated (180 degrees) each molecule about its centroid and searched the structure of 980 candidates for those with the lowest binding energy for shape complementarities and electrostatic contribution. The final docking structure was determined through GRAMM and HEX.

In vivo Delivery of Fluorescent-Labeled GQ-ODN

We administered 5′-fluorescent-labeled T40214 (10 mg/kg) plus polyethyleneimine (2.5 mg/kg) via i.p. injection into nude mice with HNSCC tumors. After injecting GQ-ODN/ polyethyleneimine complex at 24, 48, and 72 hours, the mice were sacrificed and the tumor tissues were harvested and frozen. Frozen tissues were sectioned for histopathologic analysis with a cryostat microtome; subsequently, the sections were lightly fixed and the tumors were examined via fluorescent microscopy.

In vivo Drug Tests with Xenograft Models

Athymic nude mice (Balb-nu/nu, 4 weeks old, and weighing ~20 g; Charles River Laboratories, Inc., Wilmington, MA) were injected s.c. into the right (or left) flank with 1 million HNSCC cells (MDA1986) in 200 µL PBS. After tumors were established, nude mice with HNSCC tumors were randomly assigned to five groups with five mice in each group: group 1 (placebo) was treated by polyethyleneimine alone; group 2 was treated with paclitaxel (a toxicity drug); groups 3 and 4 were treated with T40231/polyethyleneimine and T40214/polyethyleneimine, respectively; and group 5 was treated with T40214/polyethyleneimine plus paclitaxel. Polyethyleneimine and GQ-ODN were administered every 2 days and paclitaxel was injected i.p. every 4 days. Tumors were measured every 2 days; tumor sizes were calculated by using the function \( [a \times (0.5b)^2] \), where \( a \) and \( b \) are the length and width of tumors, respectively.

H&E Staining and Terminal Deoxyribonucleotidyl Transferase–Mediated dUTP Nick End Labeling Analysis

To determine potential morphologic changes in tumors derived from cells treated with GQ-ODNs, we collected tumor tissues from each treatment group, fixed them with 10% formaldehyde in paraffin, and sectioned them. For morphologic examination, 5-µm tissue sections were stained with H&E. For terminal deoxyribonucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) analysis, 5-µm tissue sections were mounted on siliconized glass slides, air-dried, and heated at 45°C overnight. After deparaffinization and rehydration, the sections were digested with proteinase K (120 µg/mL) for 20 minutes at room temperature. Following quenching of the endogenous peroxidase activity, the sections were washed in PBS and
subsequently incubated with equilibration buffer for 10 minutes at room temperature. After blotting, we applied 50 μL of a mix containing terminal deoxynucleotidyl transferase and reaction buffer containing dATP and digoxigenin-11-dUTP. The sections were covered with a plastic coverslip and placed in a humidified chamber at 37°C for 1 hour. After removal of the coverslip, the sections were washed in stop/wash buffer for 10 minutes at room temperature and subsequently in PBS. The sections were then incubated with antidigoxigenin-peroxidase for 30 minutes at room temperature and washed in PBS. Finally, color development was accomplished by immersion of the slides in 3.3′-diaminobenzidine/0.1% H₂O₂ for 3 to 7 minutes. Sections were counterstained with ethyl green, washed in butanol, cleared in xylol, and mounted with Permount.

Results

Inhibition of Stat3 DNA-Binding Activity by GQ-ODNs in Head and Neck Cancer Cells

Recently, we developed G-rich oligodeoxynucleotides, T40214 and T40231, as lead compounds that would serve as potent inhibitors of Stat3 activity (Fig. 1A); T40214 and T40231 both form G-quartet DNA structures (GQ-ODN) inside cells (32). The difference between the two structures is that T40214 is composed of two G-quartets in the center and two G-C-G-C loop domains on the top and bottom, whereas T40231 is composed of two G-quartets with one T-G-T-G loop on the bottom and one G-T loop on the top (Fig. 1B). The GQ-ODN T40214 forms very stable molecular structures, ~15 Å in length and 15 Å in width. The results obtained from electrophoretic mobility shift assay showed that IL-6 activated Stat3 DNA-binding activity within three HNSCC cells, TU167, B4B8, and MDA1986 (Fig. 1C), and that GQ-ODNs T40214 and T40231 strongly inhibited the Stat3 DNA-binding activity with IC₅₀s of 5 and 7 μmol/L, respectively (Fig. 1D). Nonspecific ODN, which does not form G-quartet structures, was used as a control; nonspecific ODN showed no inhibition of Stat3 DNA-binding activity even when the concentration of ODN was increased up to 285 μmol/L, showing that G-quartet structure is essential to the inhibition of Stat3 DNA-binding activity.

GQ-ODN Specifically Targets Stat3 among STAT Proteins

To confirm that the GQ-ODN was specific to Stat3, as opposed to Stat1, we examined the ability of GQ-ODN to inhibit the DNA-binding activity of IL-6-activated Stat3 and IFN-γ-activated Stat1, respectively, via electrophoretic mobility shift assay (Fig. 2A). The Stat1-DNA and Stat3-DNA complexes were identified using antibodies against Stat3 (Ab3) and Stat1 (Ab1). We found that T40214 strongly inhibited the DNA-binding of Stat3 (IC₅₀ 5 μmol/L) whereas 50% inhibition of Stat1 DNA-binding was not achieved under the same conditions using concentrations of T40214 up to 142 μmol/L. These results provide solid evidence that GQ-ODN preferentially targets Stat3 rather than Stat1.

Mechanism of Stat3-Specific Targeting by GQ-ODN

To determine the mechanism of selective Stat3 targeting by GQ-ODN, we randomly docked GQ-ODN T40214 (1,000 times) onto the dimer structures of both Stat3 and Stat1, without setting any constraints, and analyzed a distribution of H-bonds formed between GQ-ODN and the dimer of Stat3 or Stat1. The number of H-bonds formed between GQ-ODN and Stat3 dimer was 2-fold greater than that formed between GQ-ODN and Stat1 dimer, showing strong binding interaction between GQ-ODN and Stat3 dimer. The histograms of H-bond distribution show that the interaction between GQ-ODN and Stat3 dimer was highly concentrated on the binding site composed of amino acids 638 to 652, especially in the residues of Q643, N646, and N647. However, strong binding interaction did not occur within the region of the Stat1 dimer (Fig. 2B and C).
The lowest energy complex obtained from high-resolution GRAMM docking was selected as the initial ligand for HEX. HEX docking scanned 2,520,089,600 orientations; the complex of GQ-ODN/Stat3 dimer is depicted in Fig. 2D. The crystal structures of Stat3 and Stat1 dimers (36, 37) show that in Stat3 dimer, the residue Q643 of one monomer repels N646 of another due to negative-charged polar side chains, thus opening a channel in the SH2 domains. In contrast, in Stat1, K637 of one monomer interacts with S640 of another, which locks the dimer together. These docking results show that residues in the loop domains of GQ-ODN form seven H-bonds with the residues of Q643 to N647 and tightly bind into the site of Stat3, thereby destabilizing the dimer formation and disrupting Stat3 DNA-binding; in contrast, GQ-ODN cannot interact with Stat1.

Delivery of GQ-ODNs into Tumors of Xenograft Models

An effective drug delivery system is essential to the development of nucleic acid based drugs targeting intracellular signaling proteins as cancer therapeutic agents. The principal difficulty of delivering GQ-ODN into cells arises from the physical and structural properties of GQ-ODN because it cannot directly penetrate cell membranes (38). To determine if GQ-ODN is actually delivered to HNSCC tumors of nude mice and how long it persists within tumors, we administered, via i.p. injection, 5\(^{15}\)F-fluorescent-labeled T40214 (10 mg/kg) plus polyethylenimine (2.5 mg/kg). After these injections, we harvested the tumors at 24, 48, and 72 hours and examined the tumors under fluorescence microscopy. The level of GQ-ODN in tumors at 48 and 72 hours was ~60% and 20%, respectively, of GQ-ODN in tumors at 24 hours (Fig. 3A). The results clearly showed that GQ-ODN was effectively delivered within the tumors and that GQ-ODN had prolonged half-life within the tumors.

Suppression of HNSCC Tumor Growth by GQ-ODN

The assessment of the effectiveness of a drug in an animal model is an important step toward establishing the potential clinical utility of the drug. To this end, we used the nude mice tumor xenograft model to evaluate the potential of GQ-ODNs as anticancer agents. First, nude mice were injected s.c. with HNSCC cells (MDA1986). When tumors were established (volume of 50–150 mm\(^3\)), treatment of the nude mice with HNSCC tumors was administered by i.p. injection. The nude mice were randomly assigned to five groups: group 1 (placebo) was treated by polyethylenimine (2.5 mg/kg) alone; group 2 was treated with paclitaxel at 10 mg/kg (39); groups 3 and 4 were treated with T40231/polyethylenimine and T40214/polyethylenimine (10 + 2.5 mg/kg), respectively; and group 5 was treated with T40214/polyethylenimine (10 + 2.5 mg/kg) plus paclitaxel (10 mg/kg). Polyethylenimine and GQ-ODN were administered every 2 days and paclitaxel was injected every 4 days. Over the course of the 21-day treatment period, we found that the mean size of HNSCC tumors in the placebo-treated mice increased from 90 to 850 mm\(^3\) whereas the mean size of HNSCC tumors in the T40214-treated mice
only increased from 84 to 145 mm$^3$; in the T40214 plus paclitaxel–treated mice, tumors decreased from 86 to 56 mm$^3$ (Fig. 3B-D). Our statistical analyses indicated significant differences in tumor growth between (a) placebo-treated and combination-treated mice ($P < 0.001$), (b) placebo-treated and T40214-treated mice ($P = 0.001$), (c) placebo-treated and T40231-treated mice ($P = 0.037$), (d) paclitaxel-treated and combination-treated mice ($P < 0.001$), and (e) paclitaxel-treated and T40214-treated mice ($P = 0.005$).

**GQ-ODN Suppression of Tumor Growth through Apoptosis**

To determine the mechanism of tumor growth suppression by GQ-ODNs, we analyzed HNSCC tumors microscopically following H&E staining (Fig. 4, top) and TUNEL staining (Fig. 4, bottom). HNSCC tumors were harvested from placebo-treated and drug-treated (GQ-ODN or paclitaxel) nude mice under the same experimental conditions. Tumor cell histologic features of apoptosis on H&E-stained slides were observed only in drug-treated tumors and not in tumors from placebo-treated mice (Fig. 4A). TUNEL staining showed no TUNEL-positive cells in tumors from placebo-treated mice (Fig. 4B, left). A few TUNEL-positive cells were detected in the tumors from paclitaxel-treated mice (Fig. 4B, middle). However, the greatest number of TUNEL-positive cells was observed in tumors from GQ-ODN–treated mice (Fig. 4B, right). A count of the TUNEL-positive cells among total cells in the tumors revealed that the mean percentage of TUNEL-positive cells in the tumors from placebo-treated mice was 1.4% whereas that from T40214-treated mice was 12.3% ($P = 0.023$, Wilcoxin rank sum test).
Discussion

Stat3 has been proposed as a target for development of novel treatments for cancers including HNSCC (32, 33). Stat3 is constitutively activated in HNSCC as results of increased epidermal growth factor receptor or IL-6 receptor (gp130) activity, mediated by increased epidermal growth factor receptor expression and autocrine production of transforming growth factor-α (40, 41) or IL-6 (42), respectively. Inhibition of Stat3 activity—by targeting epidermal growth factor receptor, transforming growth factor-α (43, 44), and gp130 (42) or by directly targeting Stat3 via antisense oligodeoxynucleotides, dominant-negative Stat3 constructs, or antisense constructs—has been shown to inhibit tumor cell growth in vitro (45) and increase tumor cell apoptosis in vivo (13, 46). These findings indicate the importance of increased Stat3 activity in HNSCC and confirm that Stat3 is a critical oncogenic signaling protein involved in the proliferation and apoptosis prevention of HNSCC. Our previous studies have shown that GQ-ODN strongly suppresses the growth of prostate and breast tumors in nude mice xenografts by inhibiting Stat3 activity, which blocks transcription of the antiapoptotic proteins Bcl-xL and Bcl-2 and triggers apoptosis of cancer cells (33). Cumulatively, these findings show that Stat3 strongly influences tumor cell proliferation and apoptosis in multiple types of human cancer, making it an important therapeutic target. The development of a potent inhibitor of Stat3 may therefore be an important step toward formulating a novel chemotherapeutic treatment for HNSCC.

To determine whether targeting Stat3 with GQ-ODN will produce a novel chemotherapeutic treatment for head and neck cancers, we did in vitro and in vivo experiments with GQ-ODN using several HNSCC cell lines. The results clearly show that GQ-ODN significantly inhibits the DNA-binding activity of Stat3 but not that of Stat1. Specifically targeting Stat3 among other STAT proteins is highly desirable. In addition to Stat3, constitutive activation of Stat1 protein has been shown in certain human cancer cells and tumor tissues, including breast cancer and HNSCC. Stat1 acts in a proapoptotic and antiproliferative manner, which denotes a function that is in opposition to Stat3 (3). Consequently, specifically targeting Stat3, rather than Stat1, is advantageous in the development of a potent Stat3 inhibitor.

The mechanism for the selective targeting of Stat3 by GQ-ODN was determined using computational analyses, which are based on statistical methods at the molecular level (47). We randomly docked GQ-ODN onto Stat3 or Stat1 dimers 1,000 times without setting any restrictions. The number of H-bonds formed between GQ-ODN and Stat3 dimer was 2-fold greater than those formed between GQ-ODN and Stat1 dimer, showing a stronger binding interaction.

Figure 4. Apoptosis induced in HNSCC tumors. A, H&E images show that in placebo-treated tumors, all HNSCC cells are alive (left) whereas in GQ-ODN–treated tumors, most HNSCC cells are reduced in size and condensed, indicating apoptosis (right). B, TUNEL-stained slides show that in placebo-treated tumors, TUNEL-positive cells are not present (i.e., no cells are stained dark brown; left); a few TUNEL-positive cells are present in paclitaxel-treated tumors (middle); and in the GQ-ODN–treated tumors, the greatest number of TUNEL-positive tumor cells are presented (right).
between GQ-ODN and Stat3 dimer. The histograms of H-bond distribution clearly showed that GQ-ODN interacts predominantly with Stat3 dimer in the range of amino acid residues 638 to 652, which lie within the SH2 domains; these residues play a critical role in Stat3 dimerization. Although the sequences and structure of Stat3 dimer are very similar to the Stat1 dimer (36, 37), selective targeting of Stat3, and not of Stat1, by GQ-ODN was based on a few critical amino acid residues. The paired residues, Q643 and N646, repel one another due to their negatively charged polar side chains. The docking results showed that GQ-ODN extended into the SH2 domains and formed seven H-bonds with residues Q643 to N647, and predicted that GQ-ODN would destabilize Stat3 dimer formation and disrupt DNA-binding in Stat3. However, GQ-ODN did not interact with Stat1 due to the fact that the K637 of one monomer interacts with the S640 of the other, thereby locking the binding site and blocking the interaction of GQ-ODN with the Stat1 dimer.

The growth of HNSCC tumors in nude mice xenografts was significantly suppressed by GQ-ODN T40214, as well as by the combined treatment of GQ-ODN T40214 and paclitaxel. The tumors grew very fast when the mice were treated with polyethyleneimine alone. However, for the five mice treated with T40214 and paclitaxel, virtually all tumors disappeared over the 21-day treatment period. Paclitaxel binds to microtubules, stimulates microtubule polymerization, and blocks the ability of cells to dismantle the mitotic spindle during mitosis (48, 49). GQ-ODN has been shown to inhibit Stat3 activation and suppress the expression of antiapoptotic proteins, such as Bcl-xL and Bcl-2, inducing apoptosis in tumors (33). In our in vivo drug tests, we found that the combined treatment of T40214 and paclitaxel significantly suppressed HNSCC tumor growth during the entire study period, showing the synergy of this combination. GQ-ODN suppressed tumor growth, at least in part, because of increased apoptosis whereas paclitaxel presumably blocked tumor cell division. Possibly, the two activities, enhancing cell death and reducing cell proliferation, combined to effectively block tumor growth.

GQ-ODN, as a novel class of anticancer agent, has several specific features (47). GQ-ODNs such as T40213 and T40231 form the G-quartet structures within cells, and T40231 also inhibits and T40214 significantly suppresses HNSCC tumor growth during the entire study period, showing the synergy of this combination. GQ-ODN suppressed tumor growth, at least in part, because of increased apoptosis whereas paclitaxel presumably blocked tumor cell division. Possibly, the two activities, enhancing cell death and reducing cell proliferation, combined to effectively block tumor growth.

GQ-ODN, as a novel class of anticancer agent, has several specific features (47). GQ-ODNs such as T40213 and T40231 form the G-quartet structures within cells, which prevent single-strand endonucleases from accessing their cleavage sites, leading to a long oligonucleotide half-life inside cells (50). In addition, G-quartet ODNs show low toxicity. An analogue of T40214 (51) did not exhibit genetic toxicity in three different mutagenesis assays: the Ames Salmonella mutagenesis assay, the Chinese hamster ovary/hypoxanthine-guanine phosphoribosyltransferase mammalian cell mutagenesis assay, and the mouse micronucleus assay. In addition, this GQ-ODN had an LD₅₀ ≥ 1.5 g/kg body weight, which is 150-fold greater than the therapeutic dose used in our studies (10 mg/kg). Therefore, GQ-ODNs represent promising novel agents for cancer therapy either alone or in combination with other drugs such as paclitaxel.

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