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Spectroscopic investigation of the interaction between G-quadruplex of *KRAS* promoter sequence and three isoquinoline alkaloids

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ABSTRACT

KRAS promoter can form G-quadruplex structure and regulate gene transcription. The drugs which can bind with G-quadruplex of *KRAS* promoter may be potential remedy for treatment of cancers associated with *KRAS* mutation. The interaction mechanism between the G-quadruplex of *KRAS* promoter and three isoquinoline alkaloids (jatrorrhizine, berberine and sanguinarine) has been investigated by UV-visible, fluorescence and circular dichroism spectroscopic methods. The results showed that the three alkaloids can form complexes with G-quadruplex *KRAS* promoter with the molecular ratio of 1:1, and the binding constants were $(0.90 \pm 0.16) \times 10^6$ L mol^{-1}, $(0.93 \pm 0.21) \times 10^6$ L mol^{-1} and $(1.16 \pm 0.45) \times 10^6$ L mol^{-1} for jatrorrhizine, berberine and sanguinarine. The absorption spectra, KI quenching and fluorescence anisotropy and polarization studies suggested jatrorrhizine and berberine interacted with G-quadruplex by not only end-stacking binding mode but also grooves or loops binding mode, while sanguinarine by end-stacking binding mode. Sanguinarine was more beneficial to maintain the stability and parallel conformation of *KRAS* promoter G-quadruplex. MTT assay was performed to evaluate antiproliferation effects of the three isoquinoline alkaloids on SW620 cells, and the antiproliferation effects of the three alkaloids were sanguinarine > berberine > jatrorrhizine. All the three alkaloids can bind with *KRAS* promoter G-quadruplex, and sanguinarine had the better binding property and antiproliferation effects on SW620 cells. The results obtained are meaningful to explore potential reagents targeting the parallel G-quadruplex structure of *KRAS* promoter for gene theraphy of colorectal carcinomas.
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1. Introduction

G-quadruplex DNA is an effective target for anticancer drug development and has aroused the wide attention of researchers [1]. As a special nucleic acid secondary structure, G-quadruplex conformation of DNA was first recognized in the early 1960s [2]. For the guanine rich sequences, four guanines formed "G-quartet" by interacting with each other through Hoogsteen hydrogen bonds, which stacked on top of one another, constituted the G-quadruplex topologies, and kept stable in physiological buffer conditions.

In the human body, over 300000 sequences have the possibility of forming G-quadruplex structures [3–5]. Human telomeres contain the highest abundance of G-quadruplexes, which consist of repetitive sequences $(TTAGGG)_n$. Therefore, telomeric G-quadruplex DNA is able to prevent cancer cell immortality and drugs inducing or stabilizing quadruplex structures may be promising antitumor agents [6]. Besides, there are also a large number of G-quadruplex-forming sequences in many important regions of the human genome, such as gene promoters, immunoglobulin gene class switch and microsatellite [7]. Because of the differences in G-quartets number and the base composition and length of the loops or residues in the structure formed by non-guanine bases, there were various G-quadruplex geometries, for instance, four stands parallel structure, propeller-type, basket-type, chair-type, hybrid-type 1, hybrid-type 2, two strands antiparallel structure with diagonal loops and two strands antiparallel structure with lateral loops [8–20]. These conformations had different stabilities, and the binding abilities of small molecules with different G-quadruplexes were also different [21,22]. Blocking G-quadruplex formation may promote gene transcription, while stabilizing G-quadruplex structure may suppress gene transcription [23]. Previous research demonstrated the G-quadruplexes played an important role in cell life activity, especially in regulating gene transcription and translation, and then affected cell proliferation and cancer progression [24].

Promoter regions of some oncogenes frequently contain G-quadruplex structures which were essential for transcription, for example, *BCL-2*, c-*MYC*, c-*KIT*, *KRAS* and so on [25,26]. As a molecular switch in human body, *KRAS* gene plays a regulatory role in the signal transduction pathway of tumor cell growth and angiogenesis [27,28]. It encodes Kras 21-kD proteins, which take part in the regulation of many

Abbreviations: JTZ, jatrorrhizine; BBR, berberine; SGR, sanguinarine; CD, circular dichroism; NHE, nuclease hypersensitive element.

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important physiological processes such as cellular proliferation and differentiation [29]. *KRAS* gene is prone to having activating mutations in tumors [30]. Normal *KRAS* can suppress growth of tumor cells, however, gene mutation may induce the permanent activation of *KRAS*, leading to the production inhibition of normal Kras proteins, so that cell proliferation was out of control and then cancer occurred [31,32]. *KRAS* gene mutation widely exists in many neoplastic disease such as pancreatic adenocarcinoma, colorectal carcinomas, lung cancer and prostatic cancers [33–35]. The promoter of human *KRAS* proto-oncogene contains a nuclease hypersensitive element (NHE), and its G-rich strand can form a parallel G-quadruplex structure, which may suppress gene transcription and exhibit antiproliferation effects on pancreatic carcinoma cells [36–39]. Previous studies demonstrated that the sequence 5′-AGGGCGGTGTGGGAAGAGGGAAGAGGGGGAGG-3′ derived from the promoter region of *KRAS* formed the intramolecular parallel G-quadruplex structure with three G-tetrads and three loops in the presence of K^+, which could be stabilized by the cationic porphyrin TMPyP4 and further regulate gene transcription [37]. From this point of view, for cancers associated with *KRAS* mutations, small molecule which can bind with *KRAS* promoter G-quadruplex and stabilize its structure may play a role in cancer treatment by regulating gene transcription.

Natural products have been a fertile medical source of cancer treatment. Some of the quindoline alkaloid natural products and their derivatives have been demonstrated to stabilize the structure of G-quadruplex [40]. As shown in Fig. 1A-D, Jatrorrhizine (JTZ), berberine (BBR) and sanguinarine (SGR) are three structural analogous isoquinoline alkaloids. The former two drugs with protoberberine type isoquinoline structures are quaternary ammonium salt type alkaloids, and exist widely in the medicinal plants of *Coptischinensis* Franch, *Scutellaria baicalensis* Georgi and *Phellodendron chinense* Schneid, while sanguinarine with benzophenanthridine type isoquinoline structures exists in both non-ion form and quaternary ammonium salt form in the body [41], and comes from the medicinal plants of *Eomecon chionantha*, *Chelidonium majus* and *Macleaya cordata*. The three drugs have extensive pharmacological activities, such as anti-inflammatory, antibacterial, antiviral, and antioxidant effects [42–44]. In recent years, the antitumor activity of them has gradually been concerned. Researches showed that they performed antitumor functions in multiple cancer cells. The possible anticancer mechanisms include suppression of cell cycle, cell proliferation and tumor angiogenesis, regulation of apoptosis related genes, signal transduction pathway, tumor invasion and metastasis, as well as immune function [43,45]. Molecular mechanisms of inhibition effect on cell proliferation were mainly due to the interaction between drugs and DNA or RNA [46,47]. Binding of these drugs with human double helix DNA, c-*MYC* promoter and telomere G-quadruplex DNA has been reported [48–53].

In previous studies, we discussed the binding ability of biological polyamines and SGR with different G-quadruplexes and their influence on G-quadruplex structures, proving that the binding characteristic of drugs with G-quadruplex DNA depended on the base composition and geometrical conformation of G-quadruplex sequences [26,54]. In this work, molecular mechanisms of interactions between three structural analogue alkaloids JTR, BBR, SGR and G-quadruplex motifs of *KRAS* promoter NHE sequence were probed in depth by UV-visible, fluorescence and circular dichroism (CD) spectroscopic methods. In China, over 50% of the colorectal cancer patients have mutational *KRAS* gene. Drugs binding with promoter G-quadruplex structure may impact KRAS gene transcription and ultimately affect cell proliferation, so effects of the three drugs on the proliferation of a kind of colorectal cancer cell lines SW620 were studied, too. The purpose of this research was to comparatively study interaction mechanisms between three alkaloids and *KRAS* promoter G-quadruplex, as well as the correlation of the drug molecular structure and its binding characteristic with *KRAS* promoter G-quadruplex, in order to explore potential targeting drugs for gene theraphy of colorectal carcinomas.

2. Material and methods

2.1. Materials and Reagents

The *KRAS* promoter NHE oligonucleotide sequence 5′-AGGGCGGTGTGGG-AAGAGGGAAGAGGGGGAGG-3′ containing four runs of three or five guanines, was purchased from Sangon Biotech Co., Ltd, China (Shanghai, China), and was used without further purification. The three alkaloids JTZ, BBR and SGR as chloride salts were obtained from Aladdin Co., Ltd, China (Shanghai, China). Tris(hydroxymethyl)- aminomethane (Tris) were purchased from Beijing Dingguo Biotech Co., Ltd, China (Beijing, China). KCl, KI and HCl were purchased from Beijing Chemical Works, China(Beijing,China). 3-(4-5-Dimethylthiazol-2-yl)-2,5-d iphenyltetrazolium bromide (MTT) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All these reagents were of analytical grade purity and used as received. The cell culture grade reagents high-glucose (4.5 g/L) Dulbecco's

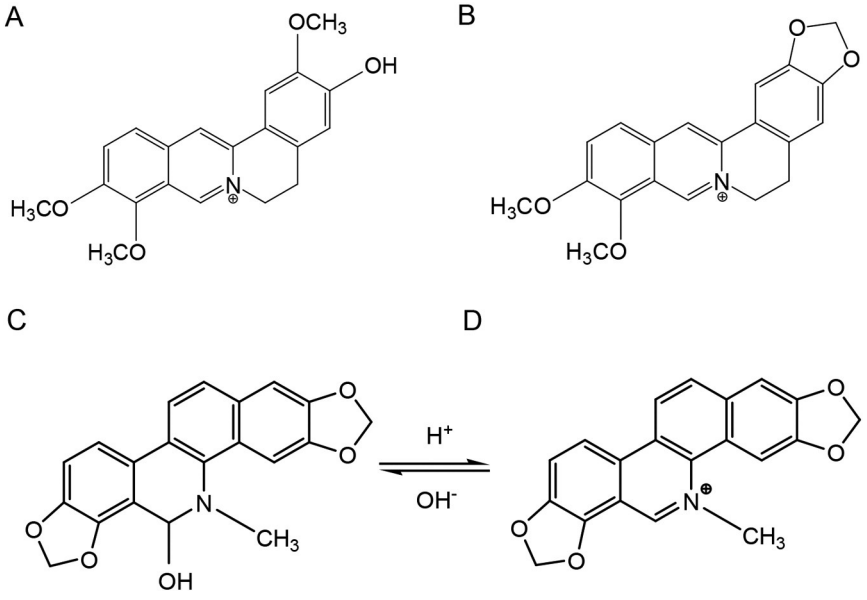

Fig. 1. Molecular structures of (A) jatrorrhizine, (B) berberine, (C) non-ion form of sanguinarine and (D) quaternary ammonium salt form of sanguinarine.

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