Original article

JS-K, a nitric oxide prodrug, induces DNA damage and apoptosis in HBV-positive hepatocellular carcinoma HepG2.2.15 cell

Zhengyun Liu\textsuperscript{a,b,d}, Guangmin Li\textsuperscript{f}, Ying Gou\textsuperscript{a,b,c}, Dongyan Xiao\textsuperscript{a,b,c}, Guo Luo\textsuperscript{a,b}, Joseph E. Saavedra\textsuperscript{e}, Jie Liu\textsuperscript{d}, Huan Wang\textsuperscript{a,b,c,*}

\textsuperscript{a}Key Laboratory of infectious disease, Provincial Department of Education, Zunyi Medical College Guizhou, 563000 China
\textsuperscript{b}Research Center for Medicine and Biology, Zunyi Medical College, Guizhou, 563000 China
\textsuperscript{c}Department of Microbiology, Zunyi Medical College, Guizhou, 563000 China
\textsuperscript{d}Key Lab for Basic Pharmacology of Ministry of Education, Zunyi Medical College, Guizhou, 563000 China
\textsuperscript{e}Leidos Biomedical Research Inc, Frederick, MD, United States
\textsuperscript{f}Department of emergency, Affiliated Hospital of Zunyi Medical College, China

A R T I C L E   I N F O

Article history:
Received 31 March 2017
Received in revised form 29 May 2017
Accepted 30 May 2017

Keywords:
JS-K
Nitric oxide donor
HBV
HCC
Apoptosis
DNA damage

A B S T R A C T

Hepatocellular carcinoma (HCC) is the most important cause of cancer-related death, and 85% of HCC is caused by chronic HBV infection, the prognosis of patients and the reduction of HBV DNA levels remain unsatisfactory. JS-K, a nitric oxide-releasing diazeniumdiolates, is effective against various tumors, but little is known on its effects on HBV positive HCC. We found that JS-K reduced the expression of HBsAg and HBeAg in HBV-positive HepG2.2.15 cells. This study aimed to further examine anti-tumor effects of JS-K on HepG2.2.15 cells. The MTT assay and colony forming assay were used to study the cell growth inhibition of JS-K; scratch assay and transwell assay were performed to detect cell migration. The cell cycle was detected by flow cytometry. The immunofluorescence, flow cytometry analysis, and western blot were used to study DNA damage and cell apoptosis. JS-K inhibited HepG2.2.15 cell growth in a dose-dependent manner, suppressed cell colony formation and migration, arrested cells in the G2 phase. JS-K (1–20 \textmu M) increased the expression of DNA damage-associated protein phosphorylation H2AX (\gamma H2AX), phosphorylation of checkpoint kinase 1 (p-Chk1), phosphorylation of checkpoint kinase 2 (p-Chk2), ataxia-telangiectasia mutated (ATM), phosphorylation of ataxia-telangiectasia mutated rad3-related (p-ATR) and apoptotic-associated proteins cleaved caspase-3, cleaved caspase-7, cleaved poly ADP-ribose polymerase (cleaved PARP). The study demonstrated JS-K is effective against HBV-positive HepG2.2.15 cells, the mechanisms are not only related to inhibition of HBsAg and HBeAg secretion, but also related with induction of DNA damage and apoptosis. JS-K is a promising anti-cancer candidate against HBV-positive HCC.

\textcopyright 2017 Elsevier Masson SAS. All rights reserved.

1. Introduction

Hepatocellular carcinoma (HCC) is the most important cause of cancer-related death [1], especially from hepatitis B virus (HBV) infection, and 85% HCC is caused by chronic HBV infection [2,3]. The anti-HBV drugs in combinations of transplantation, surgery, radiotherapy and chemotherapy are the most effective and widely performed treatment for HCC with HBV infection [4]. Although these treatments can improve the quality of life in patients of HCC with HBV positive, the prognosis of patients and the reduction of HBV DNA levels are still unsatisfactory. Therefore, the development of more effective therapeutic strategies, especially selective drugs is urgently required.

http://dx.doi.org/10.1016/j.biopharma.2017.05.141
0753-3322/\textcopyright 2017 Elsevier Masson SAS. All rights reserved.
HepG2.2.15 Antibody is a monoclonal antibody designed to be activated for nitric oxide (NO) release by glutathione S-transferase (GST) [5]. GSTs are frequently over-expressed in a broad spectrum of tumors [6]. The activation reaction is catalyzed by the GST [5]. The design of JS-K is aimed to target the over-expression of GST in malignant cells as compared to with normal tissues [7,8]. Importantly, JS-K has recently been shown to inhibit tumor growth both in vitro and in vivo [5,8]. For example, JS-K inhibits the growth of HL-60 (human myeloid leukemia) cells with IC$_{50}$ of 0.25–0.5 µM and against subcutaneous xenograft in NOD/SCID mice [5]; JS-K is effective in inhibiting the growth of multiple myeloma (MM-OPM1) cells and subcutaneous xenograft in mice [7,8]. For liver cancer cells, JS-K inhibits hepatoma cell proliferation in vitro [9–11] and increases the cytotoxicity of cisplatin and arsenite in drug-resistant liver cancer cells by increasing intracellular drug concentrations [12]. The most important mechanisms of JS-K anti-tumor effects are the induction of DNA double-strand breaks (DSBs) and apoptosis [7,9]. However, the effects of JS-K on the HBV-positive HepG2.2.15 cells have not been characterized. The HepG2.2.15 cells are a stable cell line, transfected with HBV genome into HepG2 cells, which secrete HBV DNA and express HBV antigen [13]. We have recently shown that JS-K was effective in reducing HBSAg and HBcAg secretion in HepG2.2.15 cells [14], and hypothesized that JS-K would be effective against this human carcinoma cell growth. In this study, we further investigated the cytotoxicity of JS-K on HepG2.2.15 cells and characterized molecular mechanisms by which JS-K induces tumor cell apoptosis and DNA damage. These studies will provide the preclinical evidence for the JS-K to inhibit HBV-positive hepatoma cells.

2. Materials and methods

2.1. Reagents

JS-K was purchased from Santa Cruz Biotechnology (California, USA), and the preliminary study was from Joseph E. Saavedra at NCI/Frederick. The Dulbecco’s Modified Eagle’s Medium/Nutrient Mixture F-12 Ham (DMEM/F12) was obtained from Hyclone (Utah, USA), fetal bovine serum (FBS) was purchased from Gibco (California, USA), Annexin V-FITC Apoptosis Detection Kit and Cell Cycle Detection Kit were purchased from KeyGEN BioTECH (Nanjing, china), DNA Damage Antibody Sampler Kit and Apoptosis Antibody Sampler Kit were purchased from CST (Boston, USA), Goat Anti-rabbit IgG H&L (Alexa Fluor® 488) was purchased from Abcam (Cambridge, UK), Transwell 24-well plates was purchased from Corning (New York, USA).

2.2. Cell culture

HepG2.2.15 cells were cultured in DMEM/F12 medium supplemented with 10% FBS, 0.5 mg/ml G418, 100 µg/ml streptomycin and 100 IU/ml penicillin at 5% CO$_2$, 37°C.

2.3. Cell morphology

HepG2.2.15 cells were seeded at $5 \times 10^5$ cells/well in 6-well plates for 48 h, and treated with JS-K for 24 h, and the morphology was observed with optical microscope (Olympus, Tokyo, Japan). We used IPP6.0 software to analyze the image.

2.4. MTT assay

Cell viability was analyzed by the MTT assay. Briefly, HepG2.2.15 cells were seeded at $1 \times 10^4$ cells/well in 96-well plates for 48 h, cultured in fresh medium containing various concentrations of JS-K and cisplatin. After incubated for 72 h, 10 µL MTT (5 mg/ml) was added and the plates were incubated at 37°C for 4 h. The culture medium was removed from the wells and replaced with 100 µL DMSO. Then, the value of optical density (OD) of the cells was measured at 570 nm using a microplate reader (Molecular Devices, Sunnyvale, USA). The effect of the drugs on cell viability was assessed as the percentages of cell viability compared with the control cells which were arbitrarily assigned as having 100% viability.

2.5. Cell migration scratch assay

HepG2.2.15 cells (5 × 10$^4$ cells/well) were plated and grown to confluence in 96-well plates. Then, the cell layer in each well was scratched using a 10 µL pipette tip. Once the scratch was made the media was removed and replaced with fully supplemented media or fully supplemented medium containing with JS-K 10 µM for 72 h. Images of the scratch were captured immediately after the scratch was induced and after drug administration. The migration distances were measured and quantified with IPP6.0 software. The cell migration rate (%) = (0 h scratch distances – 72 h scratch distances)/0 h scratch distances × 100%.

2.6. Colony forming assay

HepG2.2.15 cells were treated with JS-K for 24 h, and then trypsinized to collect cells, seeded 5 × 10$^3$ cells/well onto a 6-cm dish incubation for 14 days. The cells were washed with PBS twice, fixed with 4% parformaldehyde at room temperature for 10 min, washed with PBS twice, stained with 1% crystal violet for 10 min, washed with PBS 5 times and air-dried. The colonies were counted under an ordinary optical microscope (Olympus, Tokyo, Japan).

2.7. Transwell assay

Migration of HepG2.2.15 cells (3 × 10$^5$ cells/well) was analyzed by Transwell inserts. The cells were seeded to the upper chambers (200 µL/well) in 2% FBS medium with JS-K (0, 1, 10 and 20 µM). The lower chambers (500 µL/well) were filled with medium containing 20% FBS. After incubated at 37°C for 72 h, the cells that passed through the filter into the lower chamber were fixed with 4% parformaldehyde and stained with hematoxylin and eosin. The number of invaded cells was counted and photographed under a microscope (Olympus, Tokyo, Japan) for at least five fields.

2.8. Immunofluorescence

HepG2.2.15 cells (4 × 10$^4$ cells/well) were seeded in chamber slides for 24 h, and treated with JS-K. After 24 h, the cells were washed with PBS and fixed with 4% parformaldehyde at room temperature for 20 min, cells were washed twice with PBS. Permeabilization in 0.5% Triton X-100 for 20 min, cells were washed twice in PBS and blocked for 30 min in blocking buffer (3% albumin bovine V, 0.2% – Triton-X 100, 2% donkey serum). Cells were incubated with the γ-H2AX antibody diluted with blocking buffer overnight at 4°C. On the following day, cells were washed with PBS twice and incubated in Goat Anti-rabbit IgG H&L (Alexa Fluor® 488) second antibody overnight at 4°C. The slides were then washed 3 times in PBS before mounted using mounting medium with DAPI (VECTASHIELD, CA, USA). The images were obtained with a confocal microscope (Leica, Mannheim, Germany).
2.9. Flow cytometric analysis of cell cycle

Cell cycle detection Kit was used to detect the cell cycle. HepG2.2.15 cells (5 × 10^5 cells/well) were cultured in 6-well plates for 48 h, treated with JS-K for 24 h. The cell pellets were re-suspended in 70% methanol for overnight at 4 °C. On the following day, cells were washed with PBS twice and re-suspended in 100 μL RNase A buffer 30 min at 37 °C, added 400 μL propidium iodide (PI), vortexed and incubated for 10 min in the dark. The samples were analyzed on a FACSCalibur flow cytometer (Beckman, Fullerton, USA).

2.10. Flow cytometric analysis of apoptosis

Annexin V-FITC/PI Apoptosis Detection Kit was used to detect the apoptotic cells. HepG2.2.15 cells (5 × 10^5 cells/well) were cultured in 6-well plates for 48 h, treated with JS-K for 24 h, and then used trypsin without EDTA to collect cells. The cell pellets were re-suspended in 500 μL 1 × binding buffer, and 5 μL Annexin V-FITC and 5 μL PI were added. The cells were vortexed and incubated for 5 min without light. Flow cytometric analysis (Beckman, Fullerton, USA) was performed immediately after staining.

2.11. Western blot

After treated with JS-K 24 h, HepG2.2.15 cells (5 × 10^5 cells/well) were harvested in RIPA lysis buffer, cell protein concentrations were determined by the BCA assay. The protein was resolved on a SDS-polyacrylamide gel electrophoresis and transferred to polyvinylidene difluoride membrane. The membranes were blocked for 2 h at room temperature in 5% BSA and incubated with the indicated antibodies overnight at 4°C, followed by fluorescence secondary antibodies (Goat anti-rabbit IgG (H + L)/Goat anti-mouse IgG (H + L), HSA) at room temperature for 2 h. The bands were detected with the Odyssey infrared imaging system (LI-COR, Nebraska, USA).

2.12. Statistical analysis

The results were described as the mean value ± standard deviation (SD). All the data were analyzed using the SPSS 17.0 software, using independent sample T test and a one-way analysis of variance (ANOVA), followed by LSD or Dunnett’s T3. P value <0.05 was considered to indicate a statistically significant difference.

Fig. 1. JS-K inhibited the growth of HepG2.2.15 cells. (A) Cells were treated with various concentrations of JS-K for 24 h and cell morphology was observed by microscope, 400×. (B) Cells were cultured in the presence of JS-K or cisplatin for 72 h, cells viability were determined by the MTT assay. (C) and (D) In colony forming assay, before the cells plated, cells were treated with 20 μM JS-K for 24 h. Cells were stained with 1% crystal violet after incubation for 14 days. The data were expressed as the mean ± standard deviation (SD) of three experiments. *P < 0.05 indicates a significant difference from the control group.
3. Results

3.1. JS-K inhibited the growth of HepG2.2.15 cells

Treatment of HepG2.2.15 cells with JS-K at the dose of 0, 1, 10 and 20 μM reduced the cell–cell contact and produced cell cytoplasm shrinkage (Fig. 1A). To evaluate cell viability after JS-K and cisplatin administration, HepG2.2.15 cells were treated with JS-K and cisplatin at 0, 3.12, 6.25, 12.5, 25 and 50 μM, followed by the MTT assay at 72 h. As shown in Fig. 1B, significant ($P < 0.05$) decreases in cell viability were observed after a range of concentrations of JS-K and cisplatin. In the lower concentrations, JS-K was more effective compared with cisplatin, while cisplatin was more effective in higher concentrations. The colony formation assay (Fig. 1C, D) showed that JS-K suppressed HepG2.2.15 cell colony formation ($P < 0.05$), clone formation rates were 28.10% for control group and 11.92% for JS-K group. Taken together, these results indicated that JS-K inhibited HBV-positive HCC cell growth in a dose-dependent manner (Figs. 2 and 3).

3.2. JS-K inhibited cell migration in HepG2.2.15 cells

Next, we investigated the effect of JS-K administration on cell migration. In scratch assay, we observed the obvious cell migration in control group after scratch at 72 h, but this phenomenon did not occur in JS-K group (Fig. 2A,B). The cell migration rate was reduced to 3.27% (JS-K group) from 21.12% (control group) after treatment. JS-K also inhibited cell migration in transwell cell migration assay, with the increasing concentration the cells that passed through the filter into the lower chamber were dropped off in a dose-dependent manner, compared with control (Fig. 2C,D). At JS-K 20 μM, the rate of inhibition was up to 83.74% ($P < 0.05$). Taken together, these results suggest that JS-K inhibits migration of HepG2.2.15 cells.

3.3. JS-K contributed to cell cycle arrest in HepG2.2.15 cells

The effect of JS-K on cell cycle of HepG2.2.15 cells was detected by flow cytometry with Cell Cycle Detection Kit. The cell cycle was assessed to determine whether the growth and migration inhibition were due to alterations in the different phases. The data indicated that JS-K reduced the G1 phase population and increased the population in G2 phase accordingly. Following 24 h of treatment with 5 μM JS-K, population of cells in G1 phase was significantly reduced from 62.38% to 27.24%, cells in the G2 phase increased appreciably from 9.15% to 52.07%, and cells in S phase had no change, indicating that JS-K contribute to induction of G2/M arrest in HepG2.2.15 cells (Fig. 3).

3.4. JS-K induced apoptosis of HepG2.2.15 cells

The apoptotic effect of JS-K on HepG2.2.15 cells was detected by flow cytometry with Annexin V-FITC Apoptosis Detection Kit. As shown in Fig. 4, the early apoptotic cells were Annexin V-positive and PI-negative in Q2-4 region, whereas cells in the late apoptosis were positive for both Annexin V and PI in Q2-2 region, the Q2-1

![Fig. 2](image-url) J-S inhibited cell migration in HepG2.2.15 cells. (A) and (B): Migration of cells was measured by testing the rate of scratch closure at 72 h with JS-K 20 μM. (C) and (D): Cells were plated in transwell inserts and treated with different concentrations of JS-K for 72 h, and stained with hematoxylin and eosin. The data were expressed as the mean ± SD of three experiments. *$P < 0.05$ indicates a significant difference from the control group.
Fig. 3. JS-K influenced the cell cycle. Cells were treated for 24 h with JS-K in 5 μM, and cell cycle phase distribution was determined by flow cytometry. The data expressed as the mean ± SD of three experiments.

Fig. 4. JS-K induced apoptosis of HepG2.2.15 cells. Cells were treated with different concentrations of JS-K for 24 h, and stained with Annexin V-FITC and PI, apoptotic cells were quantified by flow cytometry. The data were expressed as the mean ± SD of three experiments. *P < 0.05 indicates a significant difference from the control group.
Fig. 5. JS-K induced the DNA damage. Cells were treated with 10 μM JS-K for 24 h, and immunofluorescence detection of γH2AX in HepG2.2.15 cells. Nuclei were stained using DAPI (blue) and γH2AX was visualized with Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (green).

Fig. 6. The expressions of apoptosis-related proteins. Cells were treated with various concentrations (0, 1, 10 and 20 μM) of JS-K for 24 h and cellular protein was extracted for analysis of apoptotic-associated. Left panel showed representative western blot analysis, right panel showed quantitative analysis of the relative expression levels (cleaved caspase-3, cleaved caspase-7, cleaved PARP). The data were expressed as the mean ± SD of three experiments. *P<0.05 indicates a significant difference from the control group.
region means PI positive stained the necrotic cells. The percentages of apoptotic cells (early apoptotic cells and the late apoptotic cells) following treatment with different JS-K concentrations (0, 5, 15 and 30 μM) were 0.05%, 9.48%, 10.37% and 70.83%. The result showed the percentage of apoptotic cells increased in a concentration-dependent manner.

3.5. JS-K induced DNA damage in HepG2.2.15 cells

γH2AX was a “gold marker” in DNA double strand breaks (DSBs) [15]. We used immunofluorescence to detect the expression of γH2AX in the cells. The cells were cultured with 10 μM JS-K for 24h, and results showed that JS-K induced the expression of γH2AX in HepG2.2.15 cells (Fig. 5).

3.6. JS-K induced the expression of apoptotic-associated proteins and DNA damage-associated protein

We next tested whether JS-K induced apoptotic-associated proteins and DNA damage-associated proteins. The expression levels of apoptotic-associated proteins cleaved PARP, cleaved caspase-7, cleaved caspase-3 (Fig. 6), PARP, caspase-7, caspase-9 and caspase-3 (Fig. 5). DNA damage-associated protein p-H2AX, p-Chk1, p-Chk2 (Fig. 7), Chk2, ATM and p-ATR (Fig. S2) were analyzed by western blot analysis. These data showed that JS-K increased the expression levels of cleaved caspase-3, cleaved caspase-7, cleaved PARP, p-H2AX, p-Chk1, p-Chk2, ATM, p-ATR compared with the control group. The caspase-3, caspase-7, caspase-9, PARP and Chk2 proteins were not significantly changed.

4. Discussion

In the present study, JS-K inhibited HepG2.2.15 cell growth, suppressed HepG2.2.15 cell colony formation and migration, arrested HepG2.2.15 cells gather in the G2 phase. Furthermore, JS-K induced apoptosis and DNA damage and increased the expression of DNA damage-associated protein p-H2AX, p-Chk1, p-Chk2, ATM, p-ATR and apoptotic-associated proteins cleaved caspase-3, cleaved caspase-7, cleaved PARP. JS-K was effective in reducing HBsAg and HBeAg secretion in HepG2.2.15 cells [14], and the current study further demonstrated anticancer effects of JS-K against the HBV-positive hepatoma HepG2.2.15 cells.

HBV is the most frequent underlying cause of HCC [3]. The HepG2.2.15 cells are a HBV-positive hepatoma cell line, transfected by pDoLT-HBV in HepG2 cells, and are widely used in screening anti-HBV drugs [13]. JS-K inhibited cell growth in hepatoma HepG2 and Hep3 B cells [9–11], suggesting the potential effects of JS-K against liver tumors. JS-K effectively inhibit HBsAg secretion in HepG2.2.15 cells [14]. As a HBV-positive hepatoma cell line, HepG2.2.15 cells is a perfect choice to the research of HBV-positive HCC.

Like the MTT assay, the colony formation assay also represents the cell proliferation [16]. JS-K inhibited HepG2.2.15 cell growth both in MTT and colony formation assay, and JS-K produced the G2 cell phase arrest in HepG2.2.15 cells. These results are consistent with the literature demonstrating that JS-K inhibited the proliferation of Jurkat T cells via G2 cell cycle arrest [17]. The cell cycle arrest was closely related with DNA damage, thus, we further examined the effects of JS-K on DNA damage as a potential mechanism.

Unrepaired DNA damage is known to cause cell death, including DSBs, single-strand breaks (SSBs) and specific DNA lesions [18,19]. To maintain genetic integrity and genomic stability, efficient DNA repair machinery is necessary to cope with the numerous DNA damages that occur daily in the cell. In all types of DNA lesions, DSBs is the most deleterious lesions because these lesions may cause apoptosis if left unrepaired [19,20]. The detection of DSBs is largely mediated by ATM and/or ATR, which send signals to checkpoint kinases (Chk1 and Chk2) to trigger DNA repair [18,21,22].

The activation of ATM in response to DNA damage is known to evoke coordinated cellular responses to initiate DNA repair [23]. After DNA damage, several proteins (such as H2AX) involved in
DNA repair are phosphorylated by ATM kinase and recruited to DNA break sites to form nuclear foci [24].

In previous reports, JS-K induced DNA damage in many tumor cells, including multiple myeloma cells [7], HL-60 cells [25] and Non-Small Cell Lung Cancer Cells (H1703) [26]. DNA strand break damage is often analyzed by comet assay, immunofluorescence assay and western blot. Our data showed that the expression of γH2AX was induced by JS-K, the phosphorylation level of Chk2, a substrate of ATM, and the phosphorylation level of Chk1, a substrate of ATR, were also higher in HepG2.2.15 cells compared with control after treatment with JS-K. Collectively, the results suggested that the DNA damage-induced by JS-K activated ATR-Chk1 and ATM-Chk2. The failure to repair DNA lesions may result in dysregulated transcription and replication, mutagenesis and apoptosis [27,28].

Apoptosis is an important regulatory mechanism in the development of cells, involving biological events such as chromosome condensation, DNA ladder, membrane blebbing, and cytochrome C release, which lead to the removal of unnecessary cells [29]. Apoptosis occurs through two main pathways. The first pathway is the intrinsic or mitochondrial pathway, which is mediated by Bcl-2 family of pro- and anti-apoptotic proteins [30]. The second pathway is the extrinsic apoptosis pathway or cytoplasmic pathway, which mediated through the FAS death receptor. Whatever the intrinsic apoptosis pathway or extrinsic apoptosis pathway are activated, the caspases family will be activated finally.

Both pathways converge to a final common pathway involving the activation of a cascade of proteases called caspases that cleave regulatory and structural molecules, culminating in the death of the cell. Caspases are cysteine-aspartic proteases families, which play an important role in apoptosis, necrosis, and inflammation. They are specific proteases that function to mediate apoptotic destruction of the cell [31]. Caspases are activated by extrinsic and intrinsic apoptotic pathways [32]. The difference was intrinsic apoptosis pathway activity by caspase-9, extrinsic apoptosis pathway activity by caspase-8, caspase-10.

Vidya Udipi [33] reported that JS-K induced apoptosis by activating caspase-3 in HL-60 myeloid leukemia cells. Caspase-3, called death protein, is one of the widely-studied proteases. The activation of caspase-3 promotes cells into an irreversible apoptosis pathway [34]. In the present study, treatment of HepG2.2.15 cells with JS-K resulted in a dramatic increase in the cleaved caspase-3 activity, which is the main executioner of apoptosis. JS-K induced non-small cell lung cancer cells (H1703) apoptosis by activity cleaved PARP, cleaved caspase-3, and cleaved caspase-7 [26]. In our study, we found JS-K induced the expression levels of cleaved caspase-3, and cleaved caspase-7, demonstrating that JS-K induced cell apoptosis, but the expression level of caspase-9 was not changed [See Supporting information]. Thus, a potential mechanism for JS-K-induced cells apoptosis is mediated through extrinsic apoptosis pathway.

In conclusion, our findings indicated that JS-K could inhibit the growth of HepG2.2.15 cells, possibly by producing a G2 cell cycle arrest, inducing DNA damage via the γH2AX, ATM-Chk2, ATR-Chk1, cleaved PARP and by activating cell apoptosis via the cleaved caspase-3 and cleaved caspase-7 related extrinsic apoptosis pathway. Our findings imply JS-K could be developed as an anti-cancer drug for HBV-positive HCC patients in the future.

Funding

This work was supported by the National Natural Science Foundation Committee (NSFC) of China (Grant No.81360261); Science and Technology Foundation of Guizhou (Grant No. LKZ 2014-108); Construction Project of the Educational Department of Guizhou (Grant No. KY[2015]331).


