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Research Article

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5-(2-Carboxyethenyl) isatin derivatives induces G2/M cell cycle arrest and apoptosis in human gastric cancer MGC-803 cells

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ABSTRACT

*We previously reported the potential of a novel small molecule (E)-methyl 3-(1-(4-methoxybenzyl)-2, 3-dioxoindolin-5-yl) acrylate (**HKL 2-3-15**) as an anticancer agent. This study was focused on the molecular mechanisms by which **HKL 2-3-15** exerts its cytotoxic activity in human gastric cancer MGC-803 cells. We showed that **HKL 2-3-15** induced G_2/M phase arrest in a time-dependent and dose-dependent manner. Following the treatment, **HKL 2-3-15** also caused Annexin-V staining, suggested **HKL 2-3-15** caused apoptosis in MGC-803 cells. Collectively, our findings demonstrate that **HKL 2-3-15**, a 5-(2-Carboxyethenyl) isatin derivative, notably inhibits MGC-803 cell proliferation through the induction of G_2/M cell cycle arrest and apoptosis. Its potential as a candidate anticancer agent warrants further investigation.*

Keywords: isatin derivatives, apoptosis, antitumor activity, MTT, MGC-803

INTRODUCTION

Isatin is an indole derivative widely present endogenously in both human and other mammalian tissues and fluids likely as a result of the tryptophan metabolic pathway [1-2]. Isatin derivatives possess a wide variety of biological activities [3-4], such as anticancer [5-6], antidepressant, anticonvulsant [7], antibacterial [8-9], anti-inhibitor, anti-fungal [10], anti-HIV [11], anti-inflammatory, and sedative-hypnotic, et al [12]. In the last several decades, increasing numbers of researchers from both industry and academia have embarked on the development of new isatin-based anticancer agents. Among them, 5-bromo-3-o-nitrophenyl isatin hydrazone and a series of 5-bromo-(2-oxo-3-indolinyl) thiazolidine- 2,4-diones substituted derivatives have been reported to exhibit anticancer activity against lymphocytic leukemia [1, 7]; N-benzylation of 5,7-dibromoisatin can increase the cytotoxicity against U937 lymphoma cells [5]; and SU11248 (Sutent), a 5-fluoro-3-substituted isatin derivative, have even been approved by FDA in 2006 for the treatment of advanced renal carcinoma and gastrointestinal stromal tumors carcinoma and gastrointestinal stromal tumors [13].

We previously reported [2] the design, synthesis and in vitro cytotoxicity evaluation of 44 5-(2-carboxyethenyl) isatin derivatives as anticancer agents and identified that **HKL 2-3-15** exhibits excellent cytotoxic activity against three different cancer cell lines including K562 (IC_{50}=3 nM), HepG2 and HT-29, and the molecular mechanism of the cytotoxic activity of **HKL 2-3-15** against the human chronic myelogenous leukemia K562 cells [14]. In the present study, the molecular mechanism of the cytotoxic activity of **HKL 2-3-15** against adherent cells (the human gastric cancer MGC-803 cells) was investigated.

EXPERIMENTAL SECTION

2.1. Cell lines and culture conditions

MGC-803 cell line was obtained from the Shanghai Institutes of Biological Sciences (Shanghai, China). Cells were grown at 37℃ in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum, 4mM L-glutamine, 1g/L D- glucose, 110mg/L sodium pyruvate and 1% penicillin/streptomycin. Cells were cultured in a humidified atmosphere of 5% CO_2. The medium was replaced once every third day.

2.2. Cytotoxic activity assay

The cytotoxic activity was measured using the MTT assay as described previously [2]. Briefly, 100 μL of MGC-803 cells were cultured in 96-well plates at a density of 5×10^4 cells/ml for 24 h. Different concentrations of **HKL 2-3-15** (1-10000 nM) were added to each well to culture for another 48 h. MTT assay was performed using thermo microplate reader. The DMSO-treated controls were calculated as a cell viability value of 100%. The inhibitory concentrations (IC_{50}) were obtained by nonlinear regression using GraphPad Prism 5.0. For each experiment, IC_{50} value was calculated from three independent assays.

2.3. Cell cycle analysis

For the DNA content analysis, 2×10^6 cells were cultured for 24 h and treated with **HKL 2-3-15** (300 nM) for 3, 6, 12 and 24 h, respectively. Cells were collected and fixed with 1 ml of 70% ice-cold ethanol at -20℃ overnight. Cells then were washed with PBS, incubated with propidium iodide (PI, 1 mg/ml), RNase A (20 μg/ml) for 15 min in dark at room temperature and analyzed using a FACS Calibur system (version 2.0, BD) using the CELL Quest program (Becton Dickinson). Results were representatives of at least three independent experiments.

2.4. Flow cytometric analysis of apoptosis

Apoptotic cells were assayed by the Annexin-V-FITC Apoptosis Detection Kit (BD Biosciences, USA) according to the manufacturer's instructions. In brief, MGC-803 cells were treated with DMSO or **HKL 2-3-15** (300 nM) for 1 h. Cells were harvested, washed twice with ice-cold PBS and resuspended in 1×Binding buffer at a concentration of 1×10^6 cells/ml. Cells were stained with 5 μl of Annexin-V-FITC and 5 μl of PI (50 μg/ml) for 15 min at room temperature in the dark and analyzed by flow cytometry.

RESULTS AND DISCUSSION

3.1 The MGC-803 treated with HKL 2-3-15 cell growth curve

To examine the inhibitory effects of **HKL 2-3-15** (**Fig.1A**) on MGC-803 cells, the cell number assay is used to investigate the inhibitory effect of **HKL 2-3-15** on proliferation of cancer cells. MGC-803 cells were incubated in the absence or presence of various concentrations of **HKL 2-3-15** (30, 100, 300 nM) for 24, 48, 72, 96, 120 h, respectively, and then cell growth curve of MGC-803 cells was determined. As shown in **Fig. 1B**. The MGC-803 cell growths could be significantly inhibited and these inhibitory effects were in a dose-dependent manner.

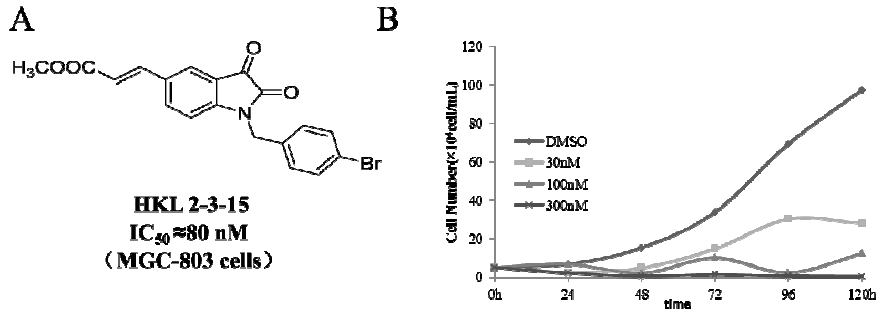

Fig. 1. The cell growth curve of HKL 2-3-15-treated MGC-803 cells. (A) Structure and *in vitro* cell proliferation inhibitory activity of HKL 2-3-15. (B) Cell growth curve induced by HKL 2-3-15 (30, 100, 300 nM) treatment for 24, 48, 72, 96 and 120 h were observed

3.2 HKL 2-3-15 induced G2/M cell cycle phase arrest in MGC-803 cells

To determine whether the influence of **HKL 2-3-15** on cell cycle distribution was related to the decrease in cell viability, flow cytometric analysis was performed. As shown in **Fig. 2A**, the exposure to **HKL 2-3-15** caused a significant increased cell fraction in G_2/M phase (peaked at 6 h) and a decreased cell fraction in G_0/G_1 phase in a time-dependent manner. The percentage of cells in G_2/M phase increased by 2.5-fold after the cells was treated with 300 nM **HKL 2-3-15** for 6 h, and the percentage of cells in G_2/M phase increased after the cells were treated from

30 nM to 300 nM **HKL 2-3-15** for 6 h (**Fig.2B**). These results demonstrated that **HKL 2-3-15** has cell proliferation inhibitory effect and can induce the cell cycle arrest of MGC-803 cells in G_2/M phase.

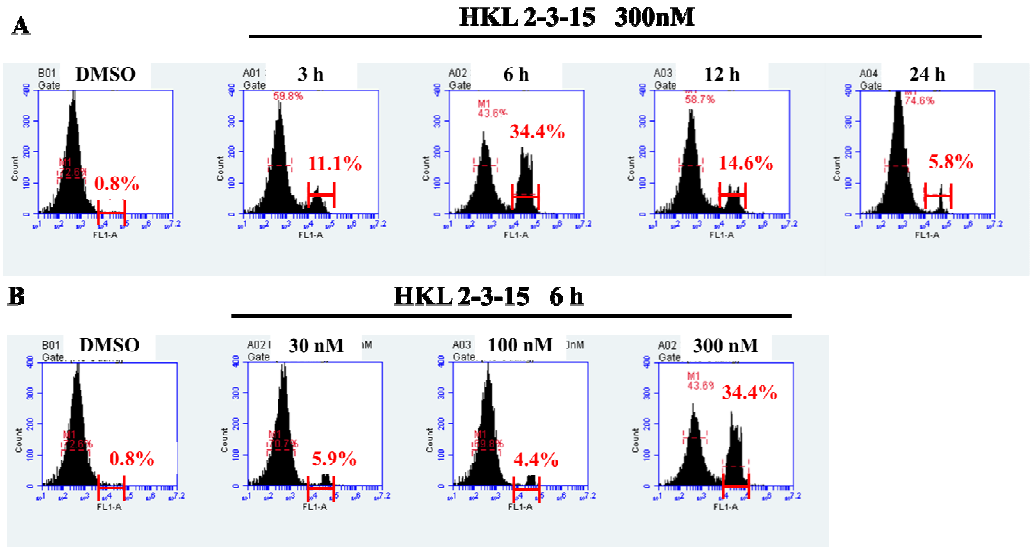

Fig. 2. HKL 2-3-15 induced the cell cycle arrest of MGC-803 cells in G_2/M phase in a time- and dose- dependent manner. (A)MGC-803 cells were treated with 300 nM HKL 2-3-15. (B) MGC-803 cells were treated with 30, 100, 300 nM HKL 2-3-15 for 6 h. At the time or concentration points indicated, cells were labelled with PI and their DNA content was determined using FACS analysis. The data was presented as mean of three independent experiments

3.3 HKL 2-3-15 induced apoptosis in MGC-803 cells

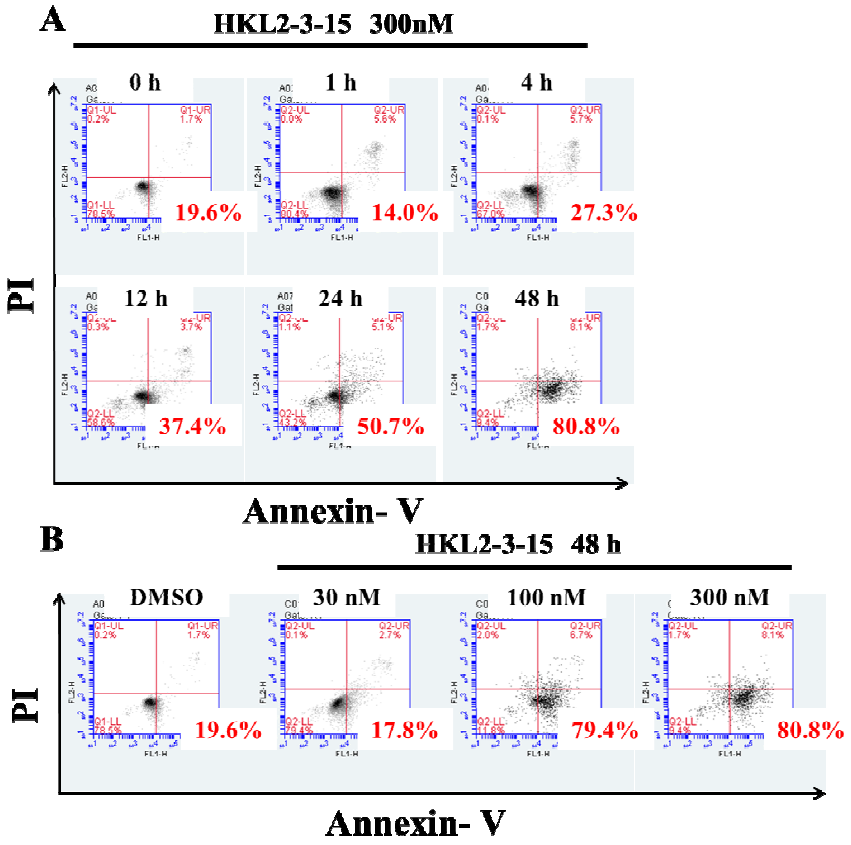

Fig. 3. HKL 2-3-15 induced apoptosis in MGC-803 cells in a time- and dose-denpendent manner. (A) MGC-803 cells were treated with 300 nM HKL 2-3-15. (B) MGC-803 cells were treated with 30, 100, 300 nM HKL 2-3-15 for 6 h. At the time and concentration points indicated, cells were labeled with Annexin-V-FITC and PI and apoptosis was determined using FACS analysis. Data was shown as the mean of three independent experiments. The cell percentage in each phase of cell cycle was indicated

Flow cytometric analysis showed that apoptotic cells were observed in **HKL 2-3-15**-treated MGC-803 cells when

double labelled with annexin-V-FITC and PI (**Fig. 3A**). The apoptotic rates (Annexin V+/PI-) in 300 nM **HKL 2-3-15**-treated MGC-803 cells for 1-48 h were increased from 14.0 to 80.8 of the total cells, furthermore, the apoptotic rates (Annexin V+/PI-) in 30-300 nM **HKL 2-3-15**-treated MGC-803 cells for 48 h were increased from 17.8 to 80.8 of the total cells, whereas only 19.6% cells were observed as apoptotic cells in the control (**Fig.3B**). These results suggested that **HKL 2-3-15** inhibited the proliferation of MGC-803 cells by inducing apoptosis in a time- and dose-dependent manner.

CONCLUSION

In our previous study, **HKL 2-3-15** has been found to be an excellent cytotoxic agent against human chronic myelogenous leukemia cells K562 (IC_{50}=3 nM) [14]. In this study, the cell growth curve, cell cycle arrest and apoptotic events of MGC-803 cells when treated with **HKL 2-3-15** were investigated. Firstly, our study showed that **HKL 2-3-15** caused MGC-803 cell number decreased significantly compared to DMSO-treated cells (**Fig. 1**), indicated that **HKL 2-3-15** might inhibit the cell proliferation of MGC-803 cells. Flow cytometric analysis showed that **HKL 2-3-15** caused the cell cycle arrest of the MGC-803 cells in G_2/M phase. G_2/M phase accumulation peaked at 6 h after **HKL 2-3-15** treatment. We also found that **HKL 2-3-15** caused typical apoptosis in MGC-803 cells. In summary, the exposure of MGC-803 cells to **HKL 2-3-15**, a novel 5-(2-carboxyethenyl) isatin derivative results in cell growth inhibition which is concomitant with reversible G_2/M cell cycle arrest and apoptosis at doses as low as 80 nM. Further optimization of the structure to improve the bioavailability and solubility is ongoing.

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