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Isoorientin triggers apoptosis of hepatoblastoma by inducing DNA double-strand breaks and suppressing homologous recombination repair

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ABSTRACT

Hepatoblastoma (HB) is the most common malignant liver tumor in children. DNA and DNA-associated processes are one of the most important targets of chemotherapeutic agents. Isoorientin (Iso), a natural flavonoid compound, can be extracted from several plant species. The effects of Iso and its molecular mechanisms on hepatic malignancies remain unclear. Herein, the anti-tumor effects of Iso in HB and its underlying mechanisms were explored. We found that Iso significantly inhibited the proliferation of HB cells both *in vitro* and *in vivo*. Mechanistic studies showed that Iso triggered cell apoptosis by inducing DNA double-stranded breaks and blocking the initiation process of homologous recombination repair, which was related to the attenuation of ataxia telangiectasia mutated (ATM) activation and inhibiting the binding of phosphorylated ataxia telangiectasia mutated (pATM) and the MRE11-RAD50-NBS1 (MRN) complex. Furthermore, Iso markedly sensitized HB cells to the anti-proliferative effects of the poly ADP-ribose polymerase (PARP) inhibitor olaparib both *in vivo* and *in vitro*. Taken together, our study first showed that Iso was a DNA-damage agent, and the combination of Iso with a PARP inhibitor might be a promising strategy for treating HB patients.

1. Introduction

Hepatoblastoma (HB) is the most common type of malignant liver tumor in children. It is usually diagnosed during the first 5 years of life [1]. The average risk of a child developing HB is about 1 in 1,000,000 in the US [2]. Approximately 70% of children with this disease are treated successfully with surgery and chemotherapy. The survival rate is > 90% for early-stage HB [3, 4]. However, the prognosis in advanced stages remains poor, and the typical chemotherapeutic agents used for treatment are still limited by significant toxicity [5]. Consequently, the current therapeutic strategy for HB is mainly focused on reducing chemotherapy-related toxicity by decreasing dose intensity and improving the clinical outcome of patients with metastatic disease by intensifying chemotherapy in combination with new drugs. To further improve the outcomes of high-risk children, in particular those with metastatic HB, new therapeutic approaches to increasing the efficiency of chemotherapy and avoiding excessive toxicity are urgently needed.

Natural compounds are regarded as a fertile source of potential cancer chemotherapeutic and chemopreventive agents that have received increasing attention in recent years because of their safety in chemotherapy and advantages in reducing the risk of mutagenicity in normal cells [6,7]. Isoorientin (3′,4′,5,7-tetrahydroxy-6-C-glucopyranosyl flavone, Iso), a chemical flavonoid-like compound, can be extracted from many plant species, such as *flax straw* [8], *aqueous leaf* [9], *Gypsophila elegans* [10], *Phyllostachys pubescens* [11], *Patrinia* [12], and *Drosophyllum lusitanicum* [13]. It has been reported that Iso has a variety of pharmacological properties, including anti-oxidant, anti-inflammatory and anti-nociceptive activities [14,15]. Recent investigations have found that Iso also induces apoptosis through an increase in the generation of reactive oxidation state (ROS) in human hepatocellular carcinoma [16,17]. However, the effects of Iso on hepatic malignancies, especially on HB, and the molecular mechanisms underlying apoptosis induced by Iso in HB cells remain unclear.

DNA double-stranded breaks (DSBs), one of the most deleterious

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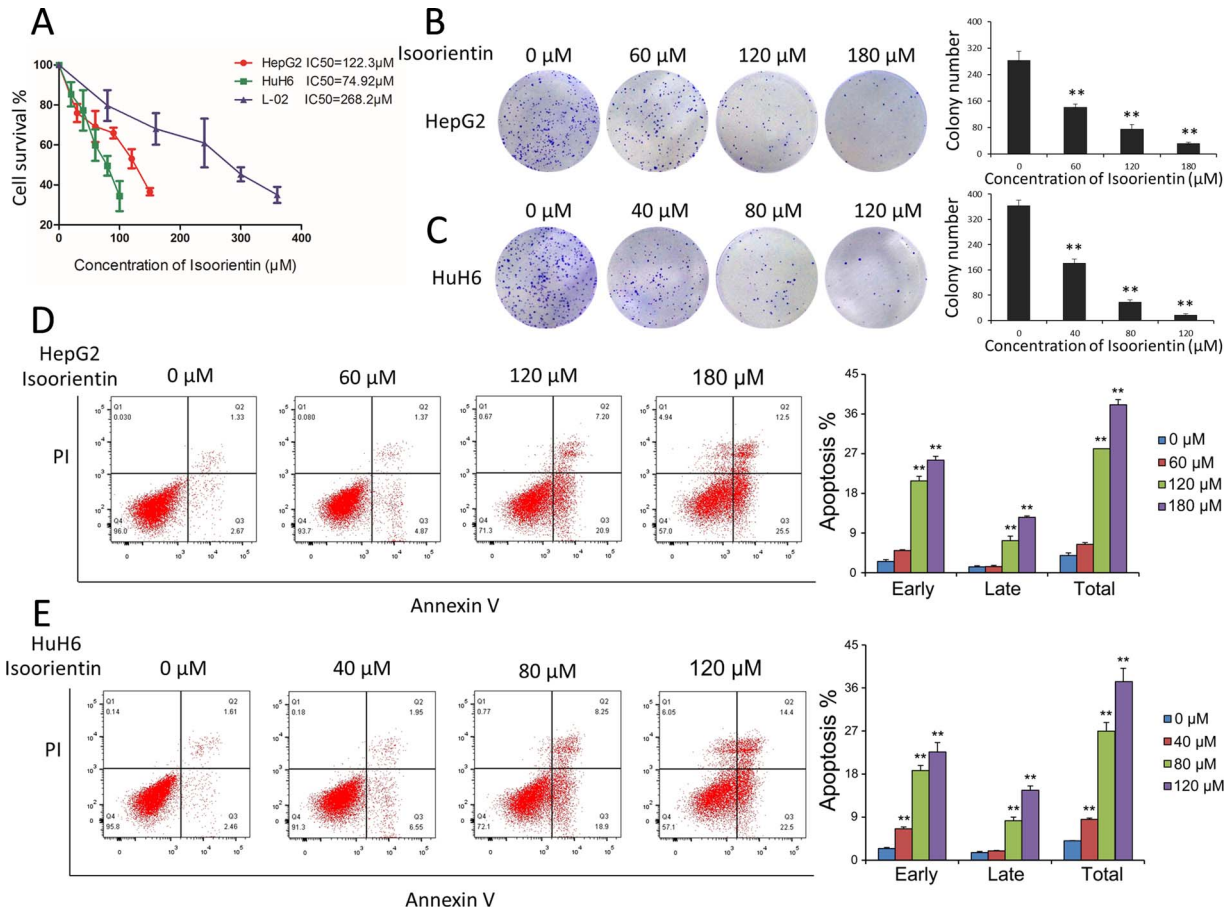

Fig. 1. Iso inhibited proliferation and induced apoptosis of HB cells *in vitro*.
A. HB cells (HepG2 and HuH6) and normal hepatocyte (L-02) were treated with increasing concentrations of Iso and the cell viability was assessed *via* CCK8 assay. B–C. Colony formation assays of inhibition roles of Iso in HepG2 (A) and HuH6 (B) cells. Columns (right panel) represent the colony numbers of HepG2 cells (B) and HuH6 cells (C) from three independent experiments, which are shown as the mean ± SD. D–E. Flow cytometry assays of cell apoptosis of HepG2 (D) and HuH6 (E) cells treated with indicated concentrations of Iso. Columns (right panel) represent the average percent of apoptosis of HepG2 cells (B) and HuH6 cells (C) from three independent experiments, which are shown as the mean ± SD. $^{**}p < 0.01$, compared to the control group.

forms of DNA damage, may cause genomic instability, apoptosis and cancer if not properly repaired [18]. The maintenance of genomic integrity needs a conserved and intricate signaling pathway in response to DSB damage [19]. DSBs are mainly repaired by homologous recombination (HR) or non-homologous end joining (NHEJ) [20]. HR is an error-free repair pathway that uses the sister chromatid as a template for the correct replacement of the DNA sequence [21]. Factors, such as the MRN complex, ATM, 53BP1, BRCA1, BRCA2, and Rad51, are involved in the HR pathway [22]. The MRN complex has been reported to be essential for the regulation of the cellular response to DSB, which is involved in multiple processes of the DNA damage response (DDR), such as initial DSB detection, signal transduction, and the promotion of DSB repair [23]. The ATM kinase activates a network of checkpoint and DNA repair proteins in response to DNA damage, thus being another keystone in maintaining genomic stability [24].

In the present study, we investigated the antiproliferative and proapoptotic effects of Iso on HB and explored its mechanism of action. Furthermore, the putative potentiation of the anti-tumor effect on HB as a result of combining Iso with olaparib, the first-in-class PARP inhibitor approved by the Food and Drug Administration (FDA) for patients with BRCA-mutated ovarian cancer, was also assessed here.

2. Materials and methods

2.1. Cell lines and cell culture

The HB cell line HepG2 and the normal liver cell line L-02 were purchased from the American Type Culture Collection (Manassas, VA, USA). The HB cell line HuH6 was purchased from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). The HB cell lines (HepG2 and HuH6) were maintained in logarithmic growth in Dulbecco's Modified Eagle Medium (DMEM) (Gibco, Carlsbad, CA, USA) with addition of 1% MEM Non-Essential Amino Acids Solution (Gibco, USA), 10% fetal bovine serum (FBS, Gemini, USA), 100 U/ml penicillin, and 100 U/ml streptomycin. The normal liver cell line L-02 was cultured in RPMI-1640 medium (Gibco, Carlsbad, CA, USA) with 10% FBS, 1% penicillin, and 1% streptomycin. All cell lines were incubated at 37°C in an atmosphere of 5% CO2.

2.2. Reagents and antibodies

Iso (purity ≥ 98%) was purchased from Sigma Chemical (St Louis, MO, USA). olaparib (AZD2281) was purchased from Selleck Chemical (Houston, TX, USA). Both drugs were dissolved in dimethyl sulfoxide (DMSO) and diluted to different concentrations. When the cells reached 70%–90% confluence, they were then treated with Iso or olaparib and then prepared for subsequent experiments. The amount of DMSO added to the cell culture was less than 0.1% in all cases. The primary