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Original Articles

Isorhamnetin augments the anti-tumor effect of capeciatbine through the negative regulation of NF-κB signaling cascade in gastric cancer

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ABSTRACT

Development of drug resistance to standard chemotherapy is a common phenomenon that leads to poor prognosis in patients. Thus, novel agents that can attenuate chemoresistance are urgently needed. Therefore, we analyzed whether isorhamnetin (IH), a 3′-O-methylated metabolite of quercetin, can enhance the potential efficacy of capecitabine in gastric cancer. The potential effect of IH on viability was analyzed by MTT assay, apoptosis by flow cytometric analysis, and NF-κB activation by DNA binding as well as Western blot assays. The *in vivo* effect of IH was also examined on the growth of subcutaneously implanted tumors in nude mice. IH inhibited the viability, potentiated the apoptotic effects of capecitabine, abrogated NF-κB activation, and suppressed the expression of various NF-κB regulated gene products in tumor cells. In a gastric cancer xenograft model, administration of IH alone (1 mg/kg body weight, i.p.) significantly suppressed the tumor growth alone as well as in combination with capecitabine. IH further reduced NF-κB activation and the expression of various proliferative and oncogenic biomarkers in tumor tissues. Overall, our results demonstrate that IH can significantly enhance the anti-tumor effects of capecitabine through the negative regulation of NF-κB regulated oncogenic genes.

Introduction

Among all solid tumors, gastric cancer is the second leading cause of cancer related morality annually [1,2]. For most gastric cancer patients, the first line of treatment is chemotherapy followed by surgery [3]. Despite the advent of various targeted therapies, only few patients respond positively to chemotherapy while others exhibit resistance to commonly employed drugs such as capecitabine [4]. Thus, novel strategies to enhance the efficacy of existing chemotherapeutic agents and attenuate the development of resistance are required.

Several prior reports have clearly established that constitutive activation of transcription factor NF-κB may play an important role in gastric cancer initiation and progression [5–9]. In gastric cancer cell lines, NF-κB is often persistently activated and is associated with their various oncogenic characteristics such as aberrant growth [10], resistance to apoptosis, and overexpression of the genes involved in cell cycle promotion [11,12]. Deregulated NF-κB activation also controls the expression of chemokine receptor *CXCR4* [13] and cyclooxygenase-2 (*COX-2*) [14] genes that regulate metastatic progression of gastric cancer.

Moreover, *H. pylori*, which is the major causative factor implicated in gastric carcinogenesis can cause substantial NF-κB activation in gastric epithelial cells [15]. In infected individuals, *H. pylori* often leads to the formation of gastric ulcers with up-regulated NF-κB activation that can gradually transform into a tumor in association with Th1 type cytokine responses, thereby disrupting the whole gut epithelial barrier function [16]. Increased expression of the *ICAM-1* gene regulated by NF-κB is also observed in *H. pylori* induced gastritis [17]. Interestingly, it has also been found that NF-κB can act as a prognostic marker for grade IV gastric cancer [18]. Thus, targeted

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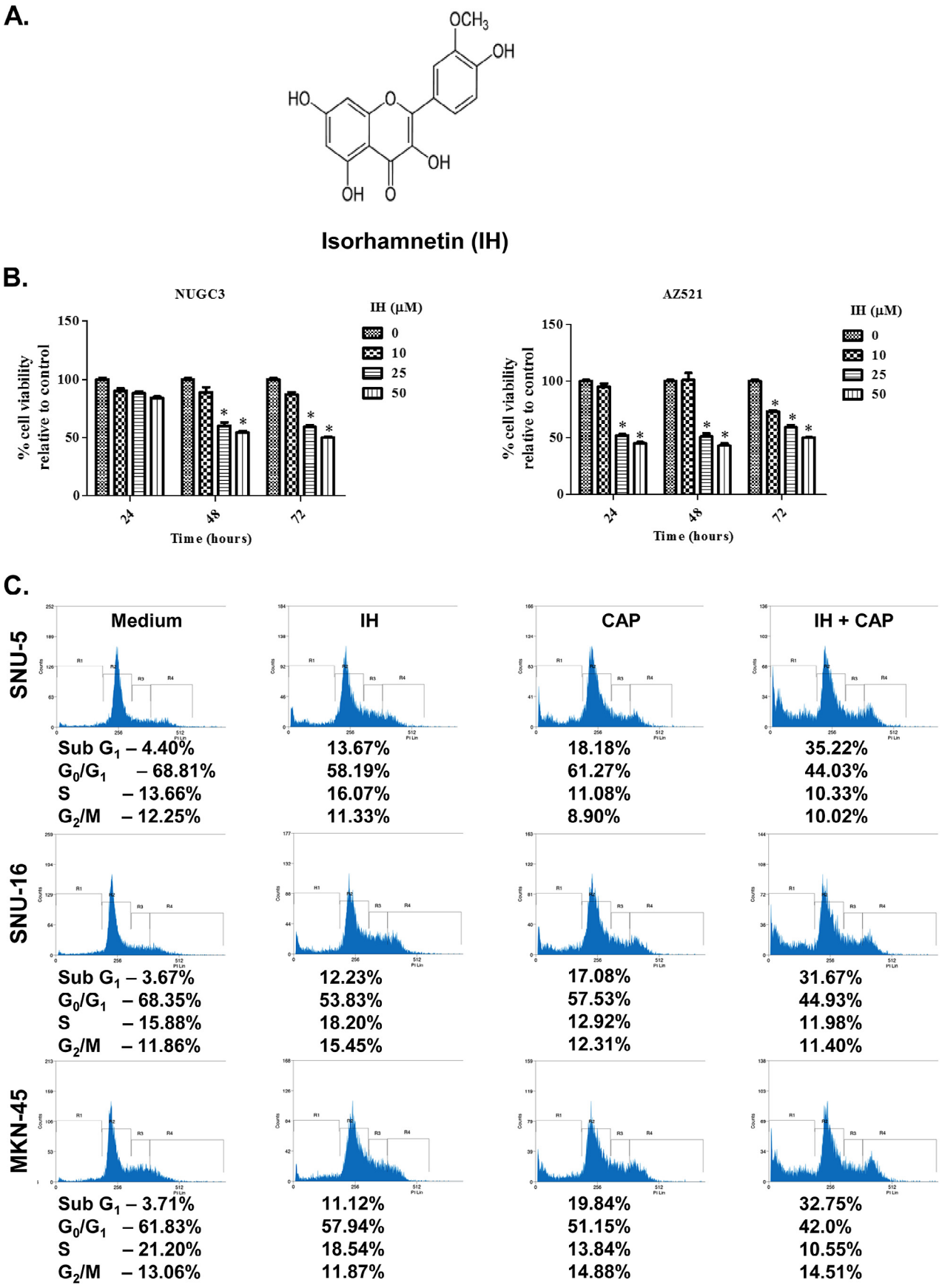

Fig. 1. IH reduces the viability and enhances the apoptotic effects of chemotherapy. (A) The chemical structure of IH. (B) Results of MTT assay indicated a dose-dependent suppression of cell viability of NUGC3 and AZ521 gastric cancer cell lines treated with IH alone. * Indicates $p < 0.05$. (C) SNU-5, SNU-16, and MKN-45 cells were treated with IH (10 μM) and capecitabine (10 μM) alone as well as in combination for 24 h. Thereafter, the cells were washed, fixed, and stained with PI and analyzed for DNA content by flow cytometry.