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Interleukin-8 is related to poor chemotherapeutic response and tumourigenicity in hepatocellular carcinoma

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Abstract ***Aim:*** Interleukin-8 (IL-8) has been suggested as a prognostic biomarker for human hepatocellular carcinoma (HCC), but its roles in HCC progression and drug resistance have not been studied. This study investigates the role and underlying mechanism of IL-8 in the chemoresistance and progressive growth of HCC.
Methods: The change of chemosensitivity and proportion of side population in hepatoma cells was examined by cell growth and flow cytometric analyses after anti-cancer treatments or knockdown of IL-8. Expression of IL-8 and ATP-binding cassette (ABC) transporters in hepatoma cells, xenograft and clinical HCC tissues was determined by Western blot and immunohistochemical analyses. Tumourigenicity of hepatoma cells was evaluated *in vivo* after silencing IL-8 gene.
Results: Treatment of hepatoma cells with anti-cancer drugs increased the production of IL-8 and its receptor, as well as the proportion of side population (SP). Exogenous IL-8 increased the SP fraction and expression of multidrug resistance-1, decreasing the drug sensitivity. Silencing of IL-8 gene decreased the ratio of SP cells and drug resistance properties. Both IL-8 and ABC transporters were highly expressed in xenograft and clinical HCC tissues, and knockdown of IL-8 significantly reduced tumour size *in vivo*.
Conclusion: Anti-cancer drug-induced IL-8 secretion increased the expression of ABC transporters and SP cells, promoting the growth of HCC *in vitro*. Thus IL-8 may be a potential therapeutic target in the treatment of HCC.

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1. Introduction

Hepatocellular carcinoma (HCC) is a difficult cancer to cure because of its high rate of distant metastases and therapy-resistant local recurrences after surgical resection or chemoembolisation [1]. The primary recurrence rate is as high as 50% within 2 years after anti-cancer treatment [2,3]. Recently, several studies suggested that interleukin-8 (IL-8) could be used as a prognostic indicator of HCC and its metastasis by showing significant correlations of serum IL-8 levels with tumour size and degree [4,5].

IL-8, also known as CXC-motif ligand 8 (CXCL8), is one of the major chemokines associated with the promotion of neutrophil chemotaxis and inflammatory responses. Previous studies have demonstrated that IL-8 signalling is involved in proliferation and survival of neoplastic cells, angiogenesis and metastatic migration of cancer cells in various solid tumours, including ovarian [6], intestine [7], prostate [8] and glioma [9]. Compared to the accumulated knowledge of IL-8 in different solid tumours, however, its potential role and underlying mechanism in the progression of HCC remain to be elucidated.

Side population (SP) cells have been shown to enrich putative cancer stem cells (CSCs) in leukaemia and several types of solid tumours, and have self-renewal, differentiating, tumour initiating and drug resistance abilities [10–12]. It has been reported that CSCs possess high levels of ATP-binding cassette (ABC) transporters and have a greater capacity to expel cytotoxic drugs, endowing cancer cells with survival mechanisms against chemotherapy [11]. Moreover, CSCs that survive after chemotherapy are more aggressive and metastatic, resulting in progressive tumour growth [13,14]. Because IL-8 is one of the major cytokines produced by cancer and stoma cells during chronic inflammatory responses, increasing evidence has suggested potential autocrine and/or paracrine effects of IL-8 on progression and drug resistance of different types of cancers [15–17].

In this study, we investigated the potential role of IL-8 in HCC and provided the first evidence that IL-8 positively regulates the expression of ABC transporters and increases SP cells, promoting the progressive growth of HCC.

2. Materials and methods

2.1. Cell culture

Huh7 and HepG2 cells were obtained from the Korean Cell Line Bank (KCLB, Seoul, Republic of Korea) and were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco BRL, Grand Island, NY, United States of America (USA)) containing 10% foetal bovine serum (FBS, Gibco BRL). All cell lines were maintained at 37 °C in a humidified 5% CO_2 atmosphere.

2.2. Side population analysis

Huh7 and HepG2 cells were treated with 10 nM doxorubicin (DOX, Sigma–Aldrich, St. Louis, MO, USA) or 10 nM paclitaxel (PTX, Sigma–Aldrich) for 72 h. DOX or PTX stock solution (1 mM) was prepared in dimethyl sulfoxide (DMSO) and diluted in distilled water (DW) before use. Analysis of the SP fraction was performed as reported previously [18]. Briefly, 1×10^6 cells/ml were incubated with 5 μg/mL Hoechst 33342 dye (Sigma–Aldrich) in DMEM with 10% FBS for 90 min at 37 °C. The other 1×10^6 cells/ml cells were treated with both Hoechst 33342 dye and 50 μM verapamil (Sigma–Aldrich), an efflux blocker, to confirm the SP fraction. After incubation, both cell groups were washed in cold PBS, and 1 μg/mL propidium iodide (PI) (Sigma–Aldrich) was added before flow cytometry analysis to identify and exclude dead cells. All samples were analysed using a FACSAria (Becton Dickinson) with FACSDiva software (version 6.1.3). Excitation of the Hoechst dye was achieved with an ultraviolet laser at 351–364 nm, and the fluorescence was measured with a 515 nm SP filter (Hoechst blue) and a 608 EFLP optical filter (Hoechst red).

2.3. siRNA treatment

Two different validated IL-8 siRNAs (Hs_IL8_5 Validated siRNA and Hs_IL8_6 Validated siRNA) and negative control siRNA were purchased from Qiagen (Qiagen Inc., Valencia, CA, USA). Hs_IL8_5 Validated siRNA was used in the IL-8 knockdown experiments for Huh7 cells and Hs_IL8_6 Validated siRNA for HepG2 cells. At 50% confluency, Huh7 and HepG2 cells were treated with negative control siRNA (si-C) or with 20 nmol/L of IL-8 siRNA (si-IL-8) using Lipofectamine for 5 h, according to the manufacturer's instructions.

2.4. In vivo xenograft experiments

To investigate the change in the expression of IL-8 and ABC transporters after DOX treatment *in vivo*, 3×10^6 Huh7 cells were subcutaneously injected into the flank of 4- to 6-week-old BALB/c nude mice. When the xenograft tumour volume reached 100 mm^3 in size, the mice were given intraperitoneal injections of DOX (8 mg/kg, $n = 3$ per group) three times each for 3 days before they were sacrificed for histological analyses. To examine the tumourigenicity *in vivo*, cells were transfected with lentivectors expressing green fluorescence protein (GFP), and determination of the efficiency of transfection was performed using FACSCalibur. Isolated GFP-positive cancer cells were treated with or without siRNA IL-8 as described above. Approximately 3×10^6 cells were subcutaneously injected into the right