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17β-estradiol exerts anticancer effects in anoikis-resistant hepatocellular carcinoma cell lines by targeting IL-6/STAT3 signaling

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ABSTRACT

17β-Estradiol (E2) has been proven to exert protective effects against HCC; however, its mechanism on HCC proliferation and suppression of invasion remains to be further explored.

Because HCC up-regulates serum Interleukin-6 (IL-6) levels and Signal Transducer and Activator of Transcription 3 (STAT3), molecular agents that attenuate IL-6/STAT3 signaling can potentially suppress HCC development. In this study, we examined involvement of E2 in anoikis resistance that induces invasion capacities and chemo-resistance. Huh-BAT and HepG2 cells grown under anchorage-independent condition were selected. The anoikis-resistant (AR) cells showed stronger chemo-resistance against sorafenib, doxorubicin, 5-fluorouracil and cisplatin compared to adherent HCC cells. AR HCC cells exhibited decreased expression of E-cadherin and increased expression of the N-cadherin and vimentin compared to adherent HCC cells. We then demonstrated that E2 suppressed cell proliferation in AR HCC cells. IL-6 treatment enhanced invasive characteristics, and E2 reversed it. Regarding mechanism of E2, it decreased in the phosphorylation of STAT3 that overexpressed on AR HCC cells. The inhibitory effect of E2 on cell growth was accompanied with cell cycle arrest at G2/M phase and caspase-3/9/PARP activation through c-Jun N-terminal Kinase (JNK) phosphorylation. Taken together, these findings suggested that E2 inhibited the proliferation of AR HCC cells through down-regulation of IL-6/STAT3 signaling. Thus, E2 can be a potential therapeutic drug for treatment of metastatic or chemo-resistant HCC.

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most lethal cancers worldwide [1,2]. Although surgical resections could be effective for HCC patients with an early stage, the cumulative 5-year recurrence rate is above 70% [3]. The HCC recurrence through local metastasis and chemo-resistance are still major causes of death [4]. Although only sorafenib has been used as first-line therapy for advanced HCC, response rate is only 30–40% [5,6]. The low efficacy may be caused by chemotherapeutic resistance of HCC [7]. Better understanding of the underlying mechanism targeting the chemo-resistant and metastatic properties of HCC is pivotal for designing improved therapeutic approaches [8].

A characteristic of HCC is the male incidence ranking from 2:1 up to 5:1 across nearly all geographical areas [9]. Estrogen has been attracting people's attention because of a male predominance in morbidity and mortality in HCC patients; thus, estrogen may play a vital role in HCC development [10]. The role of estrogen and its molecular mechanism on HCC have not been elucidated yet; however, the anti-inflammation effect of estrogen is well documented [11]. During chronic inflammation, pro-inflammatory cytokines response to initiate HCC formation [12]. IL-6 is a pro-

Abbreviations: HCC, Hepatocellular carcinoma; AR, Anoikis-resistant; E2, 17β-estradiol; IL-6, Interleukin-6; STAT3, Signal transducer and activator of transcription 3; CDKs, Cyclin-dependent kinases; JNK, C-Jun N-terminal kinase.

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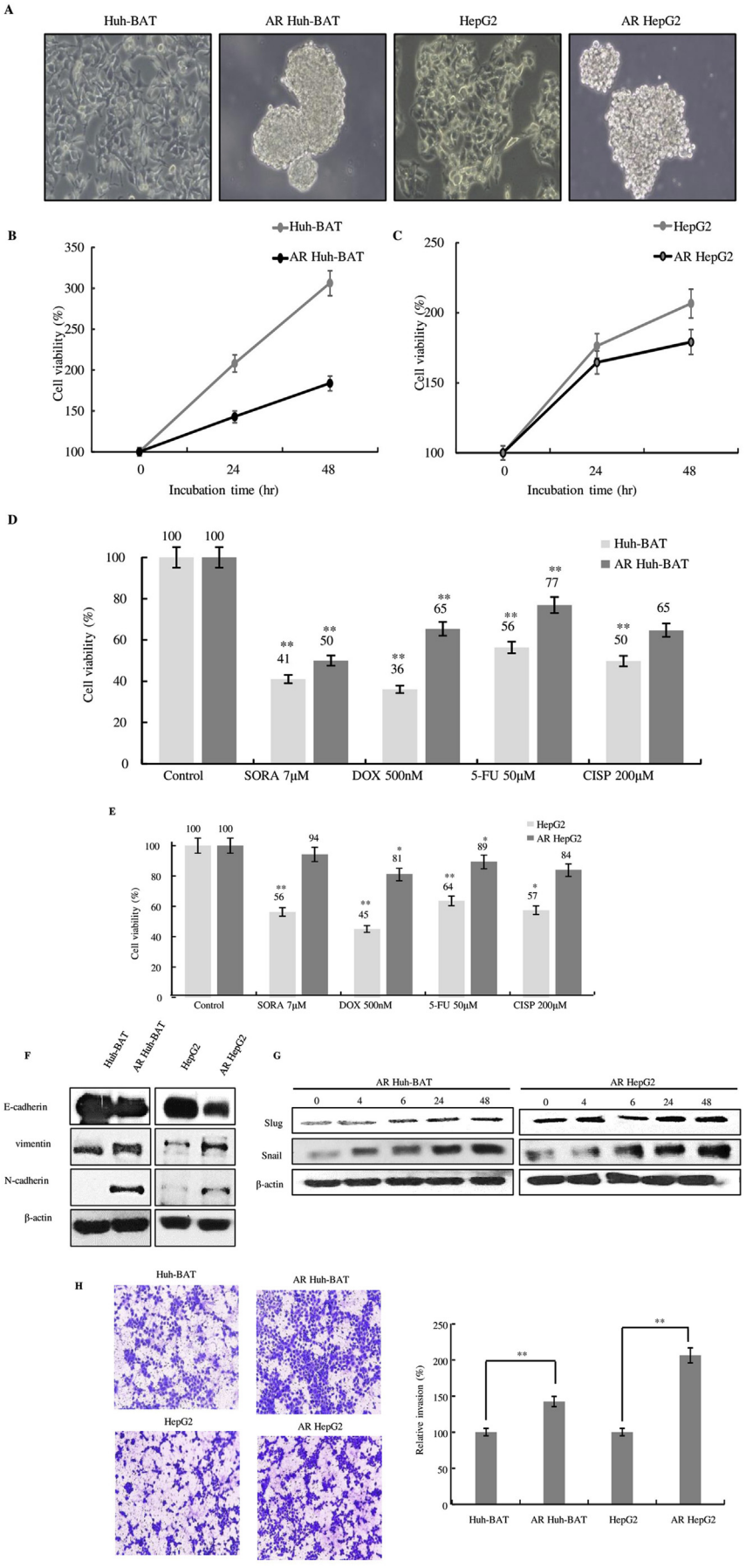
A
Huh-BAT
AR Huh-BAT
HepG2
AR HepG2
B
Huh-BAT
AR Huh-BAT
Cell viability (%)
Incubation time (hr)
C
HepG2
AR HepG2
Cell viability (%)
Incubation time (hr)
D
Huh-BAT
AR Huh-BAT
Cell viability (%)
Control
SORA 7μM
DOX 500nM
5-FU 50μM
CISP 200μM
E
HepG2
AR HepG2
Cell viability (%)
Control
SORA 7μM
DOX 500nM
5-FU 50μM
CISP 200μM
F
Huh-BAT
AR Huh-BAT
HepG2
AR HepG2
E-cadherin
vimentin
N-cadherin
β-actin
G
AR Huh-BAT
AR HepG2
Slug
Snail
β-actin
H
Huh-BAT
AR Huh-BAT
HepG2
AR HepG2
Relative invasion (%)