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Upregulation of Mcl-1 inhibits JQ1-triggered anticancer activity in hepatocellular carcinoma cells

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ABSTRACT

Bromodomains and extra-terminal (BET) proteins inhibitors are promising cancer therapeutic agents. However, tumor cells often develop resistance to BET inhibitors, greatly limiting their therapeutic potential. To study the mechanism underlying the resistance of BET inhibitors in hepatocellular carcinoma (HCC) cells, we herein investigated the impact of BET inhibitor JQ1 on the gene expression of Bcl-2 family members by RNA sequencing analysis, and found that acute treatment with JQ1 triggered upregulation of Mcl-1 in HCCLM3 and BEL7402 cell lines. This JQ1-triggered Mcl-1 upregulation was further confirmed by quantitative reverse transcription polymerase chain reaction and western blotting analysis, both at mRNA and protein levels. Inhibition of Mcl-1 by RNA interference dramatically enhanced JQ1-triggered caspase-3 activation, cleavage of poly (ADP-ribose) polymerase and apoptotic cell death induction in multiple HCC cell lines. Moreover, JQ1 in combination with cyclin-dependent kinase inhibitor flavopiridol at a subtoxic concentration that reduced expression of Mcl-1, triggered massive apoptotic cell death in HCCLM3 and BEL7402 cell lines. Together, these data suggest that Mcl-1 is a major contributor to BET inhibitor-resistance in HCC cells, and that combining drugs capable of down-regulating Mcl-1 may promote therapeutic potential in human HCC.

1. Introduction

Bromodomain and extra-terminal (BET) proteins are epigenetic readers of acetylated lysines in histones, which play an important role in regulation of the expression of genes involved in cell survival and growth [1]. Epigenetic dysfunction caused by high level of BET proteins has been found to be a critical factor for the development and progression of human cancer [2]. Accordingly, small molecule inhibitors targeting BET proteins represent a novel strategy in anticancer therapy [2,3]. Previous studies demonstrated that BET inhibitors elicited anticancer activities by suppressing a set of cancer drivers, such as cMyc, Bcl-xl, as well as Mcl-1 in hematological malignancies and a few types of solid cancers [4–7]. Clinical trials with a number of small molecule BET inhibitors have shown favorable response in hematological malignancies [8,9]. These evidences therefore suggest that BET inhibitors hold great promise for cancer patients. Nevertheless, development of resistance to BET inhibitors is a common issue, particularly in solid cancers [5–7,10]. Recent studies therefore have shifted attention to elucidate the mechanisms underlying BET inhibitors-resistance and to explore strategies for overcoming the drug resistance [5–7,10].

We have previously investigated the anticancer activity of BET inhibitor JQ1 in a panel of hepatocellular cancer (HCC) cell lines [10]. In that study, we found that JQ1 indeed triggered apoptosis in HCC cells and xenograft HCC tumor tissues, but the apoptotic effect was modest even in the most sensitive HCCLM3 cell line. Moreover, the study also showed that JQ1 only partially inhibited tumor growth in a HCC xenograft model. These evidences suggested the

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existence of BET inhibitor-resistance in HCC cells. Interestingly, our RNA-sequencing (RNA-seq) results showed that acute JQ1 treatment induced upregulation of anti-apoptotic Mcl-1 in HCC cells. This finding was inconsistent with previous research showing that BET inhibitors suppressed Mcl-1 expression in multiple myeloma (MM) and colorectal cells [5,6]. Given that Mcl-1 is a critical anti-apoptotic protein, we therefore used quantitative real time polymerase chain reaction (qRT-PCR) and western blotting analysis to further investigate the effect of JQ1 on the expression of Mcl-1, and also investigated whether this upregulation would negatively affect JQ1-mediated anticancer activity in HCC cells.

2. Materials and methods

2.1. Cell culture and reagents

HCC HCCLM3, BEL7402, MHCC-97H, HepG2 and Huh7 cell lines were obtained from China Center for Type Culture Collection (Wuhan, China), and were maintained in Dulbecco's Modified Eagle's medium (DMEM) (HyClone/Thermo Fisher Scientific, Beijing, China) supplemented with 10% heat-inactivated fetal bovine serum (Hangzhou Sijiqing Biological Engineering Materials Co., Ltd, Hangzhou, China) at 37 °C in a humidified incubator containing 5% CO2. JQ1 was kindly gifted by Professor James Bradner (Harvard Medical School). JQ1 was dissolved in Dimethyl sulfoxide (DMSO) at a stock concentration of 10 mM and stored at −20 °C.

2.2. RNA-seq analysis

Cells were treated with JQ1 or DMSO control for 4 h. RNA-Seq analysis was performed by BGI-Tech Company (Shenzhen, China) and Shanghai Biotechnology Corporation (Shanghai, China).

2.3. Quantitative real-time RT-PCR (qRT-PCR)

Total RNA from the treated cells was extracted by using the RNeasy Mini Kit (Qiagen, Inc., Shanghai, China). Total RNA (1−2 μg) was used for reverse transcription to cDNA with the SuperScript II Reverse Transcriptase system (Invitrogen, Shanghai, China). Quantitative real-time PCR (qRT-PCR) was performed with IQ Sybr-Green Supermix on a Bio-Rad ICycler Real-Time PCR machine (Bio-Rad, Shanghai, China). The following primers were used: Mcl-1: (forward) 5′-TGAAATCGTTGTCTCGAGTGATG-3′ and (reverse) 5′-TCACAA TCGCCCCAGTTT-3′. All values were normalized to the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (gapdh) (forward primer: 5′- GTCAGCCGCATCTTCTTT -3′, reverse primer: 5′- CGCCCAATACGACCAAAT-3′).

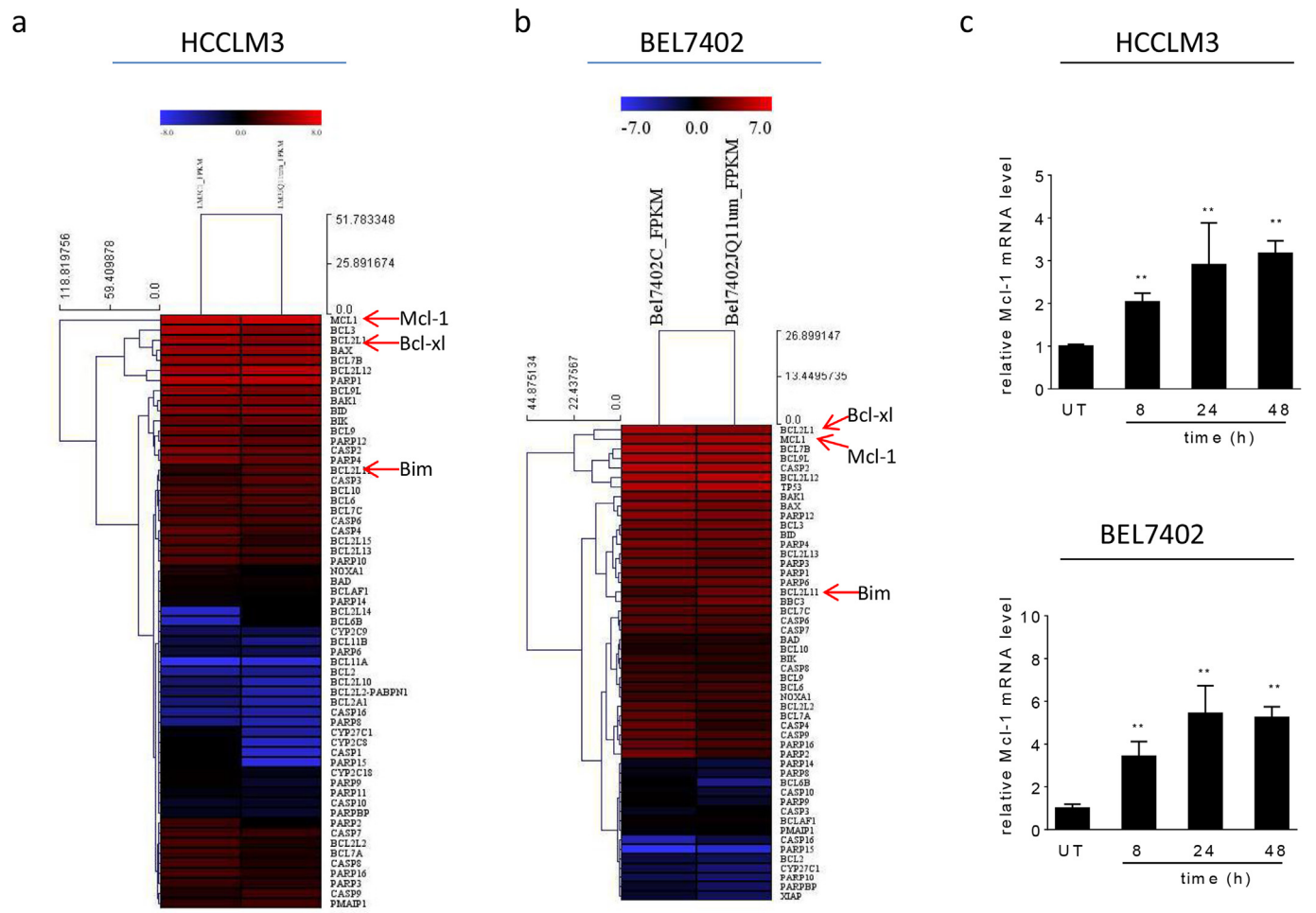

Fig. 1. JQ1 rapidly increases the mRNA level of Mcl-1 in HCC cells. (a) HCCLM3 and (b) BEL7402 cell lines were treated with JQ1 (0.5 μM for HCCLM3 and 1 μM for BEL7402) for 4 h. The alterations in gene expression in the treated cells were examined by RNA-Seq analysis. Heat maps depict the inhibited or increased ($p \leq .01$ and fold change ≥ 1.5 log2) genes involved in regulation of apoptosis. (c). HCCLM3 and BEL7402 cell lines were treated with 0.5 μM JQ1 for 8, 24 and 48 h. Mcl-1 mRNA expression was examined by qRT-PCR analysis. The data were presented by mean ± standard deviation ($n = 3$).

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