HMGB1 regulates autophagy through increasing transcriptional activities of JNK and ERK in human myeloid leukemia cells

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INTRODUCTION

High-mobility group box 1 protein (HMGB1), one of the best characterized damage-associated molecular pattern (DAMP) molecule, is a chromatin associated nuclear protein. Intracellularly, HMGB1 functions as an architectural chromatin-binding factor and promotes assembly of the proteins including p53, p73, Rel/Nf-kB and estrogen receptor on specific DNA targets. Extracellularly, HMGB1 through interacting with the receptors RAGE and TLRs contributes to several biological functions including inflammation, cell migration, cell differ-entiation and tumor metastasis (1). Current studies reveal that HMGB1 plays an important and central role in cancer (2). The upregulation of HMGB1 mRNA expression was found in tumors including gastrointestinal stromal tumor, skin tumor, colon cancer, breast cancer, lung cancer, prostate cancer, cervical cancer, gastric cancer, hepatocellular carcinoma, leukemia, lymphoma and melanoma. Therefore, targeting the HMGB1 ligand or its receptor represents an important potential application in cancer therapeutics.

Autophagy occurs at low basal levels in all cells and is postulated to play a housekeeping role (3). It is part of the normal catabolic process needed for homeostasis, which is in the quality control of cellular components by recycling long-lived proteins and dysfunctional organelles, and also keeps cell prolonged survival by providing substrates under metabolic stresses. Autophagy occurs in response to disordered microenvironment stimuli for H2O2, rapamycin, ER stress, mitochondrial toxins, hypoxia, abnormal cell growth, and nutrient deprivation (4). It is associated with multiple disease states, involving neurodegenerative disease, infection, heart disease, autophagic cell death, and cancer (5). Although the paradoxical dual effect about autophagy on tumor formation and growth awaits more verification, overwhelming investigations have suggested autophagy is an important resistance mechanism to chemotherapy in hematological malignancies (6).

Our recent studies have demonstrated that the serum levels of HMGB1 are significantly high in childhood lymphocytic leukemia (7, 8). Later data confirms HMGB1 is a direct activator of autophagy in leukemia cells through activating PI3KC3-MEK-ERK pathway (9), and autophagy is a potential mechanism for HMGB1-mediated chemotherapy resistance. However, the correlation between autophagy and HMGB1 in human myeloid leukemia cells remains unclear.

In this paper, our results suggested that HMGB1 over-expression rendered myeloid leukemia cells (K562 cells) resistant to conventional anticancer treatments through increasing autophagy rather than decreasing apoptosis. On the other hand, suppression of HMGB1 expression increased the sensitivity of leukemia cells to chemotherapeutic drugs. HMGB1 over-ex-
expression resulted in an increase in the formation of autophago-
some and autophagolysosome fusion, in mRNA levels of
Beclin-1, VSP34 and UVRAG which are key genes involved in
mammalian autophagy, and in protein levels of p-Bcl-2 and
LC3-II. The luciferase activity assays revealed that over-ex-
pression of HMGB1 increased the synergistic transcriptional
activities of JNK and ERK, respectively, and the inhibition of
HMGB1 down-regulated the transcriptional activities of JNK
and ERK. The role of HMGB1 in transcriptional regulation of
JNK and ERK required for autophagy provides a potential drug
target for therapeutic interventions in myeloid leukemia.

RESULTS

The HMGB1 expression is associated with the progression of
childhood chronic myeloid leukemia

We firstly determined mRNA levels of HMGB1 in four leukemia
cell lines (HL60, K562, Jurkat, and Raji) by RT-PCR analysis.
Levels of HMGB1 expression were high in all four human leukemia
cell lines. In contrast, the expression levels of HMGB1 were
noticeably low in non-blood cancer cell-lines (A549, Hela, and
HepG2) and the bone marrow mononuclear cells (BMMCs) from
normal children. These results suggested that the expression of
HMGB1 in leukemia tumorigenesis was up-regulated (Fig. 1A).

To evaluate the clinical relevance, we determined the
HMGB1 protein expression levels in serum from normal healthy
subjects, children with newly diagnosed chronic myeloid
leukemia (CML) and remission after treatment. The expression
of HMGB1 was significantly lower in Control group (59.06 ±
15.76 ug/L) and remission group (62.04 ± 28.98 ug/L) than
that in the refractory group (345.00 ± 123.42 ug/L), P < 0.05.
The difference of HMGB1 expressions between the control
and remission groups was not significant (P > 0.05) (Fig. 1B).
These results suggested that HMGB1 might be involved in the

Fig. 1. (A) HMGB1 was over-expressed in blood cancer cell lines. RT-PCR
analyses of HMGB1 and β-actin in various human cancer cell lines and the bone marrow mononuclear cells (BMMCs) from normal children as indicated, (B) Expression of HMGB1 in serum of different groups. The expression of HMGB1 is significantly lower in Control group (59.06 ± 15.76 ug/L) and remission group (62.04 ± 28.98 ug/L) than that in the refractory group (345.00 ± 123.42 ug/L), P < 0.05. The difference of HMGB1 expressions between the control and remission groups is not significant (P > 0.05).

Fig. 2. HMGB1 over-expressed by plasmid transfection and knocked down by siRNA in K562 cells as indicated and then treated with HBSS (Hank's), 3 hours later, capase-9 inhibitor for apoptosis was used (B and C) or not (A). (A) HMGB1 over-expression rendered K652 cells resistant to anti-cancer drug-induced apoptosis. K562 cells treated as indicated and then treat with VCR (1 ug/ml), ADM (1 ug/ul), VP-16 (1 ug/ul), AS2O3 (5 um), PBS treat as control group. Cell viability was examined at 24 h (n = 3, *P < 0.05), (B) The analysis of changes in cell cycle was quantified by flow-cytometry after PI staining in K562 cells treated 48 h after 3-h pretreatment with HBSS, there are no differences between HMGB1+ vs HMGB1- group. Values were given as mean ± SD. A: HMGB1+; B: HMGB1-. (C) Autophagic vacuoles were labeled with 0.05 mmol/L monodansylcadaverine (MDC) in phosphate-buffered saline (PBS) at 37°C for 10 min. The fluorescent density and the MDC-labeled particles in K562 cells were significantly higher in HMGB1+ than in HMGB1- group. (×400 magnifications). A: HMGB1+; B: HMGB1-. Data are the means SEM of three separate experiments.