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Induction of MHC class I-related chain B (MICB) by 5-aza-2′-deoxycytidine

Kai-Fu Tang [a,*], Cai-Xia He [a], Gui-Li Zeng [a], Jinfeng Wu [a], Guan-Bin Song [b], Yi-Song Shi [b], Wei-guang Zhang [b], Ai-Long Huang [a], Alexander Steinle [c], Hong Ren [a,*]

[a] Key Laboratory of Molecular Biology for Infectious Diseases of the State Ministry of Education, Institute for Viral Hepatitis, The Second Affiliated Hospital of Chongqing Medical University, 74# Linjiang Road, Chongqing 400010, PR China
[b] Key Laboratory for Biomechanics and Tissue Engineering of the State Ministry of Education, Chongqing University, Chongqing 400044, PR China
[c] Department of Immunology, Institute for Cell Biology, Eberhard-Karls University Tubingen, 72076 Tubingen, Germany

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ABSTRACT

5-Aza-2′-deoxycytidine (5-aza-dC), a DNA methyltransferase inhibitor, exerts antitumor activity through induction of cell cycle arrest, apoptosis and DNA damage. In this study, we showed that MHC class I-related chain B (MICB), a ligand of the NKG2D receptor expressed by natural killer cells and activated CD8(+) T cells, was upregulated following 5-aza-dC treatment. The upregulation of MICB was accompanied by promoter DNA demethylation and DNA damage. Furthermore, the upregulation of MICB was partially prevented by pharmacological or genetic inhibition of ataxia telangiectasia mutated (ATM) kinase. Our results suggest that promoter DNA demethylation, in combination with DNA damage, contribute to the upregulation of MICB induced by 5-aza-dC.

MHC class I-related chain (MIC) A and B (MICA and MICB), two polymorphic transmembrane glycoproteins, are the ligands for the immune receptor, NKG2D, which is expressed on natural killer (NK) cells, CD8 cytotoxic T cells, and γδ-T cells. MIC is critical for the susceptibility of target cells to NK cells, CD8 cytotoxic T cells, and γδ-T cells [1–5]. Expression of MIC is mostly restricted to intestinal mucosa; however, MIC is frequently associated with epithelial tumors of diverse tissue origins [1,2]. Expression of MIC can be induced by various cellular and environmental stimuli, including heat shock, virus infection, and DNA damage-inducing reagents [1–6].

Histone deacetylase (HDAC) inhibitors have been shown to induce MIC expression and sensitize cells to NK cell-mediated lysis [7–10]. In addition to activation of different signaling pathways [8,9], modulation of the chromatin structure at the promoter region is critical for the upregulation of MIC induced by HDAC inhibitors [10]. Histone acetylation and DNA methylation both play important roles in the regulation of chromatin structure and gene expression [11]. 5-Aza-2′-deoxycytidine (5-aza-dC), a pyrimidine analogue, once incorporated into DNA in place of cytosine can covalently trap DNA methyltransferase (DNMT) to DNA and cause irreversible inhibition of the enzyme [12]. Treatment with 5-aza-dC induces reversible genome-wide DNA damage [13]. The DNA damage pathway regulates innate immune system ligands of the NKG2D receptor [6]. In this study, we explored whether MICB was upregulated in response to 5-aza-dC treatment.

Materials and methods

Cell culture. HepG2 and HEK293T cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS). The NK cell line, NKL (kindly provided by M.J. Robertson of Indiana University School of Medicine, Indianapolis, IN, USA), was grown in RPMI 1640 with 15% FCS and 200 U/mL interleukin 2.

RNA interference. siRNAs were prepared by *in vitro* transcription with the Silencer siRNA Construction Kit (Ambion). Transfection was carried out using siPORT NeoFX (Ambion) according to the manufacturer's instructions. Target sequences of siRNAs were as follows:

ATM siRNA (AACATACTACTCAAAGACATT); control siRNA (AATTCTCCGAAC GTGTCACGT).

5-Aza-dC treatment. Cells were seeded in six-well plates and cultured for 8 h, then treated with 5-aza-dC (Sigma) at different concentrations; for treatment longer than 24 h, fresh 5-aza-dC was added every 24 h. To pharmacologically inhibit ATM, 2 mM caffeine (Alfa Aesar) was added 1 h before 5-aza-dC. To genetically inhibit ATM, ATM siRNA was transfected 12 h before 5-aza-dC treatment.

Flow cytometry. Surface MICB was analyzed using mouse anti-MICB. Briefly, cells were incubated with anti-MICB (R&D Systems), or the isotype control (R&D Systems) for 1 h at 4 °C. Cells were washed three times with PBS and incubated with PE-conjugated goat anti-mouse IgG (F0102; R&D Systems) for 30 min at 4 °C. Finally, fluorescence was detected with BD FACSAria (BD Biosciences) and analyzed with WinMDI software. Relative mean fluorescence intensity (MFI) was calculated as follows: (MFI of samples − MFI of isotype control antibody staining) ÷ (MFI of untreated cells − MFI of isotype control antibody staining).

Cellular cytotoxicity assay. HEK293T cells express several other activating ligands besides NKG2DL, which are recognized by the various activating receptors on polyclonal NK cells [14]. The NK cell line, NKL, expresses high levels of NKG2D, but only marginal levels of the activating receptors NKp30, NKp44, and NKp46, and is therefore well suited to study NKG2D-mediated recognition [15]. Target cells were

* Corresponding authors. Fax: +86 23 63822696.
E-mail addresses: tangkaifu@hotmail.com, tang_kaifu@yahoo.com.cn (K.-F. Tang), renhong0531@vip.sina.com (H. Ren).

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labeled with 100 μCi Na_2 $^{51}CrO_4$ (Amersham) for 2 h at 37 °C and washed three times. In blocking experiments, anti-NKG2D (R&D Systems) was added at 10 μg/mL to the target cells during the labeling procedure. Cells were washed, and effector cells were titrated on the target cells and incubated for 4 h at 37 °C. Maximum release was determined from target cells lysed in 1% Triton X-100. The percentage lysis was calculated as follows: 100 × (experimental release − spontaneous release) ÷ (maximum release − spontaneous release). Experiments were performed in triplicate.

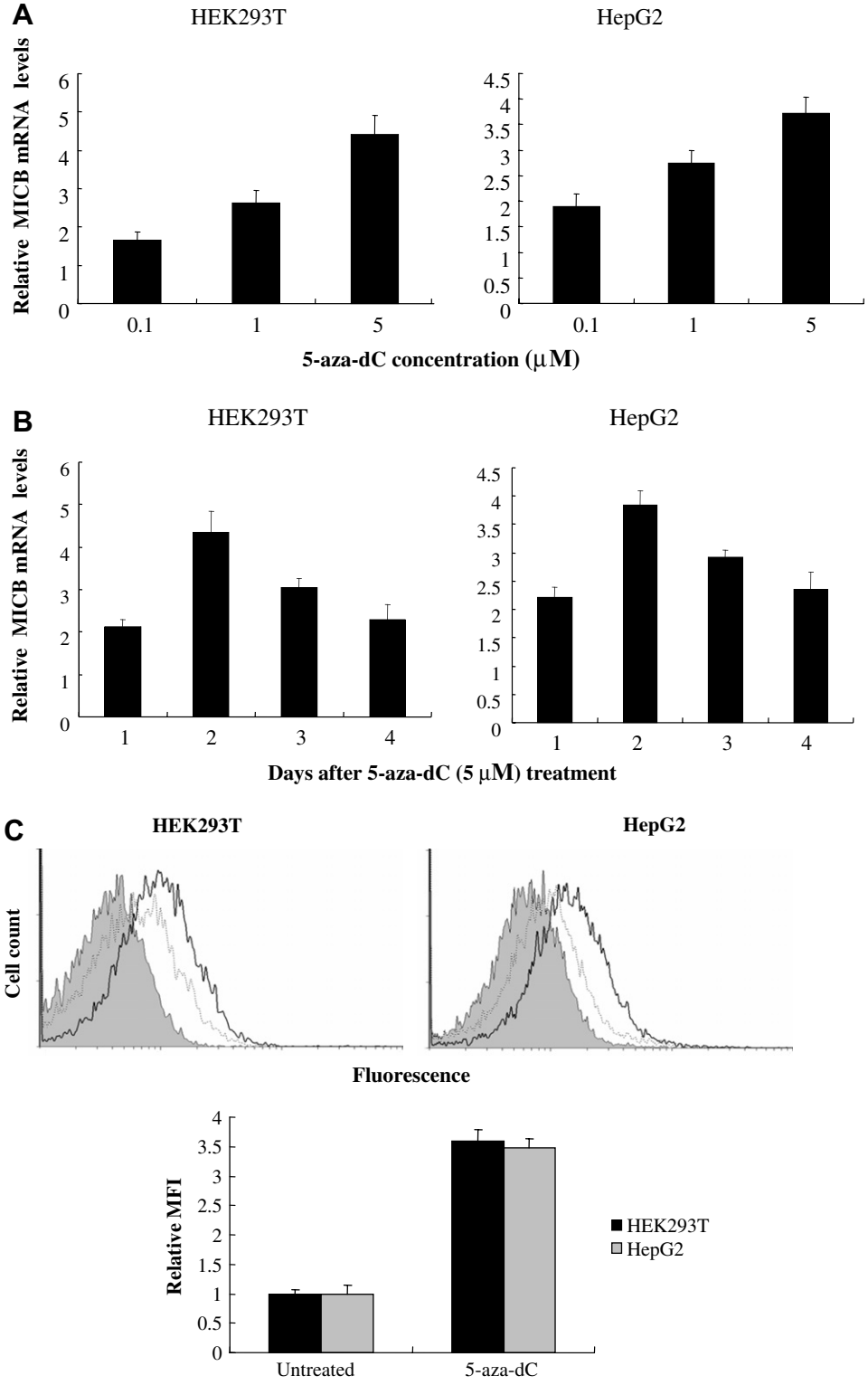

Fig. 1. 5-Aza-dC induces MICB expression. (A) Cells were treated with increasing concentration of 5-aza-dC for 48 h, and MICB mRNA was quantified by real-time RT-PCR. (B) Cells were treated with 5 μM 5-aza-dC for 4 days, and MICB mRNA levels were quantified at 24-h intervals. Data in (A) and (B) represent means ± SD from three independent experiments. The relative MICB mRNA levels are normalized to those in untreated cells, which are defined as 1. (C) Treatment with 5 μM 5-aza-dC for 48 h induced surface MICB. Upper panel: representative histograms of flow cytometry. 5-Aza-dC-treated cells (solid lines) were compared to untreated cells (dashed lines). The filled histograms indicate isotype control antibody staining. Lower panel: relative mean fluorescence intensity.