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Histone deacetylase inhibitors sensitize tumour cells for cytotoxic effects of natural killer cells

Mareike Schmudde [a,*], André Braun [a], Daniela Pende [b], Jürgen Sonnemann [c], Ulrike Klier [a], James F. Beck [d], Lorenzo Moretta [b], Barbara M. Bröker [a]

[a] *Institut für Immunologie und Immunologie, Ernst-Moritz-Arndt-Universität Greifswald, Ferdinand-Sauerbruch-Str., D-17487 Greifswald, Germany*
[b] *Istituto Nazionale per la Ricerca sul Cancro, Università degli Studi di Genova, Genoa, Italy*
[c] *Research Center of Pharmacology and Experimental Therapeutics, Ernst Moritz Arndt University Greifswald, Greifswald, Germany*
[d] *Department of Pediatric Hematology and Oncology, University Children's Hospital Jena, Jena, Germany*

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Abstract

Histone deacetylase inhibitors (HDIs) are emerging as potent anti-tumour agents which induce cell cycle arrest, differentiation and/or apoptosis in many tumour cells. Furthermore, they render tumour cells more sensitive to other therapeutic regimens like ionizing radiation, chemotherapy and recombinant tumour necrosis factor-related apoptosis-inducing ligand (TRAIL). Here, we show that the HDIs suberoylanilide hydroxamic acid (SAHA; vorinostat), sodium butyrate (NaB) and MS-275 sensitized DAOY and PC3 tumour cells for the cytotoxic effects of IL-2-activated PBMCs. In ^{51}Cr-release assays, blockade of the activating NK receptors DNAM-1, NKG2D and the NCRs completely abrogated tumour cell lysis, revealing that NK cells were the main effector cells involved. HDIs increased the tumour surface expression of ligands for the activating NK receptors NKG2D and DNAM-1 thereby facilitating tumour cell recognition by NK cells. These results suggest that the combination of HDIs and immunotherapy may be an effective strategy for anti-cancer therapy.

Keywords: Histone deacetylase inhibitors; NK cells; SAHA; Sodium butyrate; Synergy

1. Introduction

Histone deacetylase inhibitors (HDIs) are emerging as potent anti-tumour agents. They inhibit the action of histone deacetylases, thereby leading to a higher acetylation status of the chromatin. This results in an increase in transcriptionally active chromatin. By influencing the transcription of up to 22% of genes [1], HDIs induce cell cycle arrest, differentiation and/or apoptosis in tumour cells of many different histological origins. Notably, they affect cancer cells while leaving their untransformed counterparts largely unimpaired [2,3]. Several HDIs are currently tested in phase I and II trials and they have yielded favourable

* Corresponding author. Tel.: +49 3834865466; fax: +49 3834865490.
E-mail address: mareike.schmudde@uni-greifswald.de (M. Schmudde).

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results for the treatment of hematological malignancies as well as solid tumours [4–8]. Recently, SAHA has been approved by the US Food and Drug Administration for treatment of cutaneous T cell lymphoma [9].

Beside their direct anti-tumour activity, HDIs also enhance the cytotoxic effects of other therapeutic regimens like ionizing radiation, chemotherapy and recombinant tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) [10–13]. Since TRAIL is an important effector molecule of cytotoxic immune cells the question arose whether HDIs can sensitize tumour cells for the cytolytic effects of the immune system. Two main immune cell populations are involved in the defence against tumours. Tumour specific $CD8^+$ T cells of the adaptive immune system recognize tumour antigens and, if given a co-stimulatory signal, differentiate to cytotoxic T lymphocytes. On the other hand, natural killer (NK) cells are the main cytotoxic effector cells of the innate immune system. In contrast to T lymphocytes, NK cells do not express specific antigen receptors but their action is regulated by various activating and inhibitory receptors. Inhibitory signals are transmitted to an NK cell upon binding of KIRs to HLA class I molecules and/or CD94/NKG2A heterodimers to HLA-E [14]. NK cell activation depends on the engagement of triggering receptors of which the most important are NKG2D [15], DNAM-1 [16] and the "natural cytotoxicity receptors" (NCRs) NKp46, NKp44 and NKp30 [17]. While expression of the NCRs is restricted to NK cells, NKG2D is also expressed by γ/δ^+ T cells and α/β $CD8^+$ T cells.

Human NKG2D ligands are represented by MICA and MICB and the family of UL16-binding proteins (ULBPs) which are structurally related to MHC class I molecules. NKG2D ligands are up-regulated in various tumour cells and can be induced in untransformed cells upon stress [18,19]. The DNAM-1 ligands PVR (polio virus receptor, CD155) and Nectin-2 (CD112) may be over-expressed on tumour cells of different histological origins [20]. The ligands for the NCRs are not known so far.

In the present study, we assessed the impact of HDI pre-treatment on tumour cell recognition and lysis by human IL-2-activated PBMCs. We show that HDI treatment up-regulates the surface expression of NKG2D ligands on tumour cells and sensitizes them for the cytotoxic effects of NK cells.

2. Materials and methods

2.1. Cell culture

PC3 prostate carcinoma cells were obtained from ATCC (Rockville, MD, USA) and maintained in DMEM, supplemented with 10% foetal calf serum, 4 mM L-glutamine, 200 U/ml penicillin and 200 μg/ml streptomycin. DAOY medulloblastoma cells were a gift from Dr. M. Grotzer (Zurich, Switzerland) and maintained in Improved MEM Zinc Option, supplemented with 10% foetal calf serum, 200 U/ml penicillin and 200 μg/ml streptomycin. NK92C1 were from ATCC and maintained in MEM-α supplemented with 12% foetal calf serum, 12% donor horse serum, 200 U/ml penicillin and 200 μg/ml streptomycin. PBMCs were isolated from buffy coats of healthy donors by Ficoll–Hypaque density gradient centrifugation and cultured for 3 days in RPMI supplemented with 10% human serum from healthy donors, 4 mM L-glutamine, 200 U/ml penicillin, 200 μg/ml streptomycin and 6000 U/ml rIL-2. NK cells from peripheral blood of healthy donors were isolated using the RosetteSep method (StemCell Technologies, Vancouver, BC, Canada). NK cells were cultured on irradiated feeder cells in the presence of 2 μg/ml phytohemagglutinin and 100 U/ml rIL-2 to obtain proliferation of polyclonal NK cell populations. Cells were incubated in a humidified atmosphere with 5% CO_2 at 37 °C.

2.2. Reagents and antibodies

SAHA and MS-275 were purchased from Alexis (Grünberg, Germany), NaB, vincristine, phytohemagglutinin and monensin were purchased from Sigma (Deisenhofen, Germany) and rIL-2 (Proleukin) was purchased from Chiron (Emeryville, CA, USA). Fixation Medium was obtained from ADG (Kaumberg, Austria) and RNase A was obtained from Roche Diagnostics (Mannheim, Germany). Anti-MICB monoclonal antibody (mAb) was from R&D Systems (Wiesbaden, Germany), anti-TRAIL mAb was from Abcam (Cambridge, UK), anti-DR4 and anti-DR5 mAbs were from Biozol (Eching, Germany). Anti-MHC class I (clone W6-32) was from a hybridoma obtained from ATCC and grown in the laboratory. Murine IgG1 isotype control antibody was from eBioscience (San Diego, CA, USA), murine IgG2a isotype control was from DakoCytomation (Hamburg, Germany) and murine IgG2b