Cancer Letters 295 (2010) 173–181

Contents lists available at ScienceDirect

Cancer Letters

journal homepage: www.elsevier.com/locate/canlet

# Histone deacetylase inhibitors prevent activation of tumour-reactive NK cells and T cells but do not interfere with their cytolytic effector functions

Mareike Schmudde [a,*], Erika Friebe [a], Jürgen Sonnemann [b], James F. Beck [b], Barbara M. Bröker [a]

[a] Institute for Immunology and Transfusion Medicine, Ernst Moritz Arndt University Greifswald, Greifswald, Germany
[b] University Children's Hospital Jena, Department of Pediatric Hematology and Oncology, Jena, Germany

ARTICLE INFO

*Article history:*
Received 18 December 2009
Received in revised form 22 February 2010
Accepted 25 February 2010

*Keywords:*
Histone deacetylase inhibitors
SAHA
NK activation
Priming
Cytotoxicity

ABSTRACT

Histone deacetylase inhibitors (HDIs) exert direct tumour-toxic activity and sensitise tumour cells for other therapeutic regimens as well as the cytotoxic effects of activated immune cells. However, the HDI suberoylanilide hydroxamic acid (SAHA; vorinostat) interfered with the IL-2 activation of human NK cells and the priming of human tumour-specific T cells. In contrast, NK or T cells which were activated in the absence of HDIs became resistant to their immunosuppressive action. Therefore, as a therapeutic strategy, first the patient's immune system might be stimulated and then HDIs could sensitise the tumours for the attack of the pre-activated immune effector cells.



## 1. Introduction

Histone deacetylase inhibitors (HDIs) are potent antineoplastic agents that induce tumour cell death *in vitro* and yield promising therapeutic results in animal models [1] and humans [2,3]. As the first HDI, suberoylanilide hydroxamic acid (SAHA; vorinostat) has been approved by the US Food and Drug Administration for treatment of cutaneous T cell lymphoma [4]. HDIs influence the transcriptional activity of at least 22% of genes [5]: On the one hand, they increase transcriptionally active chromatin, and on the other, they regulate the activity of various non-histone proteins such as transcription factors and other transcriptional regulators [6]. HDIs thus exert direct tumour-toxic activity and also sensitise tumour cells for the cytotoxic effects of different chemotherapeutic agents, ionizing radiation, and activated immune cells [7,8].

The cellular immune system plays an important role in the defence against tumours. Two main immune cell populations are involved: NK cells, effector cells of the innate immune system, and cytotoxic $CD8^+$ T cells belonging to the adaptive immune system. NK cell activity is regulated by a multitude of activating (e.g., NKG2D, DNAM-1, natural cytotoxicity receptors) and inhibitory (e.g., KIRs, CD94/NKG2A) receptors. We and others have previously demonstrated that HDIs up-regulate the surface expression of NKG2D ligands on tumour cells and enhance tumour cell recognition and lysis by NK cells [9–11]. Therefore, the question arose whether HDIs could also sensitize tumour cells for the effector functions of tumour-specific cytotoxic T cells. T cells recognize tumour antigens presented by MHC molecules via their TCR. To become activated, naïve T cells require additional co-stimulatory signals which are normally provided by professional APCs. Antigen recognition without co-stimulatory signals results in T cell anergy, a state of antigen-specific unresponsiveness. The best characterized co-stimulatory signal is given by the ligation

* Corresponding author. Address: Institut für Immunologie, Ernst-Moritz-Arndt-Universität, Ferdinand-Sauerbruch-Str., D-17487 Greifswald, Germany. Tel.: +49 3834 865466; fax: +49 3834 865490.
*E-mail address:* mareike.schmudde@uni-greifswald.de (M. Schmudde).

0304-3835/$ - see front matter © 2010 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.canlet.2010.02.024

of CD28, expressed on both resting and activated T cells, with CD80 (B7.1) or CD86 (B7.2) on APCs [12]. Generally, tumour cells lack co-stimulatory molecules and are therefore unable to trigger a T cell response even if they present tumour antigens. However, if MHC expressing tumour cells are transfected with B7 molecules, they effectively induce T cell activation [13]. In our study, we used B7.2-transfected melanoma cells expressing both MHC class I and class II molecules to prime allogeneic T cells from unrelated donors. The alloantigens presented on the MHC molecules served as surrogate tumour antigens. In addition, the T cells that recognize such non-self MHC-peptide complexes are provided with a co-stimulatory signal directly by the melanoma cells. This results in the activation and proliferation of $CD4^+$ and $CD8^+$ T cells and in the differentiation of the latter into cytotoxic T lymphocytes.

Since in cancer histone acetylation levels have been found to be reduced [14], tumour cells are more sensitive for the cytotoxic effects of HDIs than their untransformed counterparts [15,16]. However, differentiation and function of immune cells also strongly depend on a well-regulated balance of acetylation and deacetylation [17,18]. In T cells, HDIs induce cell cycle arrest and apoptosis, and decrease the expression of co-stimulatory and adhesion molecules [19,20]. HDIs can be used as effective immune modulators: they have been shown to reduce systemic inflammation [21], enhance allograft survival [22], and reduce experimental graft-versus-host disease [23].

The current study examined the impact of HDIs on the activation and the tumour-lytic potential of both human NK and tumour-specific cytotoxic T cells. We demonstrate that although HDIs impair the activation of immune cells, they do not affect the tumour cytotoxicity of already activated immune effector cells.

## 2. Materials and methods

### 2.1. Cell culture

PC3 human prostate carcinoma cells were obtained from ATCC (Rockville, MD, USA) and maintained in DMEM, supplemented with 10% FCS, 4 mM L-glutamine, 200 U/ml penicillin and 200 μg/ml streptomycin. DAOY human medulloblastoma cells were provided courtesy of Dr. M. Grotzer (Zurich, Switzerland) and maintained in Improved MEM Zinc Option, supplemented with 10% FCS, 200 U/ml penicillin and 200 μg/ml streptomycin. Human melanoma cells SkMel63 and SkMel63-B7.2 (SkMel63 stably transfected with CD86) were a kind gift from Dr. U. Moebius (Heidelberg, Germany) and maintained in RPMI 1640, supplemented with 10% FCS, 4 mM L-glutamine, 200 U/ml penicillin and 200 μg/ml streptomycin. Culture medium of SkMel63-B7.2 cells also contained 1 mg/ml G418. Human PBMCs were isolated from buffy coats of healthy donors by Ficoll-Hypaque density gradient centrifugation. $CD3^+$ T cells were purified from nylon–wool-passaged freshly isolated PBMCs using anti-CD3-conjugated MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions. Cells were incubated in a humidified atmosphere with 5% $CO_2$ at 37 °C.

### 2.2. Reagents and antibodies

SAHA was purchased from Alexis (Grünberg, Germany), sodium butyrate, staphylococcal enterotoxin B (SEB), monensin and mitomycin C from Sigma (Deisenhofen, Germany), PHA from Virotech (Rüsselsheim, Germany), and [methyl-$^3$H]-thymidine from Amersham Biosciences (Freiburg, Germany). Nylon wool was obtained from Kisker (Steinfurt, Germany), IL-2 (Proleukin) from Chiron (Emeryville, CA, USA), and G418 (geneticin) was purchased from Invitrogen (Karlsruhe, Germany). The following antibodies were from BD Biosciences (Heidelberg, Germany): anti-CD3-PE, anti-CD56-FITC, anti-CD107a-PE-Cy5 and murine IgG1-PE-Cy5 isotype control.

### 2.3. IL-2-activation of PBMCs

To obtain activated PBMCs, cells were cultured for 3 days in RPMI 1640 supplemented with 10% human serum from healthy donors, 4 mM L-glutamine, 200 U/ml penicillin, 200 μg/ml streptomycin and 6000 U/ml IL-2. In some cases, SAHA at indicated concentrations was added to the activation culture. For further experiments, the concentration of PBMCs activated without SAHA was exactly determined and equal volumes of IL-2/SAHA-activated PBMCs were used.

### 2.4. Priming of SkMel63-specific alloreactive $CD3^+$ T cells

SkMel63-B7.2 cells at a density of $5 \times 10^{7\ ml-1}$ were inactivated with 200 μg/ml mitomycin C for 20 min at 37 °C. After washing three times, they were cultured together with purified $CD3^+$ T cells at a ratio of 1:2 in 96-well

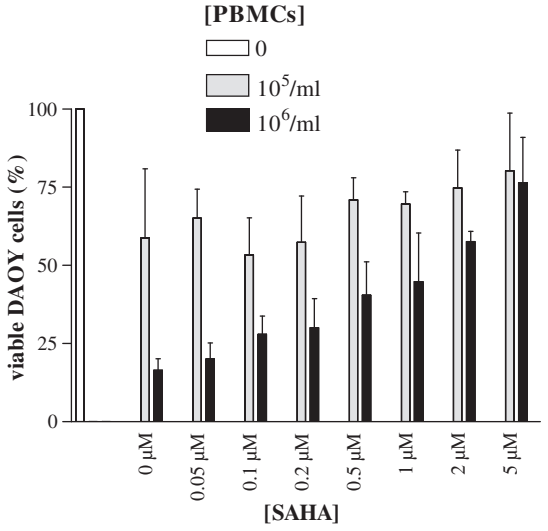


**Fig. 1.** Tumour cell death induction by PBMCs activated with IL-2 in the presence of SAHA. The presence of SAHA during pre-activation impaired the subsequent tumour-lytic potency of PBMCs. PBMCs were cultured with IL-2 and indicated concentrations of SAHA for 3 d. DAOY cells were then co-incubated with different numbers of activated PBMCs for 24 h. Cell numbers were measured by FACS. Results indicate numbers of viable tumour cells as percentages of tumour cells cultured in the absence of PBMCs. Means ± SDs of three independent experiments are shown.