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The novel HDAC inhibitor NDACI054 sensitizes human cancer cells to radiotherapy

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ABSTRACT

Background and purpose: Inhibition of histone deacetylases (HDACs) has preclinically and clinically shown promise to overcome radio- and chemoresistance of tumor cells. NDACI054 is a novel HDAC inhibitor, which has been evaluated here for its effects on cell survival and radiosensitization of human tumor cell lines from different origins cultured under more physiological three-dimensional (3D), extracellular matrix (ECM)-based conditions.

Material and methods: A549 lung, DLD-1 colorectal, MiaPaCa2 pancreatic and UT-SCC15 head and neck squamous cell carcinoma cells were treated with increasing NDACI054 concentrations (0–50 nM, 24 h) either alone or in combination with X-rays (single dose, 0–6 Gy). Subsequently, 3D clonogenic cell survival, HDAC activity, histone H3 acetylation, apoptosis, residual DNA damage (γH2AX/p53BP1 foci assay 24 h post irradiation) and phosphorylation kinetics of Ataxia telangiectasia mutated (ATM), DNA-dependent protein kinase (DNA-PK), Caspase-3 and Poly(ADP-ribose)-Polymerase 1 (PARP 1) cleavage were analyzed.

Results: NDACI054 potently decreased HDAC activity with concomitant increase in acetyl-histone H3 levels, mediated significant cytotoxicity and radiosensitization. These effects were accompanied by a significant increase of residual γH2AX/p53BP1-positive foci, slightly elevated levels of Caspase-3 and PARP 1 cleavage but no induction of apoptosis.

Conclusions: Our data show potent antisurvival and radiosensitizing effects of the novel HDAC inhibitor NDACI054 encouraging further preclinical examinations on this compound for future clinical use.

Current strategies to overcome tumor cell radio- and chemoresistance include targeted therapies with small molecule inhibitors or antibodies [1]. As epigenetic modifications are key to tumor development and progression, inhibitors for histone deacetylases (HDACs), which control epigenetic changes and gene expression in cooperation with histone acetyl transferases (HATs), arose as potential cancer therapeutics [2–5]. Radiosensitization by HDAC deactivation is thought to result from chromatin relaxation thereby ameliorating the induction of radiogenic DNA damage.

Momentarily, 18 mammalian HDACs are divided into four classes and their expression and/or activity are frequently deregulated in solid and hematological malignancies (reviewed in: [6]). Class I members share specific homologous catalytic sites and comprise family members HDAC1-3 and -8. Class II members have been further subdivided into the classes IIa (HDAC4, -5, -7, -9) and IIb (HDAC6, -10) dependent on domain structure and catalytic domain homology, while class IV HDAC11 comprises homology in the catalytic core to enzymes from both class I and II [6]. Class III HDACs are represented by SIRT1-7 family members and require the cofactor nicotinamide adenine dinucleotide (NAD^+) for activation [6].

Effects of HDAC inhibitors in tumor cells are apoptosis induction and tumor growth arrest by facilitating tumor cell differentiation and inverting the silencing of proapoptotic genes [2,6]. Moreover, HDAC inhibitors are thought to hyperacetylate transcription factors leading either to activation or inactivation of target genes [7]. Structural classes of HDAC inhibitors include hydroxamic acids (e.g. suberoylanilide hydroxamic acid (SAHA; vorinostat), trichostatin A), aliphatic acids (e.g. valproic acid) and cyclic tetrapeptides (e.g. depsipeptide (FK228)) [2,8]. While SAHA is approved for the treatment of cutaneous T-cell lymphoma, most HDAC inhibitors are still under clinical evaluation [6,9]. Recent preclinical data suggest improved tumor cell kill and control by combining HDAC inhibitors with radio-(chemo)therapy [5,10,11]. A first clinical phase I trial administering vorinostat plus pelvic palliative radiation reported good treatment tolerability [12].

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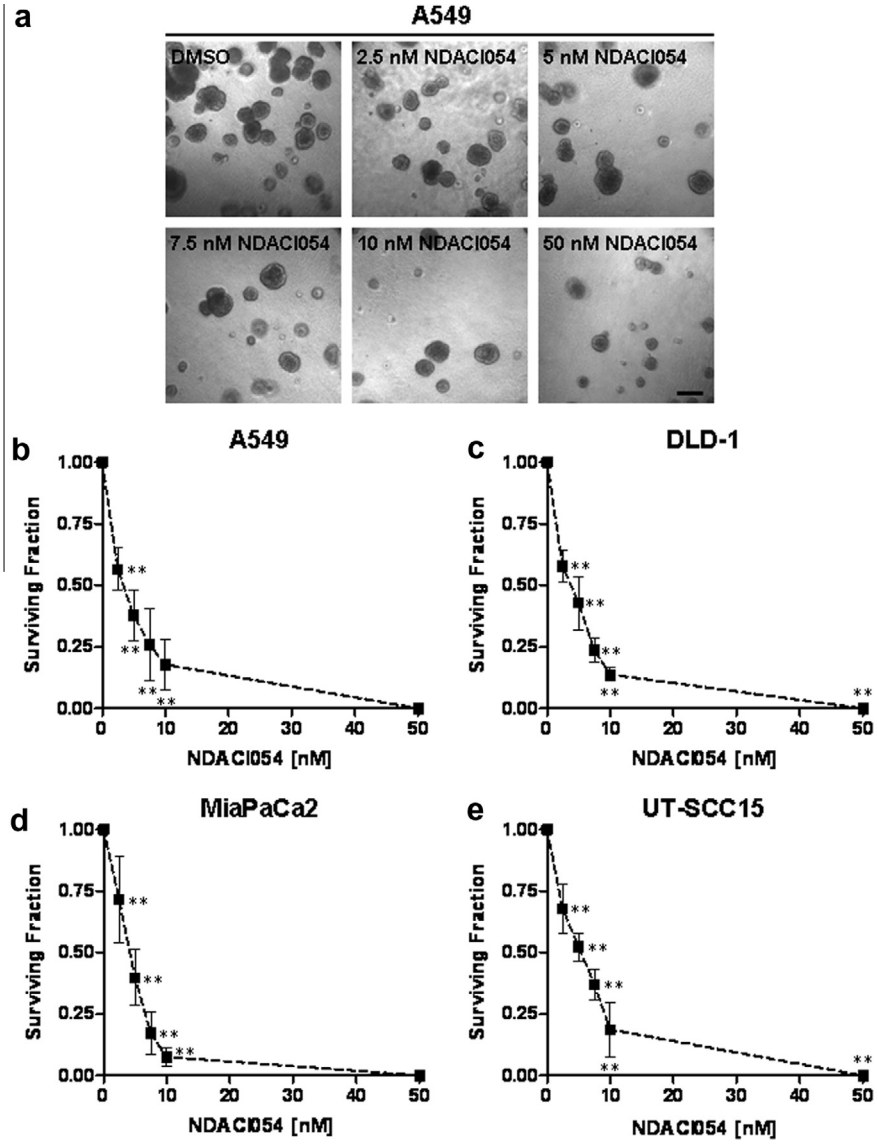

Fig. 1. The HDAC inhibitor NDACI054 strongly decreases basal 3D survival of human tumor cells in a concentration-dependent manner. (a) Phase contrast images of A549 cells illustrate decreased numbers of 3D colonies after treatment with increasing NDACI054 concentrations. Bar, 100 μm. (b–e) 3D clonogenic survival of indicated cell lines treated with increasing NDACI054 concentrations for 24 h. Results show mean ± SD ($n = 5$). Student's t-test compared NDACI054- versus DMSO-treated cells ($^{*}P < 0.05$; $^{**}P < 0.01$).

Here, we show a strong cytotoxicity of the novel class I and II HDAC inhibitor NDACI054 (Novartis DeAcetylase Inhibitor 054) already at very low concentrations of 2.5 nM and a significant radiosensitization of tumor cells grown under more physiological three-dimensional (3D; [10,13–19]) cell culture conditions. These effects were associated with elevated levels of residual DNA double-strand breaks (rDSB).

Materials and methods

Cell lines, cell culture, and irradiation

A549 human lung carcinoma, DLD-1 human colorectal carcinoma and MiaPaCa2 human pancreatic carcinoma cell lines were obtained from American Type Culture Collection (ATCC, Manassas, USA). The human head and neck squamous cell carcinoma (HNSCC) cell line UT-SCC15 was a kind gift from R. Grenman (Turku University Central Hospital, Finland). Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; PAA, Cölbe, Germany) containing glutamax-I (L-alanyl-L-glutamine) supplemented with 10% fetal calf serum (FCS; PAA) and 1% non-essential amino acids (NEAA; PAA) For 3D cell culture, cells were plated into a mixture of 0.5 mg/ml laminin-rich extracellular matrix (lrECM; Matrigel; BD, Heidelberg, Germany) and DMEM medium supplemented with 10% FCS and 1% NEAA in 24-well cell culture dishes (BD) as previously published [10,13,14,20]. All cells were cultured at 37 °C in a humidified atmosphere containing 7% CO_2. Irradiation was delivered at room temperature using single doses (2–6 Gy) of 200 kV X-rays (Yxlon Y.TU 320; Yxlon, Copenhagen, Denmark) filtered with 0.5 mm copper. The dose-rate was approximately 1.3 Gy/min at 20 mA. The absorbed dose was evaluated using a Duplex dosimeter (PTW, Freiburg, Germany).

Application of NDACI054

NDACI054 (Novartis DeAcetylase Inhibitor 054; Novartis Institutes of Biomedical Research; Cambridge, MA, USA) was dissolved in DMSO (AppliChem, Darmstadt, Germany) at a concentration of