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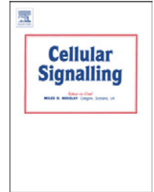

Class I histone deacetylases regulate p53/NF-κB crosstalk in cancer cells

Claudia Schäfer [a], Anja Göder [b], Mandy Beyer [b], Nicole Kiweler [b], Nisintha Mahendrarajah [b], Anke Rauch [a], Teodora Nikolova [b], Natasa Stojanovic [c], Martin Wieczorek [a], Thomas R. Reich [b], Maja T. Tomicic [b], Michael Linnebacher [d], Jürgen Sonnemann [e], Sascha Dietrich [f], Andreas Sellmer [g], Siavosh Mahboobi [g], Thorsten Heinzel [a], Günter Schneider [h,1], Oliver H. Krämer [b,*,1]

[a] Friedrich-Schiller-University Jena, Center for Molecular Biomedicine, Institute of Biochemistry and Biophysics, Department of Biochemistry, Hans-Knöll-Straße 2, 07745 Jena, Germany
[b] Department of Toxicology, University Medical Center of the Johannes Gutenberg University Mainz, Obere Zahlbacher Straße 67, 55131 Mainz, Germany
[c] Project Group "Personalized Tumor Therapy", Fraunhofer Institute of Toxicology and Experimental Medicine, Am Biopark 9, 93053 Regensburg, Germany
[d] University Medicine Rostock, Department of General Surgery, Molecular Oncology and Immunotherapy, Schillingallee 35, 18057 Rostock, Germany
[e] Department of Paediatric Haematology and Oncology, Children's Clinic, Jena University Hospital, Kochstraße 2, 07745 Jena, Germany
[f] Department of Medicine V, University Hospital Heidelberg, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany
[g] Institute of Pharmacy, Faculty of Chemistry and Pharmacy, University of Regensburg, 93040 Regensburg, Germany
[h] Technische Universität München, Klinikum rechts der Isar, II. Medizinische Klinik, Ismaninger Straße 22, 81675 München, Germany

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ABSTRACT

The transcription factors NF-κB and p53 as well as their crosstalk determine the fate of tumor cells upon therapeutic interventions. Replicative stress and cytokines promote signaling cascades that lead to the co-regulation of p53 and NF-κB. Consequently, nuclear p53/NF-κB signaling complexes activate NF-κB-dependent survival genes. The 18 histone deacetylases (HDACs) are epigenetic modulators that fall into four classes (I-IV). Inhibitors of histone deacetylases (HDACi) become increasingly appreciated as anti-cancer agents. Based on their effects on p53 and NF-κB, we addressed whether clinically relevant HDACi affect the NF-κB/p53 crosstalk. The chemotherapeutics hydroxyurea, etoposide, and fludarabine halt cell cycle progression, induce DNA damage, and lead to DNA fragmentation. These agents co-induce p53 and NF-κB-dependent gene expression in cell lines from breast and colon cancer and in primary chronic lymphatic leukemia (CLL) cells. Using specific HDACi, we find that the class I subgroup of HDACs, but not the class IIb deacetylase HDAC6, are required for the hydroxyurea-induced crosstalk between p53 and NF-κB. HDACi decrease the basal and stress-induced expression of p53 and block NF-κB-regulated gene expression. We further show that class I HDACi induce senescence in pancreatic cancer cells with mutant p53.

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1. Introduction

The NF-κB family consists of five transcription factors, namely p65/Rel-A, Rel-B, c-Rel, NF-κB1/p50, and NF-κB2/p52 [1,2]. These proteins control immunological functions, cell proliferation, transformation, and drug sensitivity [1,2]. Depending on the upstream signals, the activation of NF-κB occurs via classical or alternative pathways [3]. In addition, replicative stress and DNA damage engage atypical pathways of NF-κB activation [1,4].

Simultaneously to NF-κB activation, replicative stress and DNA damage induce the phosphorylation and acetylation of the tumor suppressor p53 [5]. These posttranslational modifications (PTMs) stabilize p53 and they induce its capacity to activate genes regulating cell cycle progression and cell death [5–7]. The growth-restricting activities of p53 explain why tumors express mutated p53 very frequently. Moreover, mutant p53 acquires new oncogenic, gain-of-function qualities, including the positive regulation of pro-tumorigenic functions of NF-κB [4,8].

In addition to individual functions of NF-κB and p53, there is an intense, context-dependent crosstalk between them [1,4]. The stress- and cytokine-dependent control of several NF-κB target genes relies on chromatin-bound p53/NF-κB p65 complexes in vivo [9,10]. Oren and colleagues confirmed our finding that mutant p53 activates NF-κB and they showed that this pathway controls chronic inflammatory responses and colorectal tumorigenesis [11]. Furthermore, Murphy and colleagues verified the interaction between p53 and NF-κB p65 and revealed that a p53 polymorphic isoform with a proline residue at position 72 (p53-P72, $p53^{P72}$) interacted more avidly with p65 than p53 carrying

Abbreviations: BCL, B cell lymphoma; CLL, chronic lymphatic leukemia; DSB, DNA double strand break; HDAC, histone deacetylase; HDACi, histone deacetylase inhibitor; MS-275, entinostat; NF-κB, nuclear factor 'kappa-light-chain-enhancer' of activated B cells; PDAC, pancreatic ductal adenocarcinoma; PTM, posttranslational modification; VPA, valproic acid.

* Corresponding author.
E-mail address: okraemer@uni-mainz.de (O.H. Krämer).
[1] Equal last author contribution.

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an arginine residue (p53-R72, $p53^{R72}$) [12]. The observation that mice with the $p53^{P72}$ variant show increased inflammatory responses [12] illustrates that p53-p65 interactions affect cancer cell growth as well as immunological functions. The finding that mutant p53 controls NF-κB, cell proliferation, and survival in other systems [4] stresses the importance of the interaction between these transcription factors.

HDACs are epigenetic modifiers that affect NF-κB and p53 at several levels [4,5,13]. HDACs fall into four classes (I, IIa/b, III, IV). Cancer-relevant functions have most frequently been reported for the subgroup of class I HDACs (HDAC1,-2,-3,-8) and for HDAC6 [13–16]. Accordingly, an increasing number of HDACi undergo clinical testing; these agents specifically block zinc-dependent HDACs [13,14]. Of note, the FDA has recently approved four HDACi for the treatment of cancer [17,18].

It is unclear how the attenuation of wild-type p53 by HDACi affects tumor cells under replicative stress, a scenario in which wild-type p53 positively regulates NF-κB-dependent anti-apoptotic genes [4,9]. Therefore, we addressed further details on the replicative stress-induced NF-κB/p53 crosstalk. We investigated whether the HDACi entinostat (MS-275), valproic acid (VPA), and Marbostat-100 affected wild-type and mutant p53 as well as NF-κB-dependent gene expression. As the ability of HDACi to decrease mutant p53 helps to eliminate cancers that depend on this tumor driver [19], we also analyzed if this beneficial activity of HDACi is associated with a modulation of the p53/NF-κB crosstalk. We report that HDACi against class I HDACs are necessary for the expression of mutant p53 and the activity of NF-κB in cancer cells. Thus, HDACi can modulate a biologically relevant crosstalk between p53 and NF-κB to shift the therapeutic outcome to a favorable response.

2. Materials and methods

2.1. Reagents

Drugs, chemicals, and buffers are described in [9,20]. Marbostat-100 is patented as *Mahboobi*, *Siavosh*; *Sellmer*, *Andreas*; *Pongratz*, *Herwig*; *Leonhardt*, *Michel*; *Krämer*, *Oliver*; *Böhmer*, *Frank-Dietmar*; *Kelter*, *Gerhard*; *Preparation of fused heterocyclic compounds as HDAC6 inhibitors and their uses*; *PCT Int. Appl. (2016)*, *WO 2016020369* A1.

2.2. Cells, transfections, luciferase, and MTT assay

For details on cells and assays see [9,20] and Supplemental Table 1; cells were regularly tested to exclude mycoplasma contamination. MCF7 cells were plated at densities of 5×10^4/mL and transfected with Lipofectamine (Invitrogen). Peripheral blood samples were obtained from patients with CLL after informed consent in accordance with the Declaration of Helsinki. Sample collection was approved by the local ethics committee of the University Hospital of Heidelberg as part of the tumor bank of the National Centre for Tumor Diseases, Heidelberg, Germany. Cases matched standard diagnostic criteria for CLL. Both patients had wild-type TP53.

2.3. Flow cytometry analyses

Cell cycle profiles were measured as stated in [9]. Intracellular staining of p53, Survivin, and BCL-XL was done according to the manufacturers' protocols with the antibodies Survivin (D-8) Alexa Fluor® 647, Santa Cruz sc-17779 AF647; BCL-XL Antibody (H-5) PE, sc-8392 PE; and TP53-FITC, BD Biosciences.

2.4. Preparation of cell lysates, immunoblotting, ABCD assays, and microscopy

For lysate preparations, Western blots, ABCD assays, and co-immunoprecipitations see [9,20,21]. Proteins were detected with Western blots generated with X-ray films (grey backgrounds) or with the Odyssey Infrared Imaging System (Licor) using IRDye® 680RD- or IRDye® 800CW-coupled secondary antibodies (white backgrounds). Antibodies were from Santa Cruz Biotechnology: p65, sc8008; p50, sc7178; p53, sc263/6243/fl-393X; STAT1, sc346/417; β-Actin, sc47778; XAF1, sc-398,012; Sigma: β-Actin, A2066/5060, acetylated Tubulin T7451, α-Tubulin T5168; Pharmingen: BCL-XL, 66461A; Novus: Survivin, 500-201L; p52, 05–361, Calbiochem: p53, OP03; Abcam: α-Tubulin, ab 176,560; Novocastra: p53 (CM5); Enzo: HSP90 ADI-SPA 830 (ac88); BD: XIAP, BD-610716; Cell Signaling: p-p65 Ser-536, #3036; Bethyl Laboratories anti-p-Ser317-CHK1 (A300-163A). For ac-*H*3/ac-H4 antibodies see [22]. Western blots were probed for housekeeping genes to ensure equal sample loading. Microscopy techniques are described in [9].

2.5. Senescence associate β-galactosidase (SA-β-gal) staining and analysis of XAF1 promoter methylation

These methods are described as Supplemental information.

3. Results

3.1. NF-κB and p53 target gene induction upon replicative stress and DNA damage

Hydroxyurea potently arrests cells in S phase via the inhibition of ribonucleoside diphosphate reductase, which provides dNTPs for DNA replication [23–25]. Etoposide inhibits topoisomerase II and thereby causes DNA double strand breaks (DSBs) [26]. After 24 h, hydroxyurea arrested HCT116 colon cancer cells in S phase and etoposide caused their accumulation in G2/M phase (Fig. 1A). After 48 h, both drugs caused cell death, which we measured as an occurrence of cells with fragmented DNA by flow cytometry (Fig. 1B).

Concomitantly with these cellular alterations, hydroxyurea and etoposide induced p53 and its direct target gene BAX. Consistent with the literature [9,10,27], hydroxyurea and etoposide evoked an accumulation of the NF-κB targets Survivin and BCL-XL (Fig. 1C).

However, the accumulation of p53 and Survivin as detected by Western blot (Fig. 1C), does not necessarily reflect a co-induction in the same cells. Therefore, we used flow cytometry to assess the putative co-expression of p53 with BCL-XL and Survivin. We stained p53 and one of these proteins with antibodies coupled to fluorescent dyes. This approach verified that hydroxyurea and etoposide caused a joint accumulation of p53 with BCL-XL and Survivin in HCT116 cell populations (Fig. 1D and data not shown).

The cytokine-induced phosphorylation of p65 at serine 536 is linked to its nuclear translocation [28]. Therefore, we analyzed whether hydroxyurea-induced replicative stress also triggers this PTM. However, we did not observe serine 536 phosphorylation of p65 in hydroxyurea-treated HCT116 cells (Supplemental Fig. S1). We consistently observed no alteration in the levels of I-κB proteins, which retain NF-κB subunits in the cytoplasm (data not shown).

These data illustrate the co-regulation of p53/NF-κB in response to various conditions evoking replicative stress and DNA damage. These data agree with our previous findings illustrating that p53 is a permissive factor for NF-κB-dependent gene expression [4,9,10,13].

3.2. Co-regulation of p53 and NF-κB in CLL cells

To test for a chemotherapy-induced p53/NF-κB crosstalk in primary cancer cells, we exposed fresh CLL cells to the DNA synthesis inhibitor fludarabine, which is a standard drug against this incurable disease [29]. We also chose this malignancy because fludarabine induces p53 signaling in CLL cells [30,31] and since such cells depend on p65 for their survival [32]. As expected [30,31], fludarabine potently induced apoptosis in CLL cells (Fig. 2A).

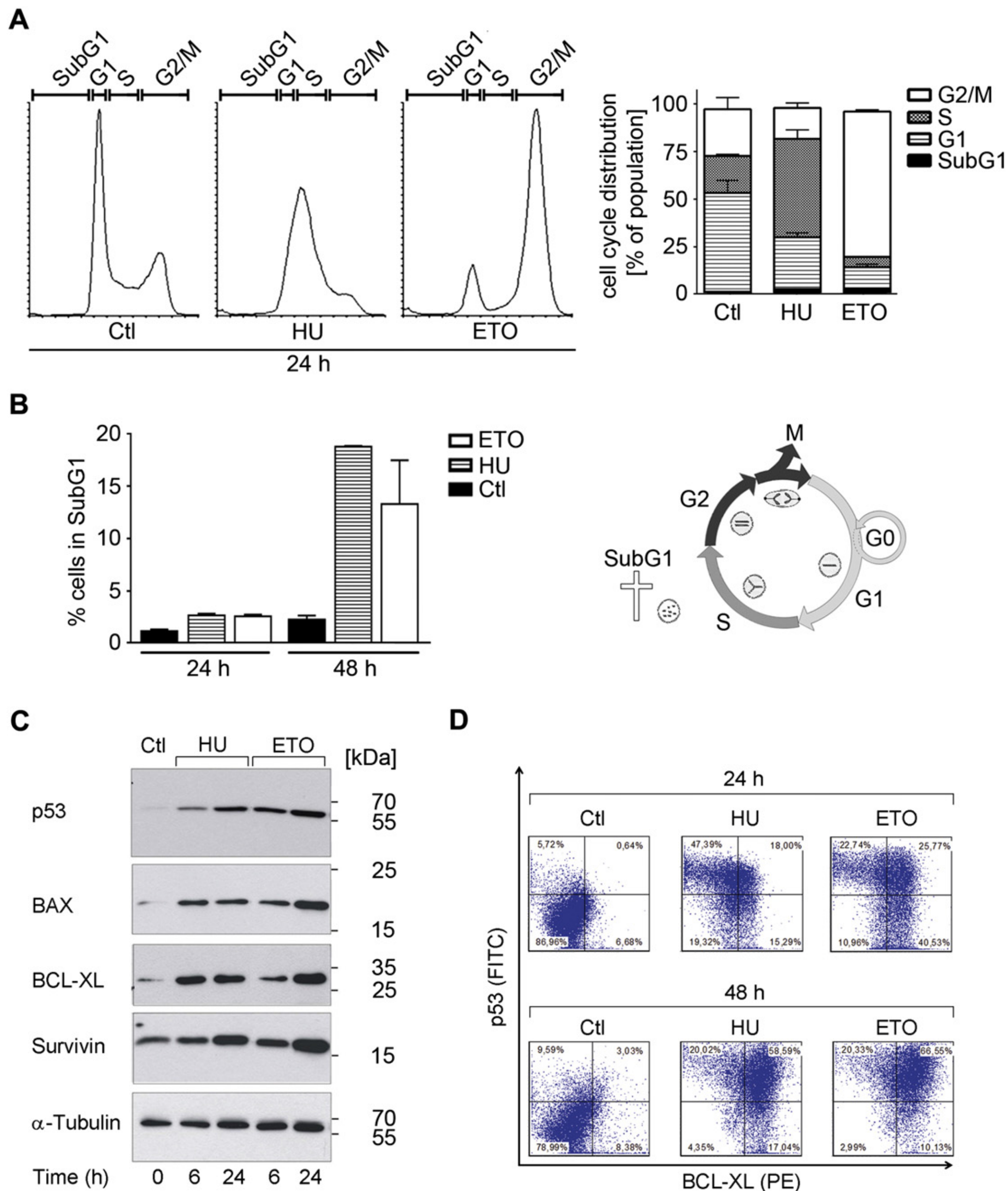

Fig. 1. Hydroxyurea and etoposide cause cell cycle arrest and induce p53/NF-κB-dependent genes (A) HCT116 cells were treated with 1 mM hydroxyurea (HU) or 10 μM etoposide (ETO) for 24 h (Ctl, untreated cells). Cell cycle distribution was evaluated by flow cytometry of fixed, PI-stained cells. Flow cytometry scans are representative for typical experimental outcomes. The graph on the right shows average results from two independent experiments. (B) Graph depicts the amount of dead HCT116 cells with DNA content below 2 N. Cells were treated as stated in (A) for 24–48 h. Illustration of the cell cycle phases is shown on the right. Dead cells in subG1 have a DNA content below 2 N. (C) HCT116 cells were treated with 1 mM hydroxyurea (HU) or 10 μM ETO for 6–24 h; Ctl, untreated cells. Expression levels of p53, BAX, Survivin, and BCL-XL were determined by immunoblot; α-Tubulin verifies equal loading; kDa, kilo Dalton. (D) 1 mM HU or 10 μM ETO was applied for 24 to 48 h to HCT116 cells. Expression of p53 and BCL-XL was determined by flow cytometry with the stated FITC- or PE-conjugated antibodies against these proteins.

We subjected primary CLL samples to immunoblotting in order to analyze the molecular changes that accompany fludarabine-induced apoptosis. We noted an induction of p53 and its target gene BAX in response to fludarabine. Moreover, the NF-κB target gene Survivin accumulated in such cells (Fig. 2B).

The functional interaction of p53 and NF-κB on NF-κB-dependent genes relies on their physical interaction in breast and colon cancer cells [4,9]. To analyze if p53 and NF-κB p65 also associate with each other in primary CLL cells, we used the ABCD assay with biotinylated NF-κB consensus oligonucleotides on DNA. Pull-down of this nucleotide with avidin-coupled beads and subsequent immunoblot analysis demonstrated the interaction of p53 and NF-κB *in cis* (Fig. 2C).

These results demonstrates the existence of a p53/NF-κB crosstalk in primary CLL cells.

3.3. Class I HDACi modulate p53

Since HDACi modulate p53 [13,33–36] and NF-κB activity in solid tumor cells [4,14], we next asked if these epigenetic modifiers could control the p53/NF-κB crosstalk. Immunoblotting of cytoplasmic and nuclear fractions demonstrated that the class I selective HDACi VPA [22] reduced the basal and hydroxyurea-induced levels of nuclear and cytoplasmic p53 (Fig. 3A). These data are consistent with the reported reduction of p53 at the mRNA and/or protein level in response to class I HDAC inhibition [10,19,28,33,37,38].

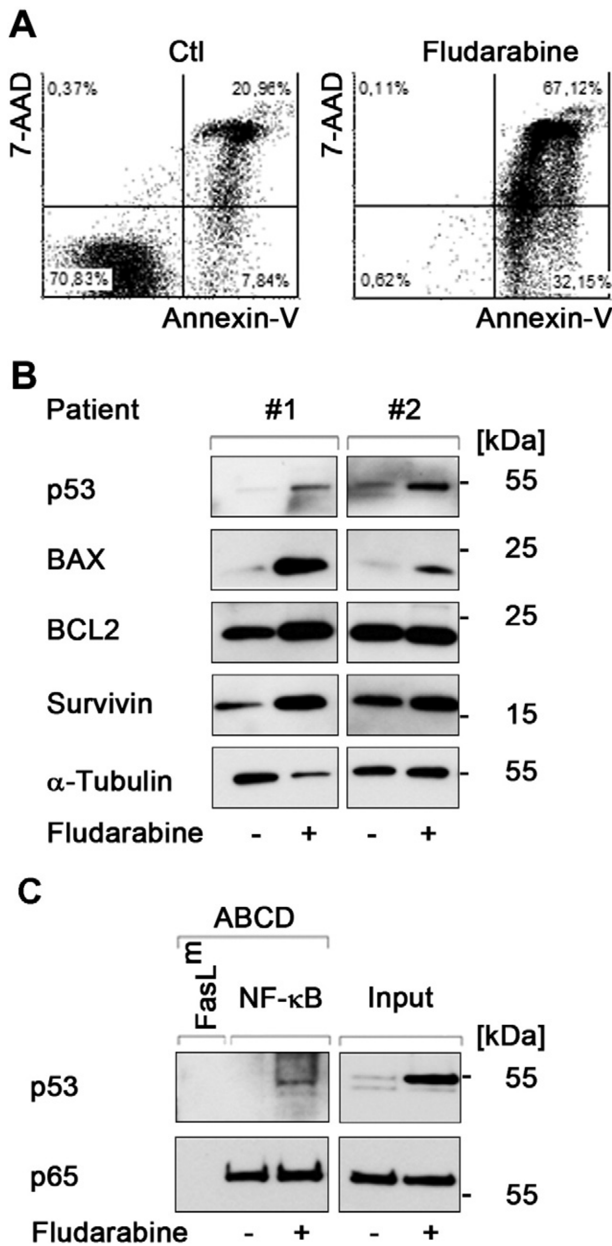

Fig. 2. Fludarabine activates p53 and NF-κB and their association on DNA in primary CLL cells (A) CLL cells were incubated with 5 μM fludarabine for 48 h and apoptosis was measured by flow cytometry assessing Annexin-V and 7-AAD positivity. (B) P53-positive CLL cells from two patients were treated with 5 μM fludarabine for 48 h (+; −, untreated control cells). Lysates were analyzed by Western blot for p53, BAX, and NF-κB-dependent factors BCL2 and Survivin; α-Tubulin was used as loading control; kDa, kilo Dalton. (C) Lysates described in (B) were subjected to ABCD assay with an NF-κB consensus oligonucleotide; mutated *FasL* oligonucleotide serves as negative control.

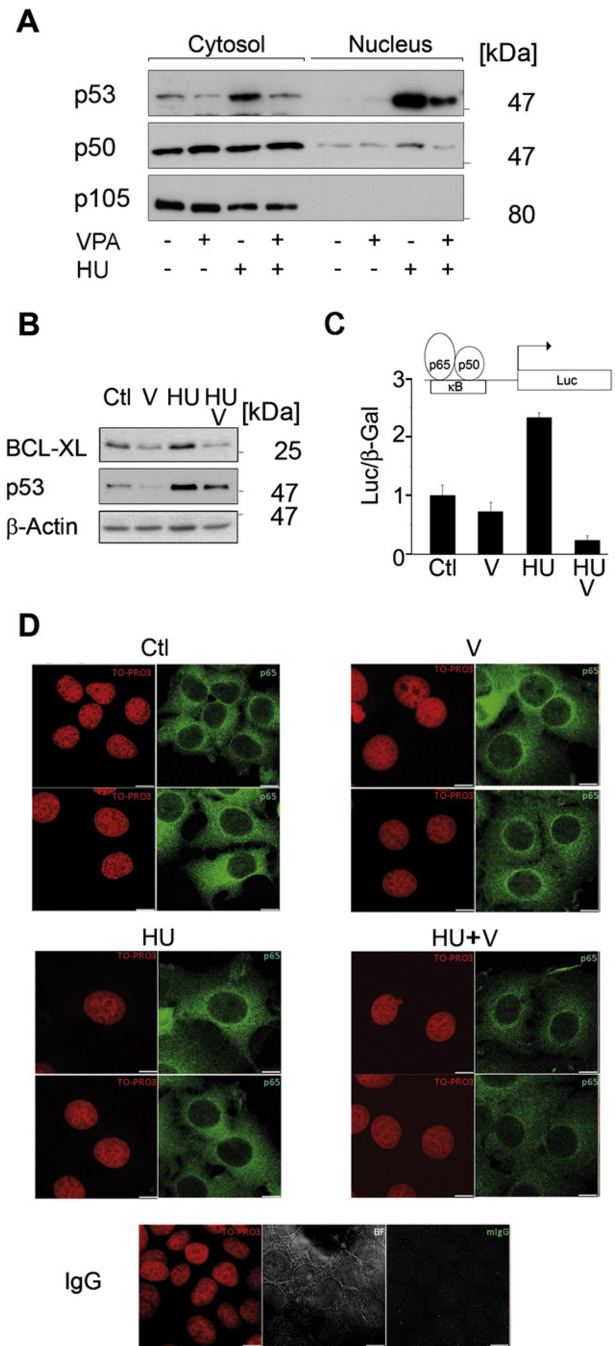

Fig. 3. HDACi attenuate p53 and NF-κB-dependent gene expression (A) Cytosolic (Cyt) and nuclear (Nuc) fractions of untreated (−) and valproic acid/hydroxyurea-treated (+; 3 mM VPA/1 mM HU for 24 h) HCT116 cells were analyzed by Western blot for p53 and the NF-κB subunits p105 and p50. The strict localization of p105 in the cytoplasm verifies the clear separation of cytoplasm from nucleus. (B) Western blot was used to detect the expression levels of BCL-XL, p53, and β-Actin (loading control) of MCF7 cells treated as stated in (A) (Ctl, untreated). (C) An NF-κB reporter was transfected into MCF7 cells. 24 h later, VPA and HU were added as stated in (A) for another 24 h. Luciferase (Luc) activity was normalized to β-Galactosidase (β-Gal) activity. (D) Immunofluorescence analysis of MCF7 cells.

Considering that HDACi inhibit NF-κB via acetylation of STAT1 [39], VPA may mediate the depletion of p53 via this mechanism. When we treated STAT1 null U3A cells with hydroxyurea and VPA, we noted that VPA also decreased p53 in these cells. Hence, HDACi likely attenuate p53 levels independent of STAT1 (Supplemental Fig. S2).

Next, we asked whether the reduction of p53 in response to VPA is a general observation. We tested cells from B cell malignancies and various solid tumors (Supplemental Table 1). The class I HDACi VPA [22, 40] attenuated the levels of p53 in all these cell lines (Supplemental Fig. S3A). Our panel of cells included SK-Mel-37 cells, which carry $p53^{P72}$ (data not shown), a wild-type p53 polymorph that interacts more avidly with NF-κB than $p53^{R72}$ [12]. Various HDACi also decreased p53 in these cells (Supplemental Fig. S3B), but we obtained no evidence for any accelerated proteasomal degradation of p53 in response to HDACi (Supplemental Fig. S3C).

These data confirm that class I HDACs support p53 expression.

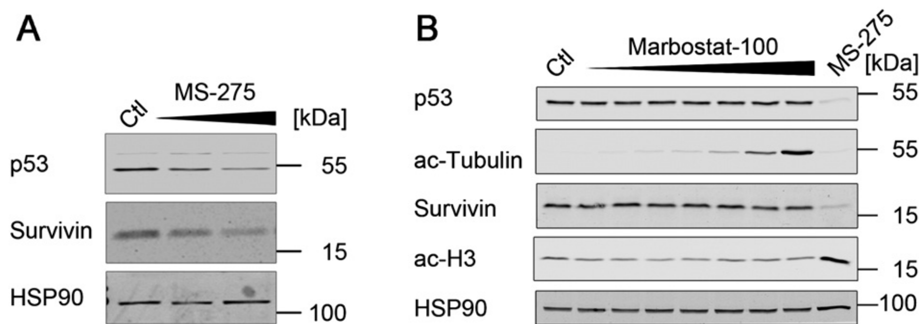

Fig. 4. Class I HDACs regulate mutant p53 (A) 5436 cells were treated with 2–5 μM MS-275 for 24 h. Levels of $p53^{R172H}$, Survivin, and β-Actin were analyzed by Western blot. (B) 5436 cells were treated with increasing concentrations of Marbostat-100 (5 nM, 20 nM, 50 nM, 100 nM, 200 nM, 500 nM, 1 μM) or 5 μM MS-275 for 48 h. Levels of $p53^{R172H}$, Survivin, α-Tubulin, and β-Actin were analyzed by Western blot. HDAC6 inhibition was verified by accumulation of acetylated Tubulin. The effectiveness of MS-275 was confirmed by the acetylation of histone H3.

3.4. Class I HDACi modulate the p53/NF-κB crosstalk

So far, our data illustrate that HDACi attenuate p53. However, we cannot exclude that the reduced levels of p53 in hydroxyurea and/or HDACi-treated cells are due to cell death; especially the combination of hydroxyurea with HDACi is highly cytotoxic [24,25,41]. To circumvent this problem, we used MCF7 breast cancer cells, for which we previously found that a therapeutically achievable dose of hydroxyurea stalled up to 70% of these cells in S phase without notable cell death [25]. Furthermore, combinations of hydroxyurea/VPA induced only about 10% killing in these caspase-3 negative cells [9,25].

Congruent with our previous data ([9] and Fig. 1), hydroxyurea increased the expression of p53 and BCL-XL in MCF7 cells. The addition of therapeutically relevant concentrations of the HDACi VPA [42] reduced BCL-XL and p53 levels and suppressed the induction of these proteins by hydroxyurea (Fig. 3B). Correspondingly, hydroxyurea induced and VPA repressed an NF-κB-dependent reporter in MCF7 cells (Fig.3C).

As anticipated [9] and consistent with the lack of effect of hydroxyurea on the phosphorylation of p65 (Supplemental Fig. S1), hydroxyurea did not significantly alter the intracellular distribution of NF-κB p65 in vivo (Fig. 3D).

We deduce that the reduction of p53 and NF-κB-dependent target genes by HDACi affects nuclear signaling.

3.5. NF-κB target gene expression and mutant p53 rely on I HDAC activity irrespective of HDAC6

Mutant $p53^{R172H}$ promotes cancer cell growth and survival through its positive effect on NF-κB-regulated survival genes [9]. We recently reported that MS-275, which is selective for HDAC1-3 [40], reduces the mRNA and protein expression of $p53^{R172H}$ in the murine pancreatic ductal adenocarcinoma (PDAC) cell line 5436 (homozygous for $p53^{R172H}$; Supplemental Table 1) [19]. 5436 cells are therefore a well-suited model to test if HDACi attenuate the $p53^{R172H}$-dependent activation of NF-κB.

As mutant p53 controls the p65-dependent expression of Survivin in 5436 cells [9], we assessed the impact of MS-275 on Survivin. The levels of Survivin decreased concomitant with the reduction of $p53^{R172H}$ (Fig. 4A), displaying an almost complete loss of both proteins after 48 h (Fig. 4B). As a control, we included the HDAC6-specific agent Marbostat-100, which produced no effect on mutant p53 as recently observed [19]. We verified the effective inhibition of HDAC6 by Western blot for acetylated Tubulin (Fig. 4B), which is a very reliable and appreciated marker for HDAC6 inhibition [16,21]. Related to its failure to reduce mutant p53, Marbostat-100 did not affect the levels of Survivin, even after 48 h (Fig. 4B).

As wild-type p53 is a negative regulator of Survivin in resting cells [4], we investigated if the reduction of wild-type p53 in HCT116 cells (Fig. 4A) may be linked to an undesired accumulation of Survivin. Analyzing the expression of Survivin in HDACi-treated HCT116 cells, we found that these agents attenuated the levels of Survivin (Supplemental Fig. S3). We considered that this reduction is associated with the reduction of X-linked inhibitor of apoptosis (XIAP), being another NF-κB target gene and interaction partner of Survivin [43–46], and with XAF1, being a negative regulator of Survivin [47]. Indeed, we noted a concomitant reduction of XIAP in HDACi-treated HCT116 cells (Supplemental Fig. S3). However, this process did not require an increase in XAF1, whose promoter remained highly methylated in the presence of MS-275 (Supplemental Table 2). Methylation of the *XAF1* promoter restricts its mRNA and protein expression (Reich and colleagues, submitted).

These data suggest that HDACi against class I HDACs target the mutant p53/NF-κB axis via a decreased expression of the oncogenic p53.

3.6. Class I HDAC activity prevents apoptosis and senescence of PDAC cells with mutant p53

Next, we analyzed whether MS-275 and Marbostat-100 could alter the morphology of 5436 cells. Flow cytometry analyses illustrated that the cell size as well as cellular granularity increased following treatment with MS-275 (Fig. 5A). Inspection of these cells by light microscopy confirmed that MS-275, but not Marbostat-100, increased cell size (Fig. 5B). Such morphological alterations are typically seen in senescent cells [48, 49], and they are accompanied by the induction of cell death (Fig. 5C). As for its lack of effect on mutant p53 and Survivin, Marbostat-100 has no effect on cell morphology and does not promote cell death in 5436 cell cultures (Fig. 5B–C). With the MTT-assay, we could confirm that MS-275 decreased the vitality of 5436 cells (Fig. 5D).

To confirm and extend these data, we stained 5436 cells for SA-β-galactosidase, which is a marker for senescence. Unfortunately, these cells stain positive for SA-β-galactosidase even in the untreated state (data not shown). We therefore used human p53 mutant PaTu8988t pancreatic carcinoma cells, in which MS-275 treatment strongly

Fig. 5. Class I HDACs regulate cell fate (A) Flow cytometry analyses of 5436 cells treated with 5 μM MS-275 or 1 μM Marbostat-100 for 24–72 h (as indicated). Representative dot blots are shown. (B) 5436 cells were exposed to 5 μM MS-275 or 1 μM Marbostat-100 for 72 h and analyzed by phase contrast microscopy. (C) 5436 cells were treated with Marbostat-100 (concentrations indicated in (B)) or 5 μM MS-275 for 72 h (Ctl, untreated cells). Cell death was measured by PI-staining and flow cytometry. Cells in subG1 fraction have DNA contents below 2 N; n = 2. (D) 5436 cells were treated with MS-275 (4 μM; 48 h) or were left as vehicle treated controls (DMSO, equal amount as MS-275). Viability was determined by MTT-assay. (E) SA-β-Gal activity and phenotypic changes of PaTu8988t cells treated for 72 h with MS-275 (4 μM) or vehicle (DMSO). Senescence-associated β-galactosidase staining (bright field) and DAPI fluorescence were merged. Pictures were taken with a monochromatic camera; arrow points to senescence-associated staining. (F) Model for the effects of HDACi on the crosstalk between wild-type p53 and NF-κB and mutant p53 and NF-κB.

attenuates the levels of p53 [19]. MS-275 evoked an accumulation of β-galactosidase in PaTu8988t cells (Fig. 5E), which supports that class I HDACi can induce senescence in pancreatic cancer cells.

These data show that HDACi reduce p53, Survivin, and XIAP, and that such processes are associated with growth arrest, apoptosis, and senescence in pancreatic cancer cells.

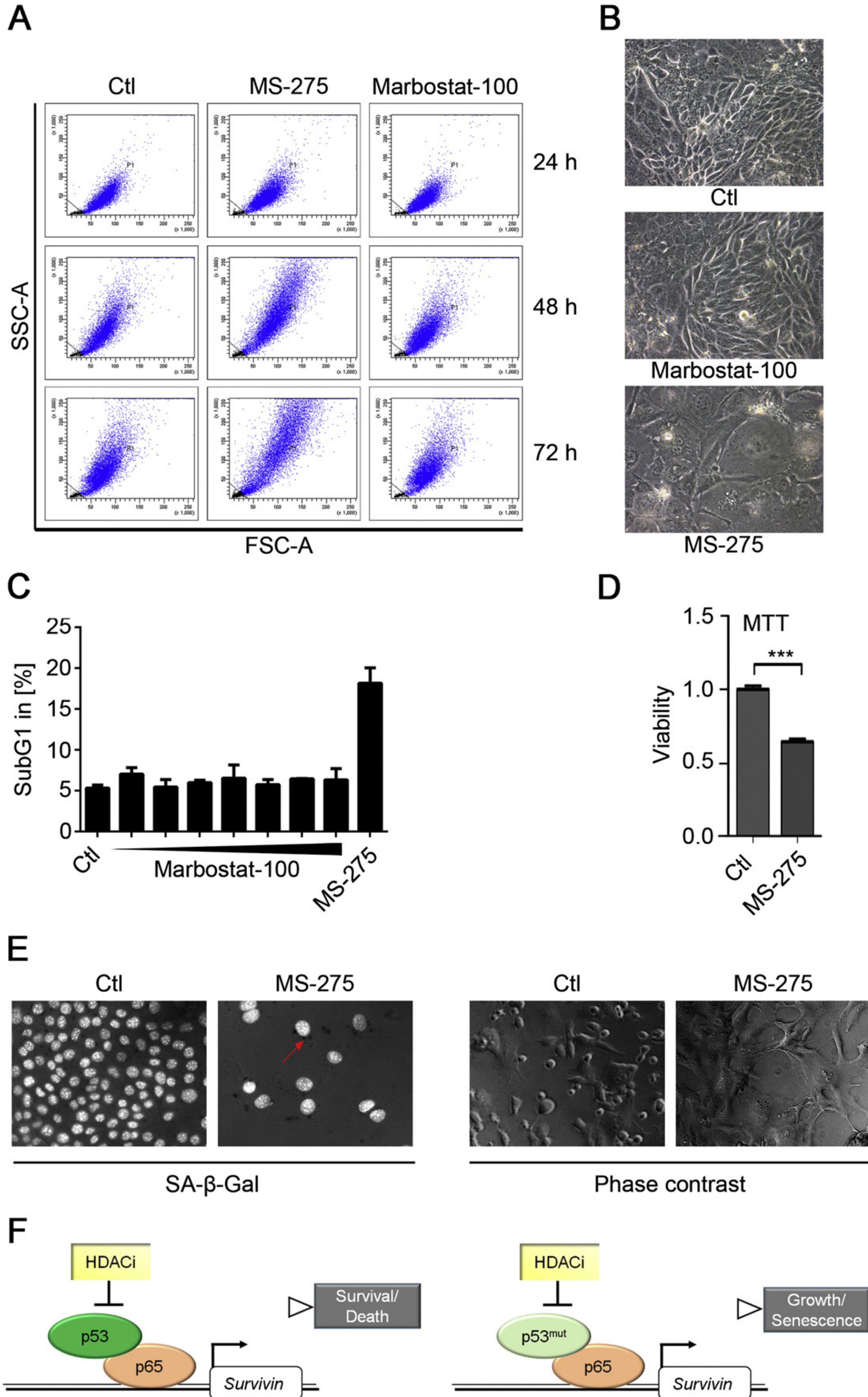

Fig. 5F shows a model that summarizes the key findings of this article.

4. Discussion

Data presented in this work demonstrate that class I HDACi attenuate p53 and NF-κB signaling in S phase, a time point when cycling cells depend on NF-κB [14,50,51]. These findings could explain why combinations of S phase toxins with HDACi cooperatively kill solid and hematopoietic cancer cells in vitro and in vivo [14,41,51]. On the other hand, our results show that HDACi reduce the levels of wild-type p53. However, the HDACi-mediated reduction of p53 does not disagree with the reported pro-apoptotic effects of HDACi, as they can induce apoptosis in various cancer cell types independently of p53 [24, 33,52–54].

We also analyzed SK-Mel-37 cells, which carry $p53^{P72}$, a polymorphism that affects interactions of p53 with NF-κB [12]. Moreover, SK-Mel-37 cells carry the $B\text{-}RAF^{V600E}$ mutation (https://cansar.icr.ac.uk/cansar/cell-lines/SK-MEL-37/mutations/), which can promote NF-κB activity in melanoma [55]. Further studies will assess the relevance of $p53^{P72}$ and $B\text{-}RAF^{V600E}$ for the good responsiveness of SK-Mel-37 cells to HDACi [39]. Additional experiments are also necessary to answer, if a p53/NF-κB crosstalk occurs in untreated tumor cells due to their high proliferation rates [56].

We recently observed that class I HDACi attenuated mutant p53 in pancreatic cancer cells and detected that HDAC1/HDAC2 maintained the expression of the p53 mutants [19]. We confirm these findings in the current study. Using the novel tool Marbostat-100, which is a highly selective inhibitor of HDAC6, we find that HDAC6 inhibition does not affect mutant p53 [19] and its positive effect on Survivin. These data differ from a previous report that showed a regulation of mutant p53 by the HDAC6-HSP90 axis [35]. Our findings rather agree with a reported regulation of wild-type and mutant p53 by class I HDACs on the mRNA and/or protein levels [19,28,33,37]. We show that the transcription factor MYC together with HDAC1 and HDAC2 promotes the expression of mutant p53 in PDAC cells [19]. Others report that HDAC8 controls the p53 promoter via HOXA5 in keratinocytes, colorectal and pancreatic cancer cells [37] or through YY1 in breast cancer cells [28]. It is plausible that different contexts with incomparable expression patterns or PTMs of these transcription factors and of HDACs cause a differential regulation of mutant p53 by different HDACs. Irrespective thereof the elimination of p53 may help to circumvent the selection of p53 mutant clones in tumors exposed to chemotherapy [41,57].

Earlier works show an intimate crosstalk between p53 and NF-κB and p53 appears to act as a licensing factor for NF-κB in stressed cells [4,9,11,12]. Consistent with a function of $p53^{R172H}$ to maintain the expression of the NF-κB target Survivin in 5436 cells, we observed a reduction of Survivin in HDACi-treated 5436 cells. Nonetheless, the attenuation of p53 by HDACi does not evoke an undesired activation of the p53-repressed expression of Survivin. In contrast, the levels of Survivin and its interaction partner XIAP decrease in the presence of HDACi. Since XIAP and Survivin impair caspase activation and apoptosis [43–46], their reduction by HDACi likely contributes to pro-apoptotic effects of HDACi.

The observations we report here may also have an impact on further systems and models. For example, in chronic inflammation, cytokines and mutant p53 promote tumorigenesis via NF-κB activation [4,11]. Thus, anti-inflammatory effects of HDACi might rely on an inhibition of the p53/NF-κB crosstalk. Attenuation of p53 may also contribute to the teratogenic potential of HDACi. Studies with p53 knock-out mice revealed that p53 null embryos exhibited a significantly increased rate of neural tube closure defects [22,58] and teratogenic effects occurred in human embryos of mothers treated with VPA and other HDACi [22,58]. These undesired effects might be linked to a reduction of p53.

In our cells, HDACi induce either cell death or senescence. Considering the current literature [48,49,59], we cannot rule out that the induction of senescence in cancer-derived cells with mutant p53 is pro-tumorigenic. Since a genetic reduction as well as a pharmacological inhibition of BCL-XL and BCL-W induces apoptosis of senescent cells [59], the reduction of NF-κB signaling by MS-275 may contribute to the elimination of cells under replicative stress. Further analyses are necessary to test this hypothesis. Despite such limitations, our data suggest that the signaling between NF-κB and p53 represents a molecularly defined therapeutic target for intervention strategies including HDACi.

The following are the supplementary data related to this article.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.cellsig.2016.11.002.

Conflict of interest

The authors declare no conflict of interest.

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