Chapter

Regulation of HDACi–Triggered Autophagy by the Tumor Suppressor Protein p53

Maria Mrakovcic and Leopold F. Fröhlich

Abstract

Cancer is a complex genetic and epigenetic-based disease that has developed a multitude of mechanisms in evading cell death. Deregulation of apoptosis and autophagy are commonly encountered during the development of human tumors. Histone deacetylase inhibitors (HDACi) have been employed to reverse epigenetically deregulated gene expression caused by aberrant post-translational protein modifications. These interfere with histone acetyltransferase- and deacetylase-mediated acetylation of histone and non-histone proteins, and thereby exert a wide array of HDACi-stimulated cytotoxic effects. Key determinants of HDACi lethality that interfere with cellular growth in a multitude of tumor cells are apoptosis and autophagy. Currently, the factors that determine the mode of HDACi-elicited cell death are mostly unclear however. Experimental evidence of the last decade convincingly reports that the frequently mutated tumor suppressor protein p53 can act either as an activator or as an inhibitor of autophagy depending on its subcellular localization, and linked to its mode of action. Consistently, we recently described p53 as a regulatory switch that governs if histone deacetylase inhibitor-administered uterine sarcoma cells undergo autophagy or apoptosis. By highlighting this novel finding, we summarize in this chapter the role of p53-mediated signaling during the activation of the autophagic pathway in tumor cells in response to HDACi.

Keywords: p53, HDACi, autophagy, apoptosis, tumor

1. Introduction

Evading cell death has been defined as a cornerstone of cancer development [1, 2]. Exploring the pathogenetic mechanisms that determine different cell death modes therefore facilitated the avenue for increased specifically directed interference with these molecular pathways. Morphologically, apoptosis, autophagy, and necrosis could be distinguished as major categories of programmed cell death very early that either act mutually exclusive or in combination involving cross talk for the elimination of tumor cells. While apoptosis and necrosis lead to inevitable cellular demise, autophagy can have either a cytotoxic or a cytoprotective function [3]. Basal autophagy in normal eukaryotic cells provide a possibility to save energy and reuse damaged or aged macromolecules or organelles by redirecting them toward lysosomal degradation via so-called autophagosomes and can be triggered by nutrient-starving conditions [4, 5]. Thus, in early phases of tumorigenesis, autophagy obviously presumes a cytoprotective or pro-survival role by suppressing necrosis and inflammation with

concomitant disruption of necrotic and apoptotic cell death induction [6, 7]. In later stages of irreversible tumor development however, autophagy may promote cell death by largely non-elucidated mechanisms that expedite the "self-degradation" program [8]. Disruption of autophagy in the latter case, which was recently enforced as novel strategy in chemotherapeutic cancer treatment, will advance the survival of tumor cells. This insight stresses the significance to confirm the context-reliant function of autophagy before initiating cancer therapy involving autophagic intervention [9, 10].

2. The molecular mechanism of autophagy

During macro-autophagy the formation of autophagosomes, representing double enveloped vesicles that enable the engulfment of targeted long-lived molecules and other cellular complexes from the remaining cytosol, are accomplished [11]. The following fusion with lysosomes containing proteolytic enzymes, i.e., the formation of autophagolysosomes, allows final degradation and reprocessing of their content [12]. The formation of the autophagosome is initiated by the phagopore assembly site (PAS) where autophagy-related (ATG) proteins are then recruited [13]. Particularly, the substantial work of the Nobel Prize laureate Ohsumi of ATG proteins in yeast has expedited our knowledge about the formation of the autophagosomes [14]. So far, 20 ATG proteins have been uncovered in mammalians that are activated during formation, enlargement, and closure of the autophagosome in a specific order. The process of autophagy has been categorized into several steps involving the ATG1/ULK kinase complex (initiation), the ATG12 conjugation system (nucleation), the ATG8/LC3 conjugation/deconjugation system (elongation), the phosphatidyl-inositol 3-kinase complex (maturation), and the ATG9/ATG9L1 cycling system (degradation).
Either tumor suppressor proteins or oncogenes resulting in activation or suppression, respectively, have been determined to control the process of autophagy [15]. Consistently, key regulators that participate in the initial phase of autophagosome formation are the nutrient-sensing serine/threonine kinase mammalian target of rapamycin (mTOR), the unc-51 like autophagy activating kinases (ULK1/ULK2), the Beclin-1 (BECN1) lipid kinase complex, and the ubiquitin-like conjugation system (**Figure 1**) [16–19]. As an overall major player, the mTOR multiprotein complex (mTORC) functions, comparable to p53, as a sensor for multiple kinds of stress signals which are of genotoxic and oxidative nature, particularly represented by reactive oxygen species (ROS), and nutrient levels such as energy, amino acids, glucose, or growth factors [19, 20]. The integration of these signals by mTOR beside autophagy also serves for the regulation of various other cellular functions such as translation, cell cycle, microtubule organization, or lipid biogenesis [21]. The mTOR complex subsequently inhibits the ATG13-ULK-FIP200 complex, consisting of ATG13, ULK1 (ATG1), and the focal adhesion kinase interacting protein of 200 kD (FIP200), which is necessary to initiate phagopore formation [22–24]; frequently, nutrient starvation-induced autophagy involves the formation of this complex. Further reports however, also noticed mTOR-mediated downregulation of the p53 family member p73 entailing the transcriptional activation of ATG5, ATG7, and UVRAG genes [25, 26]. Together with the ATG13-ULK-FIP200 complex, mTOR binds to the haplo-insufficient tumor suppressor protein Beclin-1 (ATG6) that organizes the phagopore formation and subsequently elongation and maturation of the autophagosome in a concerted action with various interacting proteins [27, 28]. For this purpose, Beclin-1 forms the Vps34 core complex consisting of Vps15 and class III phosphatidylinositol 3-kinase (PIKC3) that enables the generation of phosphatidylinositol 3-phosphate (PI3P) [28, 29]. Due to death associated protein kinase (DAPK)-mediated phosphorylation, Beclin-1 is not only controlling

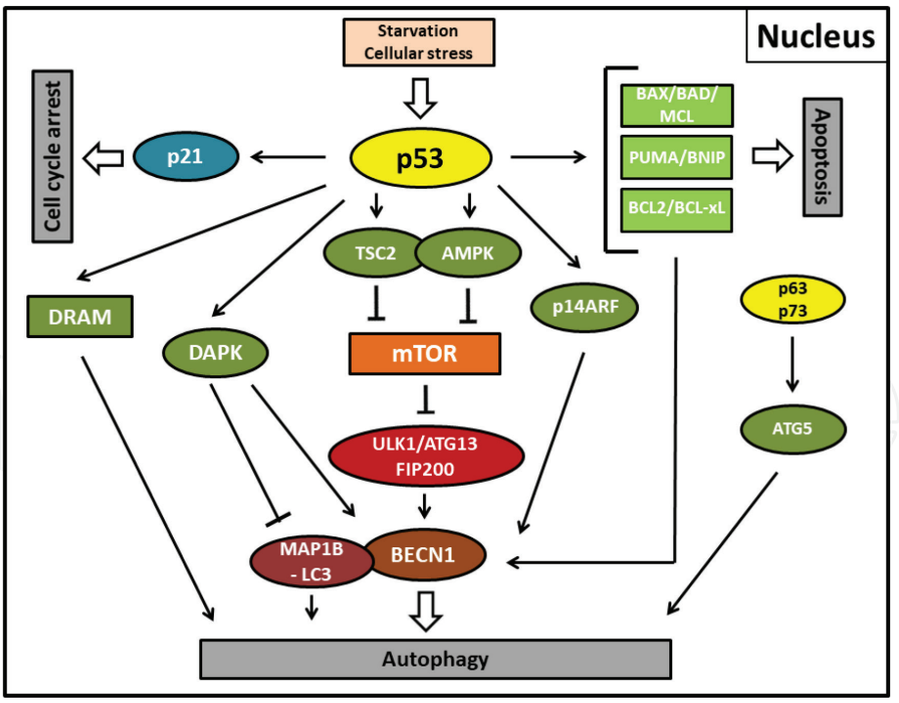

Figure 1.
Nuclear p53-mediated transcription-dependent autophagy, apoptosis and cell cycle arrest in response to stress conditions. By upregulation of tuberous sclerosis complex 2 (TSC2) or phosphatase and tensin homolog (PTEN; not shown), or AMP-activated protein kinase (AMPK) or its activators sestrins (not shown) p53 prevailingly attenuates mammalian target of rapamycin (mTOR) and the unc-51 like autophagy activating kinase 1 (ULK1) complex (consisting of autophagy-related gene 13 (ATG13) and the focal adhesion kinase interacting protein of 200 kD (FIP200)) as the autophagic canonical pathway. ULK-1 then interacts with Beclin-1 (BECN1) to initiate autophagosome formation. A shortcut for activation of autophagy involves damage-regulated autophagy modulator (DRAM), death associated protein kinase (DAPK), or autophagy-related gene 5 (ATG5) upregulation by the p53-family members, p63 and p73, or disruption of BCL2-family-or alternate reading frame protein product of the CDKN2A locus (p14ARF)-mediated release of BECN1 inhibition. In addition to autophagy, DRAM and p63/p73 are able to activate apoptosis. Arrowlines, upregulation or activation by indicated proteins; double arrow, major pathway activity. p53-mediated upregulation of the cyclin-dependent kinase inhibitor 1 (p21) enforces cell-cycle arrest. This figure is used under the terms and conditions of the creative commons attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/) derived from Mrakovcic and Fröhlich [57].

autophagy but found as a general regulator of lysosomes and endosome formation during membrane trafficking [30]. Further maturation of autophagosomes involves the interaction of the PI3P-binding proteins WIPI 1/2 with ATG12-ATG5-ATG16L and LC3-phosphatidylethanolamine (LC3-PE) complexes, both representing ubiquitin-like conjugation systems [4, 18, 31]. LC3 (microtubule-associated protein 1A/1B-light chain 3) and p62/Sequestosome-1 are two markers that are regularly employed for documenting autophagic flux as they are involved in the maturation of autophagosomes [32]. While LC3-I is processed to LC3-II, the scaffold protein p62 interacts with LC3 via its LC3-binding motif and seems to have a role in selectively guiding ubiquitinated proteins toward the autophagosome via its ubiquitin-binding domain [33]; thus, p62 levels decease during the induction of autophagy and have been found moreover to regulate protein deacetylation and is associated with tumorigenesis [34–36]. Autophagosome-lysosome fusion that requires the trans-membrane protein LAMP2 and small Rab GTPases, finally permits hydrolase and cathepsin-mediated processing of the autophagosome content [37, 38].

2.1 Positive regulation of p53-mediated autophagy

The tumor suppressor protein and transcription factor p53, which represents a "guardian" of the cell, has a fundamental role in the regulation of cell integrity

and homeostasis and consequently in tumor defense. It coordinates cellular responses such as cell cycle arrest, apoptosis, senescence, metabolism, differentiation, angiogenesis, and even modulates autophagy. Among a multitude of other post-translational modifications, acetylation assists the master regulator to sense and integrate a variety of endogenous and exogenous cellular stress signals such as DNA damage, epigenetic alterations due to DNA methylation, genotoxicity, hypoxia, oxidative stress, or oncogene activation [39, 40]. In response, p53, as a central transcription factor translocates to the nucleus by detaching from the E3 ubiquitin ligase, mouse double minute 2 homolog (MDM2), and modulates the expression of multiple downstream target genes that regulate processes such as cell cycle progression and cell death [41, 42]. In appropriate conditions, p53 induces apoptosis by transactivating, i.e., transcriptional activation of pro-apoptotic genes or in the cytoplasm by direct interaction with anti-apoptotic proteins located in the mitochondrial membrane [43].

Several signaling pathways involving the transactivational activity of p53—in normal as well as cancer cells regulate autophagy in the classical canonical mTOR pathway as specified in the previous chapter (**Figure 1**) [44]. As pro-autophagic factors emanating from p53, these pathways involve on the one hand the tumor suppressor proteins tuberous sclerosis complex 2 (TSC2) and phosphatase and tensin homolog (PTEN), and on the other hand the nutrient energy sensor AMP-activated protein kinase (AMPK) or its activators sestrins 1 and 2 [45–47]. A further path that bypasses mTOR and can directly modulate p53 stress-activated signal transduction, is damage-regulated autophagy modulator (DRAM) that can activate the autophagic as well as apoptotic program [48]. As a protein located in the lysosome, it can intervene at different steps of autophagosome formation [49]. Furthermore, by either upregulating pro-apoptotic protein expression (BAX, BAD, BNIP3, or PUMA) or downregulating anti-apoptotic protein expression (BCL-2, BCL-xL and MCL-1) of the B-cell Lymphoma-2 (BCL-2) family, p53 can enforce dual activation of autophagy and apoptosis [50, 51]. In the inactivated state these proteins directly interact with the BH3 domain of BECLIN-1 and block the direct activation of BECLIN-1-dependent autophagy [52, 53]. Direct interaction of the nuclear full-length form of p53-modulated tumor suppressor protein p14ARF (an alternate reading frame protein of the CDKN2A locus) with the BCL-xL protein is a further similar mechanism promoting the induction of autophagy although the predominant role of p14ARF seems to stabilize p53 to protect the cell against hyperproliferative growth and associated activation of oncogenes [54, 55]. Additionally, p53-elicited upregulation of DAPK has been reported to result in autophagic activation either by DAPK-mediated phosphorylation of Beclin-1 that blocks its degradation by BCL-2/BCL-xL, or by impeding the anti-autophagic LC3-interacting MAP1B protein [30, 56].

2.2 Negative regulation of p53-mediated autophagy

Beyond the nuclear-based transactivating pro-autophagic effects mediated by p53, additional inhibitory anti-autophagic responses related to cytoplasm-localized p53 protein have been uncovered by Tasdemir et al. in the past 10 years (**Figure 2**) [58]. While the transactivation-dependent nuclear autophagic response of p53 is stimulated by stress induction, the cytoplasmic blockage of autophagic induction is a steady-state function that is present under physiological conditions and seems to engage direct protein interaction. This cytoplasm-mediated inhibition of autophagy was also characterized to activate the canonical p53-AMPK-mTOR signaling cascade. In contrast to transcription-dependent pathway, however, the positive autophagic regulator AMP-dependent kinase is inhibited by p53 which

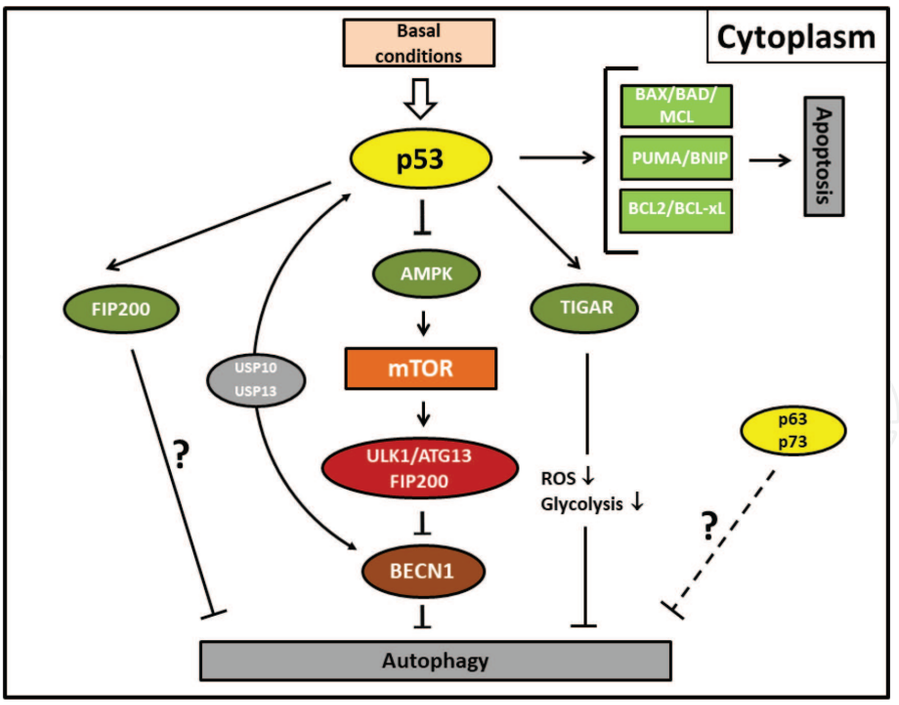

Figure 2.
Cytoplasmic p53-mediated transcription-independent autophagy (under physiological conditions) and apoptosis. By direct inhibition, wildtype p53-protein inactivates AMPK-mTOR-ULK1 transduced autophagy leading to BECN1 degradation. Similarly, BECN1 degradation can also be directly mediated by the ubiquitin-specific peptidases USP10 and USP13. Further inhibitory functions for autophagy can be mediated by TP53-induced glycolysis and apoptosis regulator (TIGAR) following the down-regulation of glycolysis and the suppression of reactive oxygen species (ROS) formation. Also, p63/p73 has been reported to presumably exert transcription-independent disruption of autophagy (dashed line); fork symbols, inhibition; arrowlines, activation by indicated proteins; downward arrow, downregulation. For abbreviations, see **Figure 1**. *This figure is used under the terms and conditions of the creative commons attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/) derived from Mrakovcic and Fröhlich [57].*

in turn activates mTOR [59]. Accordingly, either pharmacological interference, depletion of basal p53 levels, or p53 variants that possess a genetically modified nuclear export domain rendered cells more resistant toward metabolic stress through elevated autophagy. The underlying obscure mechanism that is found not only in mammalians but also in nematodes could involve direct binding of p53 to FIP200 (ATG17) as experimentally evidenced [60]. Negative regulation of autophagy by cytoplasmic p53 has been also linked to its target gene TIGAR (TP53-induced glycolysis and apoptosis regulator) that suppresses glycolysis and ROS generation when the cell is exerted to stress [61]. Nevertheless, although TIGAR mediates ROS-mediated induction of autophagy, it is not a likely candidate for the above described mechanism but rather represents an alternative path since it does not inhibit mTOR obviously. A similar but also unclear anti-autophagic mechanism could be verified in embryonal carcinoma cells, where p53-Beclin-1 interaction facilitated Beclin-1 ubiquitination and subsequent degradation, which could be de-activated by depletion of p53 [62]. Another report identified Beclin-1 as a regulator of de-ubiquitination of p53 which was mediated by USP10 and USP13 ubiquitin-specific peptidases [63]. This mechanism might therefore also relate to the previously mentioned Beclin-1-induced autophagy which enables bidirectional dual activation of apoptosis and autophagy [64].

2.3 Regulation of autophagy in p53-inactivated cells

p53 is one of the most frequently inactivated tumor suppressor genes in human tumors [65]. Particularly, single point mutations that provoke a loss of p53 function

were documented that in several cases apply a dominant-negative effect to the remaining non-mutant allele thereby enhancing their oncogenic effect [41, 66, 67]. Often, such p53 variants result in increased genomic instability, attenuated chemotherapeutic success and a poor prognosis for patients [68]. One of the underlying reasons therefore could be that many tumor-derived p53 variants also inactivate cytoprotective or cytotoxic autophagy [69–71]. Nevertheless, although nucleus-based transcription-dependent autophagy might be shut down in these cases, cytoplasm-induced activation of the autophagic program might still be available, due to p53-deficiency or functional inactivation. Interestingly, the studies of Morselli et al. demonstrated that several tumor-derived mutants of p53 that reside in the cytoplasm are still able to block autophagic induction, presumably by direct protein interaction [70]. Such experiments underline the significance why it is meaningful to discriminate p53 mutant variants with regard to their potential effects. This finding also highlights the role of context-dependent autophagy during tumorigenesis as disabled autophagy by mutant p53 was found to prolong tumor cell survival while it inactivated its tumor suppressor function. Thus, increased proliferation of pancreas and breast cancer cells could be uncovered in a report that confirmed inhibition of autophagy by mutant gain-of-function p53 proteins. This counteractivity was evidenced by stimulation of AMPK-mTOR genes with concomitant downregulation of Beclin-1, DRAM, ATG12, and sestrin genes [72].

Various investigations found also a counter-acting surveillance mechanism between autophagy and since as mutant p53 blocks autophagic induction in one way but autophagy can stimulate the sequestering of mutant p53 in order to suppress tumorigenesis in the other way. Mechanistically, this mutual crosstalk is translated by the regulatory actions of the two suppressor genes Beclin-1 and p53 on autophagy as specified in the previous chapter [63]. While p53 exerts control on Beclin-1 via the canonical autophagic pathway, Beclin-1 also directly regulates p53 via controlling its deubiquitination activity which explains the mirrored effect on the phenotype of p53- and Beclin-1 ablated mice [73]. Additionally, with respect to Beclin-1 mediated autophagy, further reports documented the possibility of autophagic activation via the tumor suppressor protein p14ARF in p53-silenced or -inhibited cells [74]. Studies using doxorubicin-treated p53-wildtype or -deficient (p53−/−) mouse embryonic fibroblasts furthermore verified that the p53 family members p63 and p73 can substitute the loss of p53 (**Figure 2**) [75]; this mechanism involved nucleus translocation of p63/p73 and the increased expression of an extensive network of ATG proteins, such as ATG4a, ATG4c, ULK1, ULK2, UVRAG, and ATG5. This finding might explain resistance in doxorubicin-mediated chemotherapeutic treatment of cancer tissues.

3. Histone deacetylases and histone deacetylase inhibitors

Histone acetylation by the families of histone acetylases (HATs) histone deacetylases (HDACs) are crucial epigenetic elements in the regulation of gene transcription of histone as well as non-histone proteins. HATs catalyze acetylation to lysine residues of proteins, which stimulates a relaxed transcriptionally accessible chromatin configuration, while HDACs facilitate their removal associated with a closed transcriptionally inaccessible chromatin structure [76, 77]. Acetylation of histones and non-histones not only interferes with gene expression but crucially governs cell signaling and cellular processes such as proliferation, differentiation, and programmed cell death [78]. Identified non-histone substrates to date are tumor suppressor proteins (e.g., p53, RUNX3), signaling mediators (e.g., STAT3, β-catenin, Smad7), steroid receptors (e.g., androgen, estrogen, SHP),

transcriptional factors, and co-regulators (e.g., c-Myc, HMG, YY1, EKLF, E2F1, GATA factors, HIF-1α, MyoD, NF-κB, and FoxB3), as well as structural (e.g., cell motility proteins), chaperone proteins, and nuclear import proteins (e.g., α-tubulin, importin-α, Ku70, HSP90) [79].

Depending on function or structure, four classes (class I–IV) have been allocated that comprise 18 members of the HDAC family [80]. The "classical HDACs" contain classes I and II and are functionally dependent on zinc as co-factor, while class III HDACs include the sirtuin proteins (Sirt1-7; homology to yeast Sir2) and require NAD+ [81]. Nevertheless, HDACs also differ in subcellular localization, and expression pattern [78]. While class I HDACs are expressed ubiquitously as they are located in the cell nucleus providing them with superior enzymatic activity, class II HDACs possess restricted tissue-specific expression pattern. Thus, they have been subdivided into class IIa HDACs (HDAC4, 5, 7 and 9) which shuttle between nucleus and cytoplasm as well as class IIb HDACs (HDAC 6 and 10) that are located mostly in the cytoplasm [82]. SIRTs exhibit specific subcellular presence in the nucleus (Sirt1, 6 and 7), in the cytoplasm (Sirt2), or in mitochondria (Sirt3, 4 and 5) which is not interchangeable. HDAC11, the single less-well explored member of class IV HDACs, has narrowed tissue expression [83].

HDAC inhibitors (HDACi) have been explored as a new category of anticancer drugs that reverses epigenetic changes established by the deregulated activities of HDACs in hematological as well as solid cancers [84]. HDACi treatment induces transcriptional de-repression of genes that are eminent regulators of tumor cell activities such as cell cycle arrest, differentiation, and programmed cell death and even the expression and stability of oncoproteins [85]. HDACi categories encompass hydroxamic acids (hydroxamates), cyclic tetrapeptides, benzamides, electrophilic ketones, and aliphatic acids that include natural but also synthetic derivatives that exhibit different structures [84]. Favored representatives of the hydroxamates are SAHA (suberoylanilide hydroxamic acid, vorinostat, and Zolinza) which is a preferred derivative of naturally occurring trichostatin A (TSA) as well as the CBHA (m-carboxycinnamic acid bishydroxamate)-derived tubacin, LAQ-824 (dacinostat), LBH-589 (panobinostat), or PXD-101 (belinostat) [86–89]. The class I-selective FK-228 (romidepsin, FR901228, istodax) belongs to the group of cyclic tetrapeptides [90]. MS-275 (entinostat) and MGCD0103 (mocetinostat) exhibiting enhanced HDAC class I selectivity are members of benzamide-based HDACi [91, 92]. The minor effective class I- and IIa-specific HDACi, VPA (valproic acid), PBA (phenylbutyrate), NaB (sodium butyrate), or AN-9 (pivaloyloxymethyl butyrate) belong to the category of aliphatic acids [93, 94]. This classification mainly depends on the chemical structure of their zinc-binding group but in addition HDACi can also be subdivided into zinc-dependent, pan- or broad-spectrum inhibitors that inhibit all class I, II and IV HDACs in contrast to primarily class I-specific HDACi [95]. Representatives of pan-inhibitors are TSA, SAHA, LBH589, and PXD-101 while valproic acid and butyrate inhibit exclusively class I HDACs. MS-275 and depsipeptide inhibits only a few members of class I HDACs, respectively. To date, the HDAC6-specific inhibitor tubacin is the only representative of an isoform-specific HDACi [86]. With the exception of nicotinamide, no clinical useful SIRT inhibitors have been uncovered yet [96].

Clinical trials of single or combined treatments of several HDACi with diverse results have been or are in the progress of being tested in hematological and solid cancers (www.clinicaltrials.gov) [97, 98]. Up to now, exclusively the evaluation of pan-inhibitors have succeeded in the admittance of four licensed HDACi, namely, SAHA, panobinostat (LBH589), belinostat (PXD-101), and romidepsin (FK228) for the treatment of cutaneous T cell lymphoma, multiple myeloma, or peripheral T cell lymphoma, respectively [99–103]. Although preclinical studies using single

treatment regimen of many HDACi were encouraging, almost all entities of solid tumors (e.g., ovarian, breast, renal, prostate, and head and neck cancer) lacked positive effects in phase II clinical trials [104, 105]. In addition, patients suffered from trivial (e.g., dehydration, anorexia, diarrhea) to toxic (e.g., cardiotoxicity, thrombocytopenia myelosuppression) non-selective side effects [85, 94, 106]. The reasons for these drawbacks are presently non-elucidated and were assumed to be due to a combination of failing blood vessel supply, endogenous molecular heterogeneity owing to epigenetic modifications, and the development of treatment resistance. In response, selective HDAC-specific inhibitors are being developed, that target only one or two isozymes [107]. The design of novel or improved specific inhibitors will allow the full exploration of individual functions of distinct HDAC activity and may furthermore provide improved therapeutic efficacy together with less toxicity.

4. Mechanisms of histone deacetylase inhibitor-induced cell death

Owing to the various posttranslational histone and non-histone protein acetylation targets, HDACi exert a multitude of anti-tumor effects that concern interference with growth, differentiation, migration, senescence, and death [108]. Although there may be tumor cell-type and HDACi-specific effects which are unclear presently, common mechanisms are shaping for different HDACi [85, 108]. Induction of apoptosis is by far the prevailing avenue of HDACi triggered cell death in transformed cells which is prepared by re-induction of cell cycle arrest and induction cell differentiation, e.g., by the downregulation of positive cell growth regulators [109–112]. G1 or G2 phase induced cell cycle arrest in the G1 or G2 phase can occur in a p53-dependent or -independent manner by stimulating the upregulation of (p21waf-1/cip1) expression which is a cyclin-dependent kinase (CDKN) inhibitor of cyclins D1/D2 [113–115]. As an underlying mechanism it is assumed that the inability to exit the cell cycle from unfinished mitosis might sensitize the activation of apoptosis due to compiled DNA damage such as double-strand breaks [116, 117]. HDACi-stimulated activation of the intrinsic (mitochondrial) pathway of apoptosis involves either down-regulation of anti-apoptotic genes (e.g., BCL-2, BCL-XL, XIAP, MCL-1, and survivin) or overexpression of pro-apoptotic genes (e.g., BAX, BAK) belonging to the B-cell Lymphoma-2 (BCL-2) family [113, 114, 118, 119]. In the extrinsic (death-receptor) pathway, HDACi predominantly re-establish the expression of death receptors such as DR4 and DR5, or their corresponding ligands (e.g., TRAIL, FAS, FAS-L, and TNF-alpha) [120–122]. Furthermore, also the induction of reactive oxygen species (ROS) by HDACi is a second important anticancer-mechanism that is also responsible for cell death induction and associated with DNA damage; presumably, ROS is scavenged in normal, but not in malignant cells due to the compiled expression of thioredoxin (TXN) which represents an endogenous cellular antioxidant [123]. HDACs have moreover been detected to control histone deacetylation at damaged DNA sites undergoing repair that involves DNA damage-related response proteins [95, 124–132]. Thus, only in tumor cells upregulated expression of a marker for DNA double strand breaks, H2AX, was detected, when these were treated with SAHA [117]. In this context also the induction of autophagy as a means to maintain genomic was noticed, for instance, following MSH2-regulated DNA mismatch repair deregulation upon HDAC6 inhibition. Cell signaling pathways that were shut down in cancer cells can be furthermore re-established by immediately modifying acetylation of non-histone proteins such as transcription factors (e.g., NF-κB, p53, and STATs) [79]. As a prominent example, half-life and stability of p53 was influenced by MDM2 E3-ligase in HDACi-treated H1299 carcinoma cells [42]. In this way also

chaperone protein function and the regulation of stress response pathways in the endoplasmic reticulum can be achieved, which affects the removal of misfolded proteins but also interference with stability and expression of oncoproteins [111, 133]. Additional mechanisms of HDACi-regulated lethality in tumor cells were evidenced in the interference with migration- and invasion capability due to re-established expression of metastasis-related genes and in the disruption of angiogenesis by altering pro- and anti-angiogenic gene expression [134–136]. In recent years, autophagy as a form of programmed cell death was added to the list of further determinants of HDACi-mediated effects that impedes cellular growth in a range of tumor cells [137–140].

5. Mechanisms of HDACi-induced autophagic cell death

An impressive diversity of mechanisms have been uncovered in cancer cells that promote HDACi-elicited autophagy which include mostly attenuation of mTOR signaling that can occur in combination combined with increased expression of LC3, Beclin-1, or ATG and can be provoked by endoplasmic reticulum stress (reviewed in [57] and **Table 1** [141]). mTOR is a well-known regulator of the canonical pathway of autophagy involving the regulation of the ULK1 complex and Beclin-1. The pivotal role of mTOR attenuated by SAHA-treatment which reestablishes ULK1 function could be initially verified by our own studies and those of Gammoh et al. using endometrial sarcoma cells, and were subsequently reiterated in many studies [71, 142]. It should be noticed that HDACi-induced autophagy is frequently accompanied by the additional induction of apoptosis.

Further predominant mechanisms of autophagic induction involve ROS accumulation, p21 upregulation, NF-κB hyperacetylation, and sirtuin-mediated acetylation of p53 [143–146]. In addition to mTOR downregulation, substantial intracellular ROS production interfering with mitochondrial function and energy metabolism has been demonstrated to facilitate SAHA-induced autophagy in tumor cells. ROS-induced autophagy can go along with additional increased expression of cathepsin D, a lysosomal protease, or decreased expression of TRX, representing its substrate and/or activation of the mitogen activated protein kinases ERK1/2 and JNK [143, 147]. Generally, enzymes related to energy metabolism, anti-oxidative stress and cellular redox control have been entangled by a proteomic study involving SAHA-administered Jurkat T-leukemia cells [147]. Cell cycle arrest, differentiation, and autophagy due to upregulated p21 expression were caused by treatment of PC-3 M and HL-60 cells with HDACi SAHA and H40 [148]. The same mechanism could be elicited by adding the novel HDACi, MRJF4, to prostate cancer cells autophagy [144]. As a further cause of SAHA/MS-275-induced autophagy in PC3 prostate cancer cells, re-activation of NF-κB associated target genes due to hyperacetylation of NF-κB RELA/p65, or downsizing of pERK/NF-κB signaling together with upregulated p21 expression, were described; however, the exact mechanism remains obscure [146].

Individual studies also noted nuclear translocation of the apoptosis inducing factor (AIF), apoptosome inactivation, FoxO1-stimulated expression, upregulation of DAPK or Nrf2, and p53-deficiency as regulatory mechanisms in HDACi-induced autophagy [71, 146, 149–153]. Thus, apoptosis, necrosis or autophagy were triggered in malignant rhabdoid tumor cells in response to FK228 (depsipeptide) treatment and upon silencing of the apoptosis inducing factor (AIF) that translocates into the nucleus for caspase-induced death, autophagy was suppressed as supported by transmission electron microscopy and LC3 measurements [149]. Following the blockage of the mitochondrial pathway of apoptosis by deleting caspase-9 or Apaf-1

Molecular mechanism	Additional mechanism	HDACi	Cell type	Ref.
mTOR inhibition	Increased p21 expression	SAHA	ESS-1	[156]
	Increase of LC3 expression; activation of ULK-1 complex	SAHA	MEFs, T98G glioblastoma	[142]
	Beclin-1 upregulation	SAHA, butyrate	HelaS3	[157]
	Induction of ER stress response	SAHA, OSU-HDAC42	HCC, Hep3B, HepG2	[158]
	ROS accumulation via Cat D, repression of TRX; BECN1 and ATG-7 upregulation.	SAHA	Jurkat T-leukemia	[147]
	BECN1 protein upregulation. and p62 downregulation	SAHA	Glioblastoma stem cells	[159]
	CASP and CPN-1 activation; reduced ATG expression*	MGCD0103	Primary CLL	[160]
	Increased ATG5 expression	Apicidin	Salivary MEC	[161]
ROS accumulation	CathD upregulation and TRX repression	SAHA	K562, LAMA 84 CMLL	[143]
	Activation of MAPK proteins: ERK1/2 and JNK; LC3 and ATG12 upregulation	FK228+ bortezomib	Gastric carcinoma (GC)	[162]
	p38 MAPK switch to apoptosis; ERK activation	M-275	HCT116	[163]
p21 $^{CIP/WAF1}$ upregulation	–	SAHA, H40	PC-3 M, HL-60	[148]
	Downregulation of pERK/ NF-κB signaling	MRJF4	PC3	[144]
NF-κB Hyper-acetylation	Induction of NF-κB target genes	SAHA, MS-275	PC3	[146]
AIF nucleus translocation	–	FK228	MRT	[149]
Apoptosome inactivation	Independent of p53; Deletion of Apaf-1/Casp-9	LAQ824, LBH589	Eμ-myc lymphomas	[152]
FoxO1 transcription	ATG expression; mTOR suppression via SESN3	SAHA, TSA	HepG2, HCT116	[150]
DAPK upregulation	–	LBH589	HCT116	[151]
Nrf2 upregulation	mTOR suppression via miR-129-3p	–	–	[153]
ATG7 acetylation*	Autophagy interactome acetylation; increased mitochondrial mass and ROS formation	SAHA, TSA, LBH589, JQ2	Megakaryoblastic leukemia	[154]
ATG gene upregulation*	Independent of p53 acetylation	Tenovin-6	CLL	[155]

Molecular mechanism	Additional mechanism	HDACi	Cell type	Ref.
p53 acetylation	Increased p53-dependent cell cycle arrest and apoptosis	Sirtinol	MCF-7	[145]
	p53 activation. by reducing MDM2 expression; cell cycle arrest and apodosis	MHY2256	MCF-7	[164]
p53-deficiency	mTOR inhibition	SAHA	ESS-1	[165]

**Leads to inhibition of autophagy; AVO (acidic vesicular organelles); (–), unknown or not determined. This modified Table is used under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/) from Mrakovcic et al. [141].*

Table 1.
Mechanisms of HDACi-induced autophagic cell death.

or in Eμ-lymphomas the autophagic pathway was activated by the HDACi LAQ824 and LBH589 as evidenced morphologically and biochemically [152]. SAHA and TSA-induced autophagic cell survival via the transcription factor FoxO1 in HepG2 and HCT116 cells was furthermore mediated by sestrin 3 (SESN3)-induced mTOR inhibition and increased ATG protein expression [150]. Protein interaction or phosphorylation of the MAPK-interacting calcium- or calmodulin-regulated DAPK at serine 308 in HCT116 colon cancer cells, rather than its enzymatic function, moreover stimulated LBH589-induced autophagy [151]. Recently, even microRNA-mediated regulation of mTOR involving the transcription factor Nrf2 (nuclear factor erythroid 2 like-2) was implicated in HDACi-induced autophagy [153]. HDACi-induced Nrf2 mRNA and protein expression thereby promoted augmented transcription of miR-129-3p which facilitated mTOR attenuation. Nonetheless, even HDACi-mediated suppression of autophagy could be documented in two studies. Negative regulation of HDACi-mediated autophagy but upregulation of autophagic flux could be induced in myeloid-leukemic cells treated with valproic acid, SAHA, TSA, panobinostat, or JQ2 by acetylation and decreased expression of ATG7, a protein important for fusion of peroxisomal and vacuolar membranes [154]. Additionally, increased ATG expression following treatment with sirtuin inhibitor tenovin-6 provoked autophagic suppression in chronic lymphocytic leukemia (CLL) cells which was evident by upregulated genes of the autophagic-lysosomal pathway and LC3-II/p62 [154, 155].

6. HDACi-induced autophagy mediated by p53

p53 as the first described representative subjected non-histone protein acetylation can in response to stress positively as well as negatively regulate cell cycle arrest, senescence as well as apoptosis and autophagy [39, 40]. Acetylated residues attached by distinct HAT-mediated acetylation can be detected for p53 at distinct sites that could not only affect DNA binding and thereby its transactivational ability but also coactivator recruitment and/or its stability via proteasomal degradation [166–168]. For example, HDAC1-specific inhibition allows p53 to stay in an accessible state associated with transcriptional activity [169]. Furthermore, by mutating a combination of C-terminal sites that undergo acetylation p53-dependent transcription of p21 can be eliminated [170]. Nevertheless, the exact modalities of these mechanisms still need clarification. As previously specified, HDACi-mediated

apoptosis that is commonly escorted by p21-mediated cell cycle arrest and ROS generation, has been documented as the most frequently encountered form of HDACi-triggered cell death [112, 119]. However, since transcription of pro-apoptotic genes, such as Bax, Noxa, and Puma, by p53 may be limited by posttranslational acetylation, the role of p53 in this relation is discussed. These assumptions are supported for example by the finding that p53-independent p21 induction and apoptosis upon HDACi administration and the anticancer effect of HDACi is not influenced by the mutational status of p53 in the tumor [109, 113]. Other reports in contrast verified p53 acetylation and stabilization in several tumor models in response to HDACi administration that presented cell cycle arrest and apoptosis [111, 171]. Conclusively, p53-dependent but also -independent signaling pathways may add to HDACi-mediated apoptotic processes and HDACi may induce p53, but do not unconditionally require p53 for providing anticancer effects. In recent time, the range of HDACi-exerted mechanisms resulting in cellular demise of cancer cells have been expanded by the induction of autophagic cell death which can alternatively or additionally to apoptosis activate autophagy (reviewed in [57, 141, 172] and **Table 1**). Also, involvement of posttranslational modification of the non-histone p53 has been linked to the control of HDACi-stimulated autophagy as evident from its key regulatory role in normal cells. This might of crucial advantage if tumor cells have developed resistance toward apoptotic cell death induction. Thus, experimental evidence from our studies of endometrial sarcoma (ESS) cells support a major regulatory function for p53 in directing cell death either toward HDACi-elicited apoptosis or autophagy [165].

In our model, the detection of HDAC2 overexpression in malignant endometrial stroma sarcoma cell lines led to the establishment of therapeutic SAHA treatment and the evaluation of its mechanism of action [173]. Significantly advanced cell death in MES-SA and ESS-1 cells was accompanied by previous p21-induced cell cycle arrest at the G1/S transition and reduced expression of HDAC2 and 7 [156, 173]. Either predominant caspase-dependent apoptotic (48%) or caspase-independent autophagic cell death (80%) was attested in SAHA-treated MES-SA and ESS cells after 24 h, respectively [156]. In line with the induction of the canonical pathway of autophagy, attenuated mTOR protein expression could be evidenced in ESS-1 cells in contrast to MES-SA cells [165]. Further screening of key regulatory molecules for apoptosis and for autophagy, upstream of mTOR, were performed to explain the differences in the modes of SAHA-induced cell death. This search uncovered entire absence of detectable p53 protein and lowered levels of PUMA protein in ESS-1 cells. Investigation of p53 gene and mRNA led to the detection of a novel nonsense mutation ($p53^{R213X}$) in the transactivating domain of p53 of ESS-1 cells that obviously provoked a degradation of the entire p53 transcript and could not be documented in MES-SA cells. Consistent with this finding, restoration of ESS-1 cells with a wild-type p53 variant restored induction of caspase-dependent apoptosis as supported by PUMA and caspase-9 upregulation as well as activation of the effector caspases-3 and -7 and final PARP-1 cleavage. Increased mTOR levels demonstrated the re-induction of basal autophagic flux in addition to apoptosis induction as verified by LC3 staining. Generalization of this finding could be obtained by several other p53-deficient tumor cell lines (such as PANC-1, Jurkat, HL-60, and U937) that are known to induce autophagy in response to SAHA and were supplied with wild-type p53.

We concluded that the molecular switch between SAHA-induced apoptosis and autophagy was thus mediated by the occurrence of functional p53 protein. Our experimental evidence thus underlines an overall major regulatory role for p53 not only in HDACi-mediated apoptosis but also in HDACi-stimulated autophagy (**Figure 3**) (reviewed in [57, 141]). As a consequence, p53-deficiency

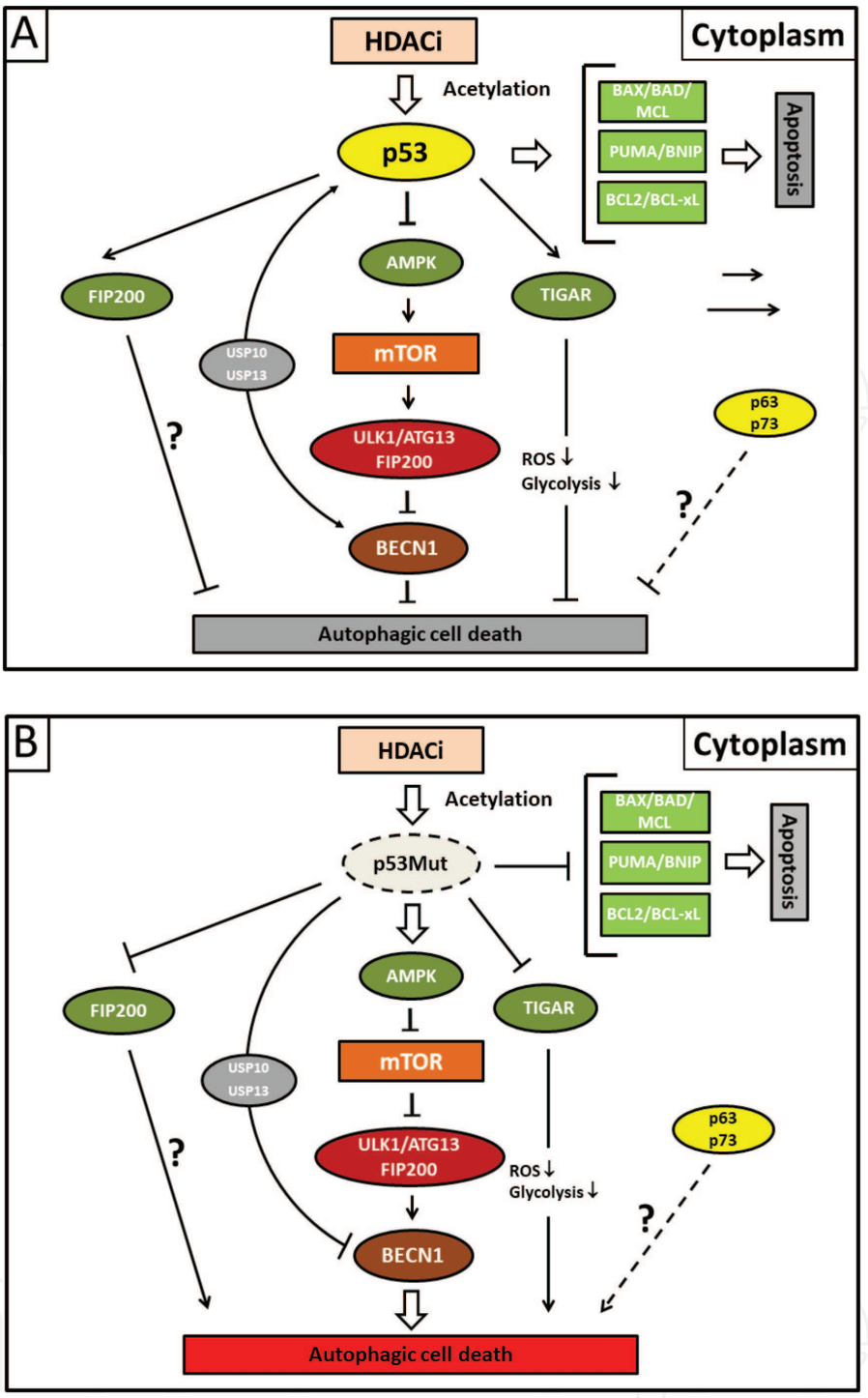

Figure 3.
Illustration depicting presumed mechanisms mediating SAHA-induced autophagy reflecting autophagic regulation by cytoplasmic mutant and wild-type p53. (A) Acetylated cytoplasmic p53 protein predominantly activates apoptotic cell death by direct binding to the BCL-2 family of pro-apoptotic proteins. Concomitantly, cytoplasmic p53 protein inhibits autophagic cell death by inducing Beclin-1 degradation via USP10/USP13 and/or inhibiting the AMPK-mTOR-ULK1 signaling pathway. It is unclear whether the canonical pathway is mediated by direct p53-FIP200 interaction or whether this represents an extra pathway. TIGAR inhibits autophagy by down-regulation of glycolysis and a suppression of ROS formation. The members of the p53 family, p63/p73, are also potential inhibitors of autophagy (dashed line). (B) Mutant p53 protein variants lose the ability of autophagic inhibition and apoptosis stimulation and activate autophagy. Fork symbols, inhibition; arrowlines, activation or interaction; double arrow, major pathway activity. This figure is used under the terms and conditions of the creative commons attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/) derived from Mrakovcic and Fröhlich [57].

could moreover explain apoptosis resistance as well predominant induction of HDACi-provoked autophagy in cancer cells. The presumptive negative regulation of autophagy by functional cytoplasmic p53 protein in SAHA-treated ESS-1 cells is

moreover very consistent with the above discussed role of p53 as a dual regulator of autophagy by Tasdemir et al. [59]. Their findings convincingly describe nuclear p53 protein as an activator of transcription-dependent autophagy, in contrast to the inhibitory autophagic control by cytoplasmic p53 protein. In addition, by our report we link SAHA-induced acetylation of p53 to the mTOR signaling pathway which has been less evaluated to date, nonetheless, future experiments are needed to directly address this question.

In line with our experiments, the class III Sirt1 and 2-specific HDACi sirtinol, that affects acetylation of p53, has also been documented to determine HDACi-induced cell fate in several reports. For instance, p53 was entangled in balancing sirtinol-mediated apoptosis and autophagy in MCF-7 breast cancer cells [145]. Sirtinol treatment on the one hand preferentially induced predominant autophagy as shown by LC3-II upregulation, while addition of the autophagic inhibitor 3-methyladenine augmented cell cycle arrest and cytochrome C-triggered apoptotic cell death caused by increased BAX and diminished BCL-2 protein expression. In a similar experiment, inhibition by sirtinol and the novel SIRT1, -2, and -3 protein inhibitor, MHY2256, a similar phenotype that included cell cycle arrest and both types of programmed cell death could be provoked [164]. As a mechanistic explanation, SIRT1 and 2-induced acetylation of p53 at lysine 382 were found to inhibit ubiquitination of p53 via MDM2 which stabilized and increased its functional activity. MDM2-mediated degradation of p53 was also documented in MHY2256-treated Ishikawa cells that are derived from endometrial cancer which elicited activation of apoptosis together with autophagy as supported by elevated levels of p21, BAX and BCL-2, cytochrome C release, and cleaved PARP-1 [174].

7. Conclusions and perspectives

In recent times, epigenetic studies gained increasing significance in reports investigating the development of cancer. For this purpose, aberrant epigenetic patterns such as DNA methylation including the misguided expression of HDACs activity has been defined to some extent in many tumors which explains their selection as targets for anticancer therapy. Posttranslational modifications of histones and non-histones in the form of acetylation and deacetylation particularly enable pharmacological interference by different kind of inhibitors such as HDACi. The ability to sensitize apoptosis-resistant tumor cells by the disruption of autophagy was considered a promising route for cancer therapy as this process heightens the pro-apoptotic effects of HDACi. In addition to restrain the extents of tumor necrosis and inflammation however, autophagy might be required for the cancer cell to deal with metabolic stress and cytotoxicity during chemotherapy. Furthermore, by expediting the autophagic pathway in advanced stages of the cancer cell, autophagy may promote cell death by mostly non-elucidated mechanisms. Consistently, it is of pivotal importance to define the factors and mechanisms that influence the balance between HDACi-elicited apoptosis, autophagy or even necrosis in the cancer cell. In this regard, considerable research efforts are in progress to investigate the molecular pathways regulating HDACi-mediated cell death in tumor cells. The expansion of the knowledge about p53 as a mediator of apoptotic and autophagic cell death may as thus help to achieve progress not only in unraveling pathogenetic insights but also in the development of novel therapeutic strategies of such disease conditions as cancer.

Author details

Maria Mrakovcic[1,2] and Leopold F. Fröhlich[1*]

1 Department of Cranio-Maxillofacial Surgery, University of Münster, Germany

2 Department of Medical Microbiology, University of Münster, Germany

*Address all correspondence to: leopold.froehlich@ukmuenster.de

IntechOpen

References

[1] Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;**100**: 57-50

[2] Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Cell. 2011;**144**:646-674

[3] Mariño G, Niso-Santano M, Baehrecke EH, Kroemer G. Self consumption: The interplay between autophagy and apoptosis. Nature Reviews. Molecular Cell Biology. 2014;**15**:81-94

[4] Mizushima N, Noda T, Yoshimori T, Tanaka Y, Ishii T, George MD, et al. A protein conjugation system essential for autophagy. Nature. 1998;**39**:395-398

[5] Yang Z, Klionsky DJ. Mammalian autophagy: Core molecular machinery and signaling regulation. Current Opinion in Cell Biology. 2010;**22**:124-131

[6] Choi AM, Ryter SW, Levine B. Autophagy in human health and disease. The New England Journal of Medicine. 2013;**368**:651-662

[7] Mathew R, Karantza-Wadsworth V, White E. Role of autophagy in cancer. Nature Reviews. Cancer. 2007;**7**:961-967

[8] He C, Klionsky DJ. Regulation mechanisms and signaling pathways of autophagy. Annual Review of Genetics. 2009;**43**:67-93

[9] Lopez G, Torres K, Liu J, Hernandez B, Young E, Belousov R, et al. Autophagic survival in resistance to histone deacetylase inhibitors: Novel strategies to treat malignant peripheral nerve sheath tumors. Cancer Research. 2011;**71**:185-196

[10] Rosenfeldt MT, Ryan KM. The role of autophagy in tumour development and cancer therapy. Expert Reviews in Molecular Medicine. 2009;**11**:e36

[11] Bento CF, Renna M, Ghislat G, Puri C, Ashkenazi A, Vicinanza M, et al. Mammalian autophagy: How does it work? Annual Review of Biochemistry. 2016;**85**:685-713

[12] Reggiori F, Ungermann C. Autophagosome maturation and fusion. Journal of Molecular Biology. 2017;**429**:486-496

[13] Rubinsztein DC, Shpilka T, Elazar Z. Mechanisms of autophagosome biogenesis. Current Biology. 2012;**22**:R29-R34

[14] Mizushima N, Yoshimori T, Ohsumi Y. The role of Atg proteins in autophagosome formation. Annual Review of Cell and Developmental Biology. 2011;**27**:107-132

[15] Maiuri MC, Tasdemir E, Criollo A, Morselli E, Vicencio JM, Carnuccio R, et al. Control of autophagy by oncogenes and tumor suppressor genes. Cell Death and Differentiation. 2009;**16**:87-93

[16] Jung CH, Ro SH, Cao J, Otto NM, Kim DH. mTOR regulation of autophagy. FEBS Letters. 2010;**584**:1287-1295

[17] Liang XH, Jackson S, Seaman M, Brown K, Kempkes B, Hibshoosh H, et al. Induction of autophagy and inhibition of tumorigenesis by Beclin 1. Nature. 1999;**402**:672-676

[18] Mizushima N, Sugita H, Yoshimori T, Ohsumi Y. A new protein conjugation system in human. The counterpart of the yeast Apg12p conjugation system essential for autophagy. The Journal of Biological Chemistry. 1998;**273**:33889-33892

[19] Noda T. Regulation of autophagy through TORC1 and mTORC1. Biomolecules. 2017;**7**:52

[20] Kim D, Sarbassov D, Ali S, King J, Latek R, Erdjument-Bromage H, et al. mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. Cell. 2002;**110**:163-175

[21] Laplante M, Sabatini D. mTOR signaling. Cold Spring Harbor Perspectives in Biology. 2012;**4**:a011593

[22] Jung CH, Jun CB, Ro S, Kim Y, Otto NM, Cao J, et al. ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. Molecular Biology of the Cell. 2009;**20**:1992-2003

[23] Ganley IG, Lam DH, Wang J, Ding X, Chen S, Jiang X. ULK1.ATG13.FIP200 complex mediates mTOR signaling and is essential for autophagy. Journal of Biological Chemistry. 2009;**284**:12297-12305

[24] Kim J, Kundu M, Viollet B, Kun-Liang G. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nature Cell Biology. 2011;**13**:132-141

[25] Rosenbluth JM, Mays DJ, Pino MF, Tang LJ, Pietenpol JA. A gene signature-based approach identifies mTOR as a regulator of p73. Molecular and Cellular Biology. 2008;**28**:5951-5964

[26] Rosenbluth JM, Pietenpol JA. mTOR regulates autophagy-associated genes downstream of p73. Autophagy. 2009;**2**:1-7

[27] Maiuri M, Criollo A, Kroemer G. Crosstalk between apoptosis and autophagy within the Beclin 1 interactome. The EMBO Journal. 2010;**29**:515-516

[28] Liang XH, Jackson S, Seaman M, Brown K, Kempkes B, Hibshoosh H, et al. Induction of autophagy and inhibition of tumorigenesis by Beclin 1. Nature. 1999;**402**:1-5

[29] He C, Levine B. The Beclin 1 interactome. Current Opinion in Cell Biology. 2010;**22**:140-149

[30] Zalckvar E, Berissi H, Eisenstein MAK. Phosphorylation of Beclin 1 by DAP-kinase promotes autophagy by weakening its interactions with Bcl-2 and Bcl-XL. Autophagy. 2009;**5**:720-722

[31] Polson HE, de Lartigue J, Ridgen DJ, Reedijk M, Urbe S, Clague MJ, et al. Mammalian Atg18 (WIPI2) localizes to omegasome-anchored phagophores and positively regulates LC3 lipidation. Autophagy. 2010;**6**:506-522

[32] Bjørkøy G, Lamark T, Pankiv S, Øvervatn A, Brech ATJ. Monitoring autophagic degradation of p62/SQSTM1. Methods in Enzymology. 2009;**452**:181-197

[33] Seibenhener ML, Geetha T, Wooten MW. Sequestosome 1/p62—More than just a scaffold. FEBS Letters. 2007;**581**:175-179

[34] Mathew R, Karp CM, Beaudoin B, Vuong N, Chen G, Chen HY, et al. Autophagy suppresses tumorigenesis through elimination of p62. Cell. 2009;**137**:1062-1075

[35] Lahiri P, Schmidt V, Smole C, Kufferath I, Denk H, Strnad P, et al. P62/sequestosome-1 is indispensable for maturation and stabilization of Mallory-Denk bodies. PLoS One. 2016;**11**:e0161083

[36] Yang H, Ni HM, Guo F, Ding Y, Shi YH, Lahiri P, et al. Sequestosome 1/p62 protein is associated with autophagic removal of excess hepatic endoplasmic reticulum in mice. The Journal of Biological Chemistry. 2016;**29**:M116.739821

[37] Xie Z, Klionsky DJ. Autophagosome formation: Core machinery and adaptations. Nature Cell Biology. 2007;**9**:1102-1109

[38] Simonsen A, Tooze S. Coordination of membrane events during autophagy by multiple class III PI3-kinase complexes. The Journal of Cell Biology. 2009;**186**:773-782

[39] Vousden KH, Lane DP. p53 in health and disease. Nature Reviews. Molecular Cell Biology. 2007;**8**:275-283

[40] Gu W, Roeder RG. Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. Cell. 1997;**90**:595-606

[41] Vousden KH, Lu X. Live or let die: The cell's response to p53. Nature Reviews. Cancer. 2002;**2**:594-604

[42] Tang Y, Zhao W, Chen Y, Zhao Y, Gu W. Acetylation is indispensable for p53 activation. Cell. 2008;**133**:612-626

[43] Chipuk JE, Green DR. Dissecting p53-dependent apoptosis. Cell Death and Differentiation. 2006;**13**:994-1002

[44] Galluzzi L, Morselli E, Kepp O, Maiuri MC, Kroemer G. Defective autophagy control by the p53 rheostat in cancer. Cell Cycle. 2010;**9**:250-255

[45] Arico S, Petiot A, Bauvy C, Dubbelhuis PF, Meijer AJ, Codogno P, et al. The tumor suppressor PTEN positively regulates macroautophagy by inhibiting the phosphatidylinositol 3-kinase/protein kinase B pathway. The Journal of Biological Chemistry. 2001;**276**:35243-35246

[46] Feng Z. p53 regulation of the IGF-1/AKT/mTOR pathways and the endosomal compartment. Cold Spring Harbor Perspectives in Biology. 2010;**2**:a001057

[47] Budanov AV, Karin M. p53 target genes sestrin1 and sestrin2 connect genotoxic stress and mTOR signaling. Cell. 2008;**134**:451-460

[48] Crighton D, Wilkinson S, O'Prey J, Syed N, Smith P, Harrison PR, et al. DRAM, a p53-induced modulator of autophagy, is critical for apoptosis. Cell. 2006;**126**:121-134

[49] Crighton D, Wilkinson S, Ryan K. DRAM links autophagy to p53 and programmed cell death. Autophagy. 2007;**3**:72-74

[50] Maiuri M, Le Toumelin G, Criollo A, Rain J, Gautier F, Juin P, et al. Functional and physical interaction between Bcl-X(L) and a BH3-like domain in Beclin-1. The EMBO Journal. 2007;**26**:2527-2539

[51] Zalckvar E, Berissi H, Mizrachy L, Idelchuk Y, Koren I, Eisenstein M, et al. DAP-kinase mediated phosphorylation on the BH3 domain of beclin 1 promotes dissociation of beclin 1 from Bcl-XL and induction of autophagy. EMBO Reports. 2009;**10**:285-292

[52] Wang EY, Gang H, Aviv Y, Dhingra R, Margulets V, Kirshenbaum LA. p53 mediates autophagy and cell death by a mechanism contingent on Bnip3. Hypertension. 2013;**62**:70-77

[53] Pattingre S, Tassa A, Qu X, Garuti R, Liang X, Mizushima N, et al. Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. Cell. 2005;**122**:927-939

[54] Pimkina J, Humbey O, Zilfou J, Jarnik M, Murphy M. ARF induces autophagy by virtue of interaction with Bcl-xl. The Journal of Biological Chemistry. 2009;**284**:2803-2810

[55] Budina-Kolomets A, Hontz RD, Pimkina J, Murphy ME. A conserved domain in exon 2 coding for the human and murine ARF tumor suppressor protein is required for autophagy induction. Autophagy. 2013;**9**:1553-1565

[56] Harrison B, Kraus M, Burch L, Stevens C, Craig A, Gordon-Weeks

P, et al. DAPK-1 binding to a linear peptide motif in MAP1B stimulates autophagy and membrane blebbing. The Journal of Biological Chemistry. 2008;**283**:9999-10014

[57] Mrakovcic M, Fröhlich LF. p53-mediated molecular control of autophagy in tumor cells. Biomolecules. 2018;**8**:E14

[58] Tasdemir E, Chiara Maiuri M, Morselli E, Criollo A, D'Amelio M, Djavaheri-Mergny M, et al. A dual role of p53 in the control of autophagy. Autophagy. 2008;**4**:810-814

[59] Tasdemir E, Maiuri MC, Galluzzi L, Vitale I, Djavaheri-Mergny M, D'Amelio M, et al. Regulation of autophagy by cytoplasmic p53. Nature Cell Biology. 2008;**10**:676-687

[60] Morselli E, Shen S, Ruckenstuhl C, Bauer M, Mariño G, Galluzzi L, et al. p53 inhibits autophagy by interacting with the human ortholog of yeast Atg17, RB1CC1/FIP200. Cell Cycle. 2011;**10**:2763-2769

[61] Bensaad K, Cheung EC, Vousden KH. Modulation of intracellular ROS levels by TIGAR controls autophagy. The EMBO Journal. 2009;**28**:3015-3026

[62] Tripathi R, Ash D, Shaha C. Beclin-1—p53 interaction is crucial for cell fate determination in embryonal carcinoma cells. Journal of Cellular and Molecular Medicine. 2014;**18**:2275-2286

[63] Liu J, Xia H, Kim M, Xu L, Li Y, Zhang L, et al. Beclin1 controls the levels of p53 by regulating the deubiquitination activity of USP10 and USP13. Cell. 2011;**147**:223-234

[64] Yousefi S, Perozzo R, Schmid I, Ziemiecki A, Schaffner T, Scapozza L, et al. Calpain-mediated cleavage of Atg5 switches autophagy to apoptosis. Nature Cell Biology. 2006;**8**:1124-1132

[65] Muller PAJ, Vousden KH. P53 mutations in cancer. Nature Cell Biology. 2013;**15**:2-8

[66] Dittmer D, Pati S, Zambetti G, Chu S, Teresky AK, Moore M, et al. Gain of function mutations in p53. Nature Genetics. 1993;**4**:42-46

[67] Santoro R, Strano S, Blandino G. Transcriptional regulation by mutant p53 and oncogenesis. Sub-Cellular Biochemistry. 2014;**85**:91-103

[68] Liu J, Ma Q, Zhang M, Wang X, Zhang D, Li W, et al. Alterations of TP53 are associated with a poor outcome for patients with hepatocellular carcinoma: Evidence from a systematic review and metaanalysis. European Journal of Cancer. 2012;**48**:2328-2338

[69] Cordani M, Butera G, Pacchiana R, Donadelli M. Molecular interplay between mutant p53 proteins and autophagy in cancer cells. Biochimica et Biophysica Acta, Reviews on Cancer. 2017;**1867**:19-28

[70] Morselli E, Tasdemir E, Maiuri MC, Galluzzi L, Kepp O, Criollo A, et al. Mutant p53 protein localized in the cytoplasm inhibits autophagy. Cell Cycle. 2008;**7**:3056-3061

[71] Fröhlich LF, Mrakovcic M, Smole C, Zatloukal K. Molecular mechanism leading to SAHA-induced autophagy in tumor cells: Evidence for a p53-dependent pathway. Cancer Cell International. 2016;**16**:68

[72] Cordani M, Oppici E, Dando I, Butturini E, Dalla Pozza E, Nadal-Serrano M, et al. Mutant p53 proteins counteract autophagic mechanism sensitizing cancer cells to mTOR inhibition. Molecular Oncology. 2016;**10**:1008-1029

[73] Qu X, Yu J, Bhagat G, Furuya N, Hibshoosh H, Troxel A, et al. Promotion of tumorigenesis by

heterozygous disruption of the beclin 1 autophagy gene. The Journal of Clinical Investigation. 2003;**112**:1809-1820

[74] Abida WM, Gu W. p53-dependent and p53-independent activation of autophagy by ARF. Cancer Research. 2008;**68**:352-357

[75] Kenzelmann Broz D, Mello SS, Bieging KT, Jiang D, Dusek RL, Brady CA, et al. Global genomic profiling reveals an extensive p53-regulated autophagy program contributing to key p53 responses. Genes and Development. 2013;**27**:1016-1031

[76] Marmorstein R, Roth SY. Histone acetyltransferases: Function, structure, and catalysis. Current Opinion in Genetics and Development. 2001;**11**:155-161

[77] Gray SG, Ekstro TJ. The human histone deacetylase family. Experimental Cell Research. 2001;**262**:75-83

[78] de Ruijter AJ, van Gennip AH, Caron HN, Kemp S, van Kuilenburg AB. Histone deacetylases (HDACs): Characterization of the classical HDAC family. The Biochemical Journal. 2003;**370**:737-749

[79] Spange S, Wagner T, Heinzel T, Krämer OH. Acetylation of non-histone proteins modulates cellular signalling at multiple levels. The International Journal of Biochemistry and Cell Biology. 2009;**41**:185-198

[80] Yang XJ, Edward S. The Rpd3/Hda1 family of lysine deacetylases: From bacteria and yeast to mice and men. Nature Reviews. Molecular Cell Biology. 2009;**9**:206-218

[81] Haigis MC, Guarente LP. Mammalian sirtuins—Emerging roles in physiology, aging, and calorie restriction. Genes and Development. 2006;**20**:2913-2921

[82] Yang X, Gregoire S. Class II histone deacetylases: From sequence to function, regulation, and clinical implication. Molecular and Cellular Biology. 2005;**25**:2873-2884

[83] Gao L, Cueto MA, Asselbergs F, Atadja P. Cloning and functional characterization of HDAC11, a novel member of the human histone deacetylase family. The Journal of Biological Chemistry. 2002;**277**:25748-25755

[84] Dokmanovic M, Clarke C, Marks PA. Histone deacetylase inhibitors: Overview and perspectives. Molecular Cancer Research. 2007;**5**:981-989

[85] Eckschlager T, Plch J, Stiborova M, Hrabeta J. Histone deacetylase inhibitors as anticancer drugs. International Journal of Molecular Sciences. 2017;**18**:1414

[86] Haggarty SJ, Koeller KM, Wong JC, Grozinger CM, Schreiber SL. Domain-selective small-molecule inhibitor of histone deacetylase 6 (HDAC6)-mediated tubulin deacetylation. Proceedings of the National Academy of Sciences of the United States of America. 2003;**100**:4389-4394

[87] Richon V, Emiliani S, Verdin E, Webb Y, Breslow R, Rifkind RA, et al. A class of hybrid polar inducers of transformed cell differentiation inhibits histone deacetylases. Proceedings of the National Academy of Sciences of the United States of America. 1998;**95**:3003-3007

[88] Coffey DC, Kutko MC, Glick RD, Butler LM, Heller G, Rifkind RA, et al. The Histone deacetylase inhibitor, CBHA, inhibits growth of human neuroblastoma xenografts in vivo, alone and synergistically with all-trans retinoic acid. Cancer Research. 2001;**61**:3591-3594

[89] Plumb JA, Finn PW, Williams RJ, Bandara MJ, Romero MR, Watkins CJ, et al. Pharmacodynamic response

and inhibition of growth of human tumor xenografts by the novel histone deacetylase inhibitor PXD101. Molecular Cancer Therapeutics. 2003;**2**:721-728

[90] Furumai R, Matsuyama A, Kobashi N, Lee K, Nishiyama M, Nakajima H, et al. FK228 (depsipeptide) as a natural prodrug that inhibits class I histone deacetylases. Cancer Research. 2002;**62**:4916-4921

[91] Maggio SC, Rosato RR, Kramer LB, Dai Y, Rahmani M, Paik DS, et al. The histone deacetylase inhibitor MS-275 interacts synergistically with fludarabine to induce apoptosis in human leukemia cells. Cancer Research. 2004;**64**:2590-2600

[92] Boumber Y, Younes A, Garcia-Manero G. Mocetinostat (MGCD0103): A review of an isotype-specific histone deacetylase inhibitor. Expert Opinion on Investigational Drugs. 2011;**20**:823-829

[93] Minucci S, Zhu P, Kramer O, Schimpf A, Giavara S, Sleeman JP, et al. Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. The EMBO Journal. 2001;**20**:6969-6978

[94] Rasheed WK, Johnstone RW, Prince HM. Histone deacetylase inhibitors in cancer therapy. Expert Opinion on Investigational Drugs. 2017;**16**:659-678

[95] Li Y, Seto E. HDACs and HDAC inhibitors in cancer development and therapy. Cold Spring Harbor Perspectives in Medicine. 2016;**6**:a026831

[96] Avalos JL, Bever KM, Wolberger C. Mechanism of sirtuin inhibition by nicotinamide : Altering the NAD+ cosubstrate specificity of a Sir2 enzyme. Molecular Cell. 2005;**17**:855-868

[97] Wagner JM, Hackanson B, Lübbert M, Jung M. Histone deacetylase (HDAC) inhibitors in recent clinical trials for cancer therapy. Clinical Epigenetics. 2010;**1**:117-136

[98] Tan J, Cang S, Ma Y, Petrillo RL, Liu D. Novel histone deacetylase inhibitors in clinical trials as anti-cancer agents. Journal of Hematology and Oncology. 2010;**3**:1-13

[99] Duvic M, Vu J. Update on the treatment of cutaneous T-cell lymphoma (CTCL): Focus on vorinostat. Biologics. 2007;**1**:377

[100] Duvic M, Talpur R, Ni X, Zhang C, Hazarika P, Kelly C, et al. Phase 2 trial of oral vorinostat (suberoylanilide hydroxamic acid, SAHA) for refractory cutaneous T-cell lymphoma (CTCL). Blood. 2007;**109**:31-39

[101] Bertino EM, Otterson GA. Romidepsin: A novel histone deacetylase inhibitor for cancer. Expert Opinion on Investigational Drugs. 2011;**20**:1151-1158

[102] Rashidi A, Cashen AF. Belinostat for the treatment of relapsed or refractory peripheral T-cell lymphoma. Future Oncology. 2015;**11**:1659-1664

[103] Greig SL. Panobinostat: A review in relapsed or refractory multiple myeloma. Targeted Oncology. 2016;**11**:107-114

[104] Slingerland M, Guchelaar HJ, Gelderblom H. Histone deacetylase inhibitors: An overview of the clinical studies in solid tumors. Anti-Cancer Drugs. 2014;**25**:140-149

[105] Rasheed W, Bishton M, Johnstone RW, Prince HM. Histone deacetylase inhibitors in lymphoma and solid malignancies. Expert Review of Anticancer Therapy. 2008;**8**:413-432

[106] O'Connor OA, Heaney ML, Schwartz L, Richardson S, Willim R, MacGregor-Cortelli B, et al. Clinical

experience with intravenous and oral formulations of the novel histone deacetylase inhibitor suberoylanilide hydroxamic acid in patients with advanced hematologic malignancies. Journal of Clinical Oncology. 2017;**24**:166-173

[107] Balasubramanian S, Verner E, Buggy JJ. Isoform-specific histone deacetylase inhibitors: The next step ? Cancer Letters. 2009;**280**:211-221

[108] Xu WS, Parmigiani RB, Marks PA. Histone deacetylase inhibitors: Molecular mechanisms of action. Oncogene. 2007;**26**:5541-5552

[109] Richon VM, Sandhoff TW, Rifkind RA, Marks PA. Histone deacetylase inhibitor selectively induces p21 WAF1 expression and gene-associated histone acetylation. Proceedings of the National Academy of Sciences of the United States of America. 2000;**97**:1-6

[110] Sandor V, Senderowicz A, Mertins S, Sackett D, Sausville E, Blagoskonny MV, et al. P21-dependent G1 arrest with downregulation of cyclin D1 and upregulation of cyclin E by the histone deacetylase inhibitor FR901228. British Journal of Cancer. 2000;**83**:817-825

[111] Butler LM, Zhou X, Xu WS, Scher HI, Rifkind RA, Marks PA, et al. The histone deacetylase inhibitor SAHA arrests cancer cell growth, up-regulates thioredoxin-binding protein-2, and down-regulates thioredoxin. Proceedings of the National Academy of Sciences of the United States of America. 2002;**99**:11700-11705

[112] Frew AJ, Johnstone RW, Bolden JE. Enhancing the apoptotic and therapeutic effects of HDAC inhibitors. Cancer Letters. 2009;**280**:125-133

[113] Vrana J, Decker R, Johnson C, Wang Z, Jarvis W, Richon V, et al. Induction of apoptosis in U937 human leukemia cells by suberoylanilide hydroxamic acid (SAHA) proceeds through pathways that are regulated by Bcl-2/Bcl-XL, c-Jun, and p21CIP1, but independent of p53. Oncogene. 1999;**18**:7016-7025

[114] Nawrocki ST, Carew JS, Douglas L, Cleveland JL, Humphreys R, Houghton JA. Histone deacetylase inhibitors enhance lexatumumab-induced apoptosis via a p21 Cip1-dependent decrease in survivin levels. Cancer Research. 2007;**67**:6987-6995

[115] Harper JW, Adami GR, Wei N, Keyomarsi K, Elledge SJ. The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. Cell. 1993;**75**:805-816

[116] Qiu L, Burgess A, Fairlie D, Leonard H, Parsons P, Gabrielli B. Histone deacetylase inhibitors trigger a G2 checkpoint in normal cells that is defective in tumor cells. Molecular Biology of the Cell. 2000;**11**:2069-2083

[117] Lee J, Choy ML, Ngo L, Foster SS, Marks PA. Histone deacetylase inhibitor induces DNA damage, which normal but not transformed cells can repair. Proceedings of the National Academy of Sciences of the United States of America. 2010;**107**:14639-14644

[118] Ruefli AA, Ausserlechner MJ, Bernhard D, Sutton VR, Tainton KM, Kofler R, et al. The histone deacetylase inhibitor and chemotherapeutic agent suberoylanilide hydroxamic acid (SAHA) induces a cell-death pathway characterized by cleavage of bid and production of reactive oxygen species. Proceedings of the National Academy of Sciences of the United States of America. 2001;**98**:10833-10838

[119] Rosato R, Almenara J, Dai Y, Grant S. Simultaneous activation of the intrinsic and extrinsic pathways by histone deacetylase (HDAC) inhibitors and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)

synergistically induces mitochondrial damage and apoptosis in human leukemia cells. Molecular Cancer Therapeutics. 2003;**2**:1273-1284

[120] Carlisi D, Lauricella M, D'Anneo A, Emanuele S, Angileri L, Di Fazio P, et al. The histone deacetylase inhibitor suberoylanilide hydroxamic acid sensitises human hepatocellular carcinoma cells to TRAIL-induced apoptosis by TRAIL-DISC activation. European Journal of Cancer. 2009;**45**:2425-2438

[121] Nakata S, Yoshida T, Horinaka M, Shiraishi T, Wakada M, Sakai T. Histone deacetylase inhibitors upregulate death receptor 5/TRAIL-R2 and sensitize apoptosis induced by TRAIL/APO2-L in human malignant tumor cells. Oncogene. 2004;**23**:6261-6271

[122] Fulda S, Küfer MU, Meyer E, van Valen F, Dockhorn-Dworniczak B, Debatin KM. Sensitization for death receptor- or drug-induced apoptosis by re-expression of caspase-8 through demethylation or gene transfer. Oncogene. 2001;**20**:5865-5877

[123] Ungerstedt JS, Sowa Y, Xu W, Shao Y, Dokmanovic M, Perez G, et al. Role of thioredoxin in the response of normal and transformed cells to histone deacetylase inhibitors. Proceedings of the National Academy of Sciences of the United States of America. 2005;**102**:673-678

[124] Li Z, Zhu WG. Targeting histone deacetylases for cancer therapy: From molecular mechanisms to clinical implications. International Journal of Biological Sciences. 2014;**10**:757-770

[125] Miller KM, Tjeertes JV, Coates J, Legube G, Polo SE, Britton S, et al. Human HDAC1 and HDAC2 function in the DNA-damage response to promote DNA nonhomologous end-joining. Nature Structural and Molecular Biology. 2010;**17**:1144-1151

[126] Bhaskara S, Knutson SK, Jiang G, Chandrasekharan MB, Wilson AJ, Zheng S, et al. Hdac3 is essential for the maintenance of chromatin structure and genome stability. Cancer Cell. 2010;**18**:436-447

[127] Thurn KT, Thomas S, Raha P, Qureshi I, Munster PN. Histone deacetylase regulation of ATM-mediated DNA damage signaling. Molecular Cancer Therapeutics. 2013;**12**:2078-2087

[128] Kao GD, McKenna WG, Guenther MG, Muschel RJ, Lazar MA, Yen TJ. Histone deacetylase 4 interacts with 53BP1 to mediate the DNA damage response. The Journal of Cell Biology. 2003;**160**:1017-1027

[129] Kotian S, Liyanarachchi S, Zelent A, Parvin JD. Histone deacetylase 9 and 10 are required for homologous recombination. The Journal of Biological Chemistry. 2011;**286**:7722-7726

[130] Gorospe M, de Cabo R. AsSIRTing the DNA damage response. Trends in Cell Biology. 2008;**18**:77-83

[131] Kaidi A, Weinert BT, Choudhary C, Jackson SP. Human SIRT6 promotes DNA end resection through CtIP deacetylation. Science. 2010;**329**:1348-1353

[132] Motoslavsky R, Chua KF, Lombard DB, Pang WW, Fischer MR, Gellon L, et al. Genomic instability and aging-like phenotype in the absence of mammalian SIRT6. Cell. 2006;**124**:315-329

[133] Rosato RR, Almenara JA, Grant S. The histone deacetylase inhibitor MS-275 promotes differentiation or apoptosis in human leukemia cells through a process regulated by generation of reactive oxygen species and induction of p21 CIP1/WAF1. Cancer Research. 2003;**63**:3637-3645

[134] Deroanne CF, Bonjean K, Servotte S, Devy L, Colige A, Clausse N, et al. Histone deacetylases inhibitors as anti-angiogenic agents altering vascular endothelial growth factor signaling. Oncogene. 2002;**21**:427-436

[135] Jeong JW, Bae MK, Ahn MY, Kim SH, Sohn TK, Bae MH, et al. Regulation and destabilization of HIF-1alpha by ARD1-mediated acetylation. Cell. 2002;**111**:709-720

[136] Pulukuri S, Gorantla B, Rao J. Inhibition of histone deacetylase activity promotes invasion of human cancer cells through activation of urokinase plasminogen activator. The Journal of Biological Chemistry. 2007;**282**:35594-35603

[137] Fournel M, Bonfils C, Hou Y, Yan P, Trachy-Bourget MC, Kalita A, et al. MGCD0103, a novel isotype-selective histone deacetylase inhibitor, has broad spectrum antitumor activity in vitro and in vivo. Molecular Cancer Therapeutics. 2008;**7**:759-768

[138] Vigushin DM, Ali S, Pace PE, Mirsaidi N, Ito K, Adcock I, et al. Trichostatin a is a histone deacetylase inhibitor with potent antitumor activity against breast cancer in vivo. Clinical Cancer Research. 2001;**7**:971-976

[139] Sato N, Ohta T, Kitagawa H, Kayahara M, Ninomiya I, Fushida S, et al. FR901228, a novel histone deacetylase inhibitor, induces cell cycle arrest and subsequent apoptosis in refractory human pancreatic cancer cells. International Journal of Oncology. 2004;**24**:679-685

[140] Kwon HJ, Owa T, Hassig CA, Shimada J, Schreiber SL. Depudecin induces morphological reversion of transformed fibroblasts via the inhibition of histone deacetylase. Proceedings of the National Academy of Sciences of the United States of America. 1998;**95**:3356-3361

[141] Mrakovcic M, Kleinheinz J, Fröhlich LF. Histone deacetylase inhibitor-induced autophagy in tumor cells: Implications for p53. International Journal of Molecular Sciences. 2017;**18**:1883

[142] Gammoh N, Lam D, Puente C, Ganley I, Marks PA, Jiang X. Role of autophagy in histone deacetylase inhibitor-induced apoptotic and nonapoptotic cell death. Proceedings of the National Academy of Sciences of the United States of America. 2012;**109**:6561-6565

[143] Carew JS, Nawrocki ST, Kahue CN, Zhang H, Yang C, Chung L, et al. Targeting autophagy augments the anticancer activity of the histone deacetylase inhibitor SAHA to overcome Bcr-Abl-mediated drug resistance. Blood. 2007;**110**:313-323

[144] Di Giacomo V, Di Valerio V, Rapino M, Bosco D, Cacciatore I, Ciulla M, et al. MRJF4, a novel histone deacetylase inhibitor, induces p21 mediated autophagy in PC3 prostate cancer cells. Cellular and Molecular Biology. 2015;**61**:17-23

[145] Wang J, Kim TH, Ahn MY, Lee J, Jung JH, Choi WS, et al. Sirtinol, a class III HDAC inhibitor, induces apoptotic and autophagic cell death in MCF-7 human breast cancer cells. International Journal of Oncology. 2012;**41**:1101-1109

[146] Shulak L, Beljanski V, Chiang C, Dutta SM, Van Grevenynghe J, Belgnaoui SM, et al. Histone deacetylase inhibitors potentiate vesicular stomatitis virus oncolysis in prostate cancer cells by modulating NF-kappa B-dependent autophagy. Journal of Virology. 2014;**88**:2927-2940

[147] Li J, Liu R, Lei Y, Wang K, Lau QC, Xie N, et al. Proteomic analysis revealed association of aberrant ROS signaling with suberoylanilide

hydroxamic acid-induced autophagy in Jurkat T-leukemia cells. Autophagy. 2010;**6**:711-724

[148] Long J, Zhao J, Yan Z, Liu Z, Wang N. Antitumor effects of a novel sulfur-containing hydroxamate histone deacetylase inhibitor H40. International Journal of Cancer. 2009;**124**:1235-1244

[149] Watanabe M, Adachi S, Matsubara H, Imai T, Yui Y, Mizushima Y. Induction of autophagy in malignant rhabdoid tumor cells by the histone deacetylase inhibitor FK228 through AIF translocation. International Journal of Cancer Research. 2009;**67**:55-67

[150] Zhang J, Ng S, Wang J, Zhou J, Tan S, Yang N, et al. Histone deacetylase inhibitors induce autophagy through FOXO1-dependent pathways. Autophagy. 2015;**11**:629-642

[151] Gandesiri M, Chakilam S, Ivanovska J, Benderska N, Ocker M, Di Fazio P, et al. DAPK plays an important role in panobinostat-induced autophagy and commits cells to apoptosis under autophagy deficient conditions. Apoptosis. 2012;**17**:1300-1315

[152] Ellis L, Bots M, Lindemann RK, Bolden JE, Newbold A, Cluse LA, et al. The histone deacetylase inhibitors LAQ824 and LBH589 do not require death receptor signaling or a functional apoptosome to mediate tumor cell death or therapeutic efficacy. Blood. 2009;**114**:380-393

[153] Sun W, Yi Y, Xia G, Zhao Y, Yu Y, Li L, et al. Nrf2-miR-129-3p-mTOR axis controls an miRNA regulatory network involved in HDACi-induced autophagy. Molecular Therapy. 2019;**27**:1039-1050

[154] Stankov MV, El Khatib M, Kumar Thakur B, Heitmann K, Panayotova-Dimitrova D, Schoening J, et al. Histone deacetylase inhibitors induce apoptosis in myeloid leukemia by suppressing autophagy. Leukemia. 2014;**28**:577-588

[155] Maccallum SF, Groves MJ, James J, Murray K, Appleyard V, Prescott AR, et al. Dysregulation of autophagy in chronic lymphocytic leukemia with the small-molecule sirtuin inhibitor tenovin-6. Scientific Reports. 2013;**3**:1275

[156] Fröhlich LF, Mrakovcic M, Smole C, Lahiri P, Zatloukal K. Epigenetic silencing of apoptosis-inducing gene expression can be efficiently overcome by combined SAHA and TRAIL treatment in uterine sarcoma cells. PLoS One. 2014;**9**:e91558

[157] Shao Y, Gao Z, Marks PA, Jiang X. Apoptotic and autophagic cell death induced by histone deacetylase inhibitors. Proceedings of the National Academy of Sciences of the United States of America. 2004;**101**:18030-18035

[158] Liu YL, Yang PM, Shun CT, Wu MS, Weng JR, Chen CC. Autophagy potentiates the anti-cancer effects of the histone deacetylase inhibitors in hepatocellular carcinoma. Autophagy. 2010;**6**:1057-1065

[159] Chiao M, Cheng W, Yang Y, Shen C, Chiao M, Cheng W, et al. Suberoylanilide hydroxamic acid (SAHA) causes tumor growth slowdown and triggers autophagy in glioblastoma stem cells. Autophagy. 2013;**9**:1509-1526

[160] El-Khoury V, Pierson S, Szwarcbart E, Brons NHC, Roland O, Cherrier-De Wilde S, et al. Disruption of autophagy by the histone deacetylase inhibitor MGCD0103 and its therapeutic implication in B-cell chronic lymphocytic leukemia. Leukemia. 2014;**28**:1636-1646

[161] Ahn MY, Ahn JW, Kim HS, Lee J, Yoon JH. Apicidin inhibits cell growth by downregulating IGF-1R in salivary mucoepidermoid carcinoma cells. Oncology Reports. 2015;**33**:1899-1907

[162] Hui KF, Yeung PL, Chiang AKS. Induction of MAPK- and ROS-dependent autophagy and apoptosis in gastric carcinoma by combination of romidepsin and bortezomib. Oncotarget. 2015;**7**:1-14

[163] Zhan Y, Gong K, Chen C, Wang H, Li W. P38 MAP kinase functions as a switch in MS-275-induced reactive oxygen species-dependent autophagy and apoptosis in human colon cancer cells. Free Radical Biology and Medicine. 2012;**53**:532-543

[164] Park EY, Woo Y, Kim SJ, Kim DH, Lee EK, De U, et al. Anticancer effects of a new SIRT inhibitor, MHY2256, against human breast cancer MCF-7 cells via regulation of MDM2-p53 binding. International Journal of Biological Sciences. 2016;**12**:1555-1567

[165] Fröhlich LF, Mrakovcic M, Smole C, Zatloukal K. Molecular mechanism leading to SAHA-induced autophagy in tumor cells: Evidence for a p53-dependent pathway. Cancer Cell International. 2016;**16**:1-15

[166] Sykes SM, Mellert HS, Holbert MA, Li K, Lane WS, Mcmahon SB. Acetylation of the p53 DNA binding domain regulates apoptosis induction. Molecular Cell. 2006;**24**:841-851

[167] Tang Y, Luo J, Zhang W. Tip60-dependent acetylation of p53 modulates the decision between cell-cycle arrest and apoptosis. Molecular Cell. 2006;**24**:827-839

[168] Barlev NA, Liu L, Chehab NH, Mansfield K, Harris KG, Halazonetis TD, et al. Acetylation of p53 activates transcription through recruitment of coactivators/histone acetyltransferases. Molecular Cell. 2001;**8**:1243-1254

[169] Luo J, Li M, Tang Y, Laszkowska M, Roeder RG, Gu W. Acetylation of p53 augments its site-specific DNA binding both in vitro and in vivo. Proceedings of the National Academy of Sciences of the United States of America. 2003;**101**:2259-2264

[170] Zhao Y, Lu S, Wu L, Chai G, Wang H, Chen Y, et al. Acetylation of p53 at lysine 373/382 by the histone deacetylase inhibitor depsipeptide induces expression of p21 Waf1/Cip1. Molecular and Cellular Biology. 2006;**26**:2782-2790

[171] Seo SK, Jin HO, Woo SH, Kim YS, An S, Lee JH, et al. Histone deacetylase inhibitors sensitize human non-small cell lung cancer cells to ionizing radiation through acetyl p53-mediated c-myc down-regulation. Journal of Thoracic Oncology. 2011;**6**:1313-1319

[172] Mrakovcic M, Bohner L, Hanisch M, Fröhlich LF. Epigenetic targeting of autophagy via HDAC inhibition in tumor cells: Role of p53. International Journal of Molecular Sciences. 2018;**19**:3952

[173] Hrzenjak A, Moinfar F, Kremser ML, Strohmeier B, Petru E, Zatloukal K, et al. Histone deacetylase inhibitor vorinostat suppresses the growth of uterine sarcomas in vitro and in vivo. Molecular Cancer. 2010;**9**:49

[174] De U, Son JY, Sachan R, Park YJ, Kang D, Yoon K, et al. A new synthetic histone deacetylase inhibitor, MHY2256, induces apoptosis and autophagy cell death in endometrial cancer cells via p53 acetylation. International Journal of Molecular Sciences. 2018;**19**:2743