Review Article

Autophagic and Apoptotic Effects of HDAC Inhibitors on Cancer Cells

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Because epigenetic alterations are believed to be involved in the repression of tumor suppressor genes and the promotion of tumorigenesis in cancers, novel compounds endowed with histone deacetylase (HDAC) inhibitory activity are an attractive therapeutic approach. Indeed, the potential of HDAC inhibitors for cancer therapy has been explored in preclinical models, and some agents approved for hematologic malignancies have reached the clinical setting. HDAC inhibitors are able to mediate the induction of both apoptosis and autophagy, which are related to anticancer activity in a variety of cancer cell lines. Given the inherent resistance to apoptosis that characterizes cancer, the targeting of alternative pathways is an attractive strategy to improve anti-tumor therapy. The activation of autophagy represents novel cancer treatment targets. This paper aims to critically discuss how the anticancer potential of HDAC inhibitors may elicit a response to human cancers through different cell pathways leading to cell death.

1. Introduction

An ideal anticancer agent should be toxic to malignant cells with minimum toxicity towards normal cells. Currently, there are only a limited number of such agents available for clinical use; thus, the development of novel cancer-selective drugs is an important and challenging task. Caspase-mediated apoptosis, involving both intrinsic and extrinsic pathways, is the main mechanism of action of most current anticancer treatments. Several anticancer drugs have been shown to induce autophagy as well as apoptosis. Autophagy is a catabolic pathway whereby cytoplasmic proteins and organelles are sequestered in vacuoles and delivered to lysosomes for degradation and recycling; however, the role of autophagy in cancer development and in the response to therapy is still controversial, requiring further investigation.

Chemoresistance may be due to epigenetic alterations leading to defects in the apoptotic pathway; therefore, modulating epigenetic changes may increase the efficacy of chemotherapy. It is possible to interfere with epigenetic modifications, such as histone acetylation, using relatively specific and reversible inhibitors. In addition, the induction of autophagy has been also observed in malignant cells following treatment with histone deacetylase (HDAC) inhibitors [1]. HDAC inhibitors are promising new agents for the treatment of multiple myeloma as they promote hyperacetylation and activate chromatin remodeling. HDAC inhibition leads to many changes at the molecular and cellular levels. HDAC inhibitors preferentially kill transformed cells or cancer cells in both cell cultures and animal models. Although further investigation is required to fully elucidate the consequences of both autophagy and apoptosis induction by treatment with HDAC inhibitors, the pharmacological inhibition of HDAC provides a new therapeutic basis for targeting cell death processes.

2. HDAC Family

In addition to DNA mutations (genetic changes), most cells in multicellular organisms result from aberrations in chromatin-modifying proteins, such as HDAC and DNA methyltransferase (epigenetic changes). It became

increasingly evident that cancer formation and persistence may be caused not only by genetic mutations but also by changes in the patterns of epigenetic modifications [2]. Epigenetic modifications are of profound importance to cells and appear to play roles in many, if not all, biological processes throughout life. In particular, acetylation and deacetylation of the lysine residues on histone proteins play a key role in the regulation of gene transcription [3]. Whereas histone acetyltransferase catalyzes the acetylation of histones and relaxes chromatin to increase the accessibility of transcription factors to the promoters of the target genes, HDACs remove the acetyl groups from histones and repress transcription [4]. In the discovery of drugs, HDAC has become a novel target for the treatment of cancer and other diseases. Inhibition of HDAC activity leads to the acetylation of histones and nonhistone proteins, and alterations in target gene expression are related to changes induced by HDAC inhibition.

According to phylogenetic analyses and sequence homology, the HDAC family consists of at least 18 members divided into two families and four classes in eukaryotic cells. These two families are the classical and sirtuin families. HDACs are categorized into class I (HDACs 1, 2, 3, and 8), class IIa (HDACs 4, 5, 7, and 9), class IIb (HDACs 6 and 10), class III (SIRTs), and class IV (HDAC11) [5]. Class I and II have zinc as a cofactor, so they are hydrolases which contain zinc. Class III, which is a series of NAD-dependent Sir2 families, differs from other HDAC classes. Class I and II HDACs are mainly found to be involved in cancer pathogenesis. Class I are found exclusively in the nucleus, whereas in certain cellular signals, class II shuttle between the nucleus and cytoplasm. While human HDACs are well characterized in terms of their catalytic domains, the discovery of type-specific inhibitors is important for anticancer therapies.

3. HDAC Inhibitors

HDAC inhibitors are emerging as potent anticancer agents that can reactivate gene expression and restore the capability of malignant cells to undergo programmed cell death. Several natural and synthetic compounds are currently known to inhibit HDACs (Table 1). Since HDAC inhibitors do not inhibit all HDAC isoforms to the same extent, these agents can be grouped into pan- and class I-specific inhibitors [5]. Hydroxamic acids (TSA, SAHA, LAQ824, and CBHA) and pyroxamic acids (PXD101 and CRA-026440) are pan-HDAC inhibitors targeting class I, II, and IV HDACs in the nanomolar range [17]. In contrast, carboxylic acids (valproic acid and sodium butyrate) and benzamides (MS275, CI-994, and MGCD0103) or cyclic tetrapeptides (trapoxin, depsipeptide and spiruchostatin A) are rather class I-specific HDAC inhibitors [18].

HDACs are often overexpressed in various types of cancers, compared with the corresponding normal tissues, and their overexpression is correlated with a poor prognosis [19]. Currently, at least 15 different HDAC inhibitors are being used in clinical trials as a part of mono- or combination therapies. For example, the orally available pan-inhibitor LBH589 (panobinostat) is currently being developed for several hematological malignancies. SAHA (vorinostat) has been approved for marketing based on data from a large Phase II trial of patients with cutaneous T-cell lymphoma that demonstrated a 30% objective response rate [6]. The class I-selective intravenously delivered agent FK228 (romidepsin), has also shown promising efficacy in patients with cutaneous T-cell lymphoma, with an overall objective response rate of 32%. The class I-selective orally available compound, MGCD0103 (mocetinostat), is in development for multiple solid and hematologic cancers [13]. Clinical trials of several HDAC inhibitors include patients with a wide variety of hematologic and solid neoplasms, including chronic lymphatic leukemia, Hodgkin's lymphoma, myeloproliferative disorder, B-cell lymphoma, acute myeloid leukemia, multiple myeloma, head and neck cancer, brain tumors, melanoma, lung cancer, hepatocellular cancer, breast cancer, ovarian cancer, renal cell carcinoma, and pancreatic adenocarcinoma. However, the main side effects of HDAC inhibitor, SAHA, are fatigue, nausea, anorexia, diarrhea, thrombus formation, thrombocytopenia, neutropenia, anemia, myalgia, hypokalemia and hypophosphatemia. Reversible thrombocytopenia has been seen with almost all HDAC inhibitors in the clinic and appears to be a mechanism-related class effect. By targeting only the most relevant HDAC isoform, it may be possible to greatly improve the efficacy by removing certain toxicities that may be associated with the inhibition of multiple isoforms.

4. Programmed Cell Death (PCD)

Cell death is a result of an unsuccessful cytoprotective mechanism against intracellular and extracellular stressors, and it is broadly divided into three forms: apoptosis, autophagy, and necrosis (Table 2). Morphologically, type I cell death (apoptosis) is characterized by chromatin condensation and DNA fragmentation. Biochemically, apoptosis is characterized by double-stranded cleavage at the linker regions between nucleosomes, resulting in the formation of multiple DNA fragments, and phosphatidylserine externalization accompanied by a series of gene and protein expressions. In the extrinsic pathway, apoptosis is mediated by death receptors on the cell surface, while in the intrinsic pathway, mitochondria play an important role. In both pathways, activated caspases (cysteine aspartic acid-specific proteases) cleave their substrates and activate other downstream cellular substrates. Type II cell death (autophagy) is characterized by the massive accumulation of double-membrane vesicles, commonly referred to as autophagosomes. In general, autophagy promotes the survival of stress; however, there is increasing evidence that when autophagy is overstimulated, it can progress to autophagic cell death. It has been also documented that malignant cell types undergo autophagic cell death when responding to anticancer agents, indicating the potential utility of autophagic cell death induction in cancer therapy. Type III cell death (necrosis) is characterized by oncosis and plasma membrane rupture [20]. Necrosis is an uncontrolled cell death manifesting osmotic dispersion of cells and organelles. While apoptosis involves the rapid destruction of all cellular structures and organelles,

TABLE 1: Characteristics of HDAC inhibitors in clinical trials.

Chemistry	Compounds	HDAC Targets	Clinical trials	Ref
Hydroxymates	SAHA (vorinostat)	Classes I, II, and IV	Phase III*	[6]
	PXD101 (belinostat)	Classes I, and IIa, HDAC6	Phase II	[7]
	Trichostatin A	Classes I and II	Toxic	[8]
	LAQ824 (dacinostat)	Classes I and II	Phase I	[9]
	PCI24781	Classes I and IIb	Phase I	[10]
	LBH589 (panobinostat)	Classes I and IIa	Phase II	[11]
Cyclic tetrapeptides	FK228 (romidepsin)	HDAC1, 2, 4, 6	Phase II	[12]
Benzamides	MGCD0103 (mocetinostat)	HDAC1, 2, 3, 11	Phase II	[13]
	MS275 (entinostat)	HDAC1, 2, 3, 9	Phase II	[14]
Short-chain fatty acids	Valproic acid	Classes I and IIa	Phase II	[15]
	Butyrate	Classes I and IIa	Phase II	[16]

* Approved (cutaneous T-cell lymphoma).

autophagy is a slow, spatially restricted phenomenon in which parts of the cytoplasm are sequestered within double-membraned vacuoles and finally digested by lysosomal hydrolases [21].

5. Apoptosis Induced by HDAC Inhibitors

All HDAC inhibitors have been reported to activate either an extrinsic or intrinsic pathway or both of these cell death pathways in many cancer models [22]. The death-receptor pathway is activated when ligands, such as Fas or TRAIL, bind to their death receptors. It was observed that MS275 (entinostat) as well as SAHA induced TRAIL expression without altering DR4 or DR5 levels in breast cancer cells, which was mediated via SP1 and markedly enhanced adriamycin cytotoxicity in these cells [23]. This resulted in the recruitment of an adaptor protein, FADD, and the activation of caspase-8. The mitochondrial pathway is activated by stress stimuli (chemotherapeutic agents) that disrupt the mitochondrial membrane, causing the release of proteins, including cytochrome c and SMAC. Cytochrome c release leads to apoptosome formation and activation of caspase-9. Caspase-8 and caspase-9 can then cleave caspases-3, -6, and -7, culminating in apoptosis. SAHA and MS275 induced mitochondrial dysfunction and apoptosis through enhanced ROS generation, XIAP downregulation and JNK1 activation [24]. Various pro- (Bax, Bak, Bim, Bid, etc.) and antiapoptotic (Bcl-2, Bcl-xL, Mcl-1, etc.) proteins regulate these cell death pathways. HDAC inhibitors, including TSA, FK228, SAHA, and LBH589, decrease the expression of Bcl-2, Bcl-xL and XIAP, and enhance the expression of pro-apoptotic proteins, such as Bax and Bak, thereby enhancing TRAIL-mediated cytotoxicity in a variety of cancer cells via the amplification of intrinsic as well as extrinsic apoptotic pathways [25]. Using siRNA approaches targeting Bim, the contribution of Bim to TSA-induced mitochondrial apoptosis was confirmed [26]. In addition, serine protease-dependent and caspase-independent apoptosis is induced by treatment with the pan-HDAC inhibitor, TSA, in pancreatic ductal adenocarcinoma cell lines [27]. This process correlates with the initially increased expression of the multidomain proapoptotic Bcl-2 family member, Bax, and the subsequent release of AIF and Omi/Htr-A2 from mitochondria. The Bax tumor suppressor gene is downregulated by SIRT1 through the DNA end-binding factor, Ku70. In the absence of SIRT1, Ku70 might be hyper-acetylated, leading to the disruption of Ku70-Bax interaction. This in turn releases more Bax, which promotes apoptosis [28]. HDAC2 inhibition opens the locus of the epigenetically silenced NOXA gene, a BH3-only protein and apical initiator of apoptosis [29]. Given the multitude of cellular effects triggered by HDAC inhibitors, it is probable that several different mechanisms contribute to their anticancer activity. Another mechanism of cancer cell death resulting from HDAC inhibitor treatment is discussed below.

6. Autophagy Induced by HDAC Inhibitors

While autophagy has become one of the most attractive topics in cancer research, the current autophagy studies are often viewed as confusing, because of its association with apparently contradictory roles, such as survival and cell death, depending on the model used [30]. A number of anticancer therapies, including HDAC inhibitors, have been observed to induce autophagy in human cancer cell lines.

6.1. Prosurvival Functions. Autophagy is an evolutionarily conserved cell survival pathway that enables cells to recoup ATP and other critical biosynthetic molecules during nutrient deprivation or exposure to hypoxia, which are hallmarks of the tumor microenvironment. The disruption of autophagy with the antimalarial drug, chloroquine, strongly enhanced SAHA-mediated apoptosis in colon cancer cells, knockdown of the essential Atg7 also sensitized cells to SAHA-induced apoptosis [31]. HDAC6, a class II HDAC isotype, is directly involved in the autophagic degradation of the cell; therefore, drugs that impair autophagy could theoretically potentiate the anticancer efficacy of SAHA and other HDAC inhibitors by disabling this important cell survival mechanism.

6.2. Prodeath Functions. The cytotoxic effects of autophagy may be explained by the extensive autophagic degradation of intracellular content or by the interfacing of the autophagic process to proapoptotic signals. Persistent autophagy in response to cellular stress states serves as a potent death signal, as in the case of therapy-induced autophagy, a specific nonapoptotic death pathway triggered upon exposure to chemotherapeutic compounds. In general, autophagic cell death is caspase independent and does not involve classic DNA laddering; however, the mechanisms underlying autophagic cell death are mostly undefined. A link between autophagy and related autophagic cell death has been demonstrated using pharmacological (3-methyladenine) and genetic (silencing of *Atg5*, *Atg7*, and *Beclin-1*) approaches for the suppression of autophagy. In chondrosarcoma cell lines, SAHA induced autophagy-associated cell death, as shown by the detection of autophagosome-specific protein and specific ultrastructural morphology in the cytoplasm [32]. In HeLa cells, HDAC inhibitors (butyrate and SAHA) can induce both mitochondria-mediated apoptosis and caspase-independent autophagic cell death. HeLa cells with APAF-1 knockout or Bcl-xL overexpression were induced to undergo autophagic cell death when cultured with SAHA or butyrate [1]. Therefore, it is reasonable to propose that the induction of autophagic cell death may be used as a therapeutic strategy to treat cancer.

7. Molecular Mechanisms of HDAC-Induced Autophagy

Many signaling pathways, including mTOR, AIF, ROS, CDKs, and HDAC1/6, play important roles in regulating HDAC-induced autophagy (Table 3). Their molecular mechanisms and regulation of autophagy are discussed below.

7.1. mTOR. mTOR plays a role in the initiation and maturation of autophagy by controlling signal transduction cascades involved in this process, acts as a good gatekeeper in autophagy, and exerts an inhibitory effect on autophagy [40]. Both mTOR and phospho-mTOR expressions were strongly decreased in SAHA-treated endometrial stromal sarcoma (ESS-1) cells in a concentration-dependent manner [33]. It seems that SAHA modulates the stability and/or the degradation mechanisms of mTOR at the protein level. SAHA also exerted an influence on mTOR phosphorylation. The level of phospho-S6rp, which plays a regulatory role in the mTOR pathway, was also affected by SAHA. S6rp phosphorylation is directly involved in the control of autophagic processes. The same results were found in ESS-1 treated by rapamycin, a well-known mTOR inhibitor. Further investigations are needed to elucidate the exact pharmacological mechanisms of different HDAC inhibitors in different experimental systems and to clarify whether HDAC inhibitors modulate the expression and phosphorylation of mTOR directly or act somewhere upstream of mTOR.

7.2. AIF. It was examined in malignant rhabdoid tumor cells whether HDAC-inhibitor- (FK228-) mediated autophagy involved AIF, which acts as a safeguard executioner in cancer cells with faulty caspase activation [34]. FK228 treatment induced the redistribution of AIF to the nucleus, in either the presence or absence of zVAD-fmk. siRNA against AIF prevented the redistribution of LC3 into punctate cytoplasmic structures and blocked FK228-induced autophagy. AIF siRNA also decreased the conversion of LC3-I to LC3-II, as analyzed by Western blotting. Treatment with autophagy inhibitors (chloroquine or 3-methyladenine) increased FK228-induced cell death. These findings suggest that AIF contributes to the autophagy induced by FK228, and disrupting autophagy enhances FK228-induced apoptosis.

7.3. ROS. Targeting the autophagy pathway might enhance the anticancer activity of SAHA, and autophagy inhibitors would synergistically potentiate the proapoptotic effects of SAHA. SAHA has pleiotropic effects that contribute to its mechanism of action in malignant cells, including the generation of ROS, and this has been shown to be a critical event in SAHA-induced cell death [41]. To determine whether chloroquine may modulate SAHA-induced ROS generation, the intracellular levels of the oxygen radical superoxide ($O_2{}^-$) were quantified after 12 h treatment with chloroquine, SAHA, and the combination of chloroquine and SAHA in K562 and LAMA 84 cells [35]. In both cell lines, there were marked increases in the generation of $O_2{}^-$ in cells treated with a combination of chloroquine and SAHA compared with cells treated with either single agent. Cells pretreated with N-acetyl-cysteine displayed significantly reduced apoptosis induction in response to chloroquine and SAHA. It is possible that the initial ROS release triggered autophagy as a protective defense mechanism of cancer cells to deal with SAHA, but the temporal order of protective autophagy is followed by apoptosis, as if autophagy sets the stage for apoptosis to kill prostate cancer cells after a futile attempt to rescue them from death.

7.4. $p21^{CIP/WAF1}$. Both a novel sulfur-containing hydroxamate HDAC inhibitor, H40, and SAHA induced autophagy in prostate cancer PC-3M cells in a concentration-dependent manner, which correlated well with their cytotoxic effects. These HDAC inhibitors significantly increased CDK inhibitor $p21^{CIP/WAF1}$ mRNA and protein expression in both PC-3M and HL-60 cells in concentrations within their cytotoxic ranges [36]. The upregulation of $p21^{CIP/WAF1}$ correlated with reduced sensitivity and blocked the apoptotic mechanism, and these cells seemed to die by autophagy. Recently, silencing of HDAC3 has been found to induce the expression of $p21^{CIP/WAF1}$ and cell cycle arrest in the G2/M phase in colon cancer cells [42]. HDAC4 was shown to suppress $p21^{CIP/WAF1}$ in ovarian carcinoma cells, cervical cancer cells, glioblastoma cells and breast cancer cells in a nonredundant fashion [43]. These results suggest that the presence of $p21^{CIP/WAF1}$ determines which type of PCD the cell undergoes.

7.5. HDAC1 and HDAC6. HDAC inhibition leads to many changes at the molecular and cellular levels. Class I HDAC

TABLE 2: Characteristic features of programmed cell death.

Programmed cell death (PCD)	Feature	Key regulators	Measurements
Apoptosis (type I PCD)	Chromatin condensation	Caspases	Caspase activation
	DNA laddering	Cytochrome *c*	DNA fragmentation
	Blebbing (nuclear, cytoplasmic) Apoptotic bodies	Bcl-2 family members	Annexin V
Autophagy (type II PCD)	Blebbing	Autophagy. genes: *beclin*, *LC3*, *Atg1*, *Atg5*, and *Atg7*	LC3 localization
	Autophagic vesicles		
	Degradation of Golgi		Autophagic vesicles
	No DNA laddering		
Necrosis (type III PCD)	Swollen organelles	RIPK1, TRAF2, PARP, and Calpains	Extracellular HMGB1
	Random DNA degradation		
	Cytoplasmic membrane rupture		S100 family members
	Potent inflammatory response		

TABLE 3: Effects of HDAC inhibitor on the process of autophagy.

Mechanisms	HDAC inhibitors	Cell lines*	Detection methods	Ref
mTOR	SAHA	ESS-1	Phosphorylation (mTOR)	[33]
AIF	FK228	MRT	siRNA (AIF)	[34]
ROS	SAHA	K562, LAMA84	N-acetyl-cysteine, chloroquine	[35]
p21$^{CIP/WAF1}$	H40, SAHA	PC-3M, HL-60	mRNA, protein expression	[36]
HDAC1	FK228	HeLa	siRNA (HDAC1)	[37]
HDAC6	Tubacin	MEF	siRNA (HDAC6)	[38]
Mitochondria	LBH589, LAQ824	E*μ-myc* lymphoma	Knockout (Apaf-1, caspase-9)	[39]

*ESS-1: endometrial stromal sarcoma cells; LAMA84: human chronic myeloid leukemia; MEF: mouse embryonic fibroblasts; MRT: malignant rhabdoid tumors.

inhibitor, FK228, led to autophagic activities through the formation of autophagic vacuoles and the increase of acidic compartments in HeLa cells. When HDAC1 siRNA was used, the amount of HDAC1 mRNA was significantly decreased in knocked-down cells, and HDAC1 inhibition induced the conversion of LC3-I to LC3-II which leads to autophagy in HeLa cells [37]. Thus, HDAC1 may play a role in autophagy because the inhibition of HDAC1 with a specific inhibitor or siRNA can induce autophagy.

HDAC6 is a member of the class II HDAC family and is known to deacetylate α-tubulin and increase cell motility. The ubiquitin-binding deacetylase, HDAC6, was also identified as a central component of basal autophagy that targets protein aggregates and damages mitochondria; however, HDAC6 is not required for autophagy activation; rather, it controls the fusion of autophagosomes to lysosomes [38]. HDAC6 promotes these fusion events by recruiting a cortactin-dependent, actin-remodelling machinery to ubiquitinated protein aggregates, where the assembly of F-actin facilitates autophagosome-lysosome fusion and clearance of autophagic substrates. In addition, the observation that tubacin, a selective inhibitor of HDAC6 deacetylase activity, blocks LC3 recruitment suggests that deacetylation is essential for this process.

7.6. Mitochondrial Function. HDAC inhibitors, LAQ824 (dacinostat) and LBH589, induce the apoptosis of E*μ-myc* lymphomas via the intrinsic apoptotic pathway. Cells that inhibited the later stages of apoptosis signaling, through the knockout of *apaf-1* or *caspase-9*, displayed morphologic features of autophagy but did not diminish the effects of LAQ824 and LBH589 on mitochondrial membrane permeabilization [39].

8. Relationship between Autophagy and Apoptosis

Recently, several anticancer drugs have been shown to induce not only apoptosis, but also autophagy in cancer cells [44]. The relationship between autophagy and apoptosis is complex and varies with cell types and the specific stress placed upon the cell [45]. While the molecular mechanisms leading to apoptosis have been elucidated to some extent during the past 15 years, autophagic cell death is not well characterized at the molecular level yet [46]. The induction of autophagic cell death may be an ideal approach for cancers that are resistant to apoptosis by anticancer therapies.

It is clear whether autophagy and apoptosis are strictly interconnected, as highlighted by the finding that the two

pathways share key molecular regulators. These are summarized as follows.

(1) Caspase-3 inhibitor zDEVD-fmk upregulated autophagy in the mouse lung cancer cells [47].

(2) Following genotoxic stress or oncogene activation, the p53 tumor-suppressor protein is stabilized and activated as a transcription factor, capable of inducing apoptosis. Nuclear localization of p53 induces autophagy, whereas cytoplasmic accumulation inhibits autophagy [48].

(3) Serum- and amino acid-starved LAMP2-negative cells exhibited an accumulation of autophagic vacuoles and then succumbed to cell death with hallmarks of apoptosis, such as loss of mitochondrial transmembrane potential, caspase activation, and chromatin condensation. Together, the accumulation of autophagic vacuoles can precede apoptotic cell death [49].

(4) Beclin-1 may be a critical molecular switch that plays an important role in fine-tuning autophagy and apoptosis through caspase-9 [50].

(5) JNK and p53 may constitute an amplifying loop of autophagic and apoptotic responses. JNK activation results in phosphorylation of Bcl-2, which enhances autophagy by disrupting the interaction between Bcl-2 and Beclin-1 [51].

(6) The enforced expression of Atg5 not only promotes autophagy but also enhances susceptibility toward apoptotic stimuli irrespective of the cell type, indicating that Atg5 is a molecular switch factor between autophagy and apoptosis [52].

(7) Full-length Atg5 is an inducer of autophagy, whereas an Atg5 fragment due to cleavage by calpain is pro-apoptotic [53].

(8) The formation of autophagosomes occurred early in the sequence of dexamethasone-induced events, before Bak activation, loss of mitochondrial membrane potential, and nuclear fragmentation, showing that dexamethasone-induced autophagy not only lies upstream of apoptosis but is also required for the latter to occur [54].

(9) A novel Bcl-xL inhibitor, Z36, efficiently induces autophagic cell death in HeLa cells through blocking the interaction between Bcl-xL/Bcl-2 and Beclin-1 [55]. The antiapoptotic protein Bcl-2 is also an antiautophagic protein through its interaction with Beclin-1 [56].

(10) The stress induced by N-(4-hydroxyphenyl) retinamide triggers autophagy at a lower dose and apoptosis at a higher dose in glioma cells. At that time, ERK was specifically activated at a lower concentration but not at a higher concentration of retinamide [57].

(11) The kinetics of Ca^{2+} influx correlated well with the onset of apoptosis and autophagy, suggesting that Ca^{2+} might play a direct role in these processes [58].

BAPTA abolished calcium influx and significantly reduced autophagy and apoptosis.

(12) Employing 3-methyladenine, a PI3K inhibitor and specific inhibitor of the autophagy pathway, enhanced the apoptotic level [59].

These findings argue against a definitive distinction between type I (apoptotic) and type II (autophagic) cell death. The two pathways are linked in a see-saw manner, such that when apoptosis is inhibited, autophagy is initiated as a backup mechanism and *vice versa* [60, 61].

9. Conclusions

HDAC enzymes affect the acetylation status of histones and other important cellular proteins, which have been recognized as potentially useful therapeutic targets for a broad range of human disorders. In particular, HDAC inhibitors have emerged as major pharmacologic agents for cancer therapy. Most likely, these agents will be used in combination with standard treatment regimens. Efforts to further develop these agents should focus on the thorough evaluation of HDAC expression in different human cancers, the comprehensive analysis of the mechanisms of action of various classes of HDAC inhibitors *in vitro*, and the confirmation of recently identified prognosticators of response in clinical settings. However, most HDAC inhibitors target either all or at least a wide range of HDACs. This creates the problem of the unspecific inhibition of several HDACs, while the targeted blockade of specific single HDACs might be more desirable. Class-specific side effects of pan-HDAC inhibitors have been reported, supporting the requirement of selective inhibitor development. The synthesis of HDAC inhibitors that selectively target HDACs relevant to cancer initiation/progression may enhance the antitumor effects while decreasing the systemic toxicities of HDAC inhibition in cancer patients. It can be expected that the tissue- and time-specific disruption of single HDACs will uncover even more the physiological functions of particular HDACs. Although initial results examining HDAC inhibitors, administered either alone or in combination, have been disappointing, attention has now turned to more potent and selective compounds that may prove to be more effective.

Abbreviations

AIF: Apoptosis-inducing factor
APAF-1:   Apoptotic protease-activating factor 1
Atg: Autophagy-related gene
BAPTA: 1,2-bis(o-aminophenoxy)ethane-N,N,N′, N′-tetraacetic acid
Beclin-1:   Bcl-2-interacting protein
CDKs: Cyclin-dependent kinases
ERK: Extracellular signal-regulated kinase
FADD: Fas-associated death domain
HDAC: Histone deacetylase
Htr-A2:   High temperature requirement factor A2
JNK1:   c-Jun N-terminal kinase 1
LAMP2:   Lysosome-associated membrane glycoprotein 2

LC3: Microtubule-associated protein 1 light chain 3
mTOR: Mammalian target of rapamycin
PCD: Programmed cell death
PI3K: Phosphatidylinositol 3-kinase
ROS: Reactive oxygen species
SAHA: Suberoylanilide hydroxamic acid
siRNA: Small interfering RNA
SMAC: Second mitochondria-derived activator of caspase
S6rp: S6 protein of the 40S ribosomal subunit
TRAIL: TNF-related apoptosis-inducing ligand
TSA: Trichostatin-A
XIAP: X-linked inhibitor of apoptosis protein.

Acknowledgments

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