HDAC inhibitors as radiosensitizers

<Review>

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ABSTRACT

Radiation therapy is one of the main anticancer therapies. However, there are considerable numbers of radioresistant carcinoma cells and severe adverse effects occasionally occur after radiation therapy. Therefore, there have been many attempts to identify radiosensitizers. Histone hyperacetylation has begun to receive considerable attention as a potential target for radiosensitization. Inhibitors of histone deacetylase (HDAC) activity are becoming a major tool for modifying histone acetylation status. This article reviews the classification of HDAC inhibitors and their potential as radiosensitizers. We also discuss possible molecular mechanisms by which the HDAC inhibitors radiosensitize cancer cells.

Key words : Histone deacetylase (HDAC) inhibitors, Histone acetylation, JNK, Radiosensitivity, Apoptosis

INTRODUCTION

Radiotherapy is a standard treatment for various malignant tumors. Ionizing radiation (IR) strongly induces growth arrest and/or apoptosis in a variety of cells.[1,2)] However, responses to IR differ markedly among cell types. Importantly, most malignant cells do not show distinct apoptosis immediately after IR exposure. In these cells, even 48 hours after IR exposure, little if any apoptosis is detectable, and thereafter weak apoptosis may occur following multiple cell divisions.[3-5)] Thus, radiotherapy appears to be ineffective for these radioresistant carcinoma cells.

To date, many attempts have been made to explore the roles of radiotherapy in the management of locally advanced tumors. To understand molecular radiobiology, strategies for enhancing tumor radiosensitivity have begun to focus on targeting the molecules regulating cellular responses after irradiation. Chemotherapeutic agents that enhance the effectiveness of radiation treatment are termed radiosensitizers,

which make cancer cells more susceptible to IR -induced death than surrounding normal cells, and several such compounds are now available for the treatment of solid tumors[6].

Modification of histone acetylation is one of the potential targets to enhance radiosensitivity, since histone hyperacetylation leads to loose chromatin structure which may affect susceptibility to DNA damage (Fig. 1)[7, 8]. Since histone acetylation levels are determined by the balance of histone acetylases (HATs) and histone deacetylases (HDACs) activities, inhibition of HDAC activity may enhance radiosensitivity. In this context, considerable efforts have been put into the development of novel HDAC inhibitors, which often strongly induce apoptosis in tumor cells by themselves. Although extensive preclinical studies have already revealed that many HDAC inhibitors have antitumor activity and promising anticancer effects[9], a limited numbers of recent studies discovered that some HDAC inhibitors can significantly enhance radiosensitivity. Importantly, a structurally diverse set of HDAC inhibitors has similar radiosensitizing effects in a variety of cancer cell types. Moreover, their radiosensitizing effects actually increased the anticancer effects of radiotherapy in several human tumor xenografts. Therefore, HDAC inhibitors are not only promising anticancer agents as single modalities or in combination with chemotherapeutic agents, but also promising radiosensitizers. We here review biological activities of HDAC inhibitors as radiosensitizers.

CLASSIFICATIONS OF HDAC INHIBITORS

There are ranges of structurally diverse HDAC inhibitors which are either products isolated from natural sources or synthetically produced compounds. These compounds can be divided into six groups based on their structure (Table 1). HDAC inhibitors include the short chain fatty acids, sodium butyrate (NaB), phenyl butyrate and valproic acid (VPA) [10], the hydroxamic acids, suberoylanilide hydroxamic acid (SAHA), trichostatin A (TSA)[11], LAQ824, LBH 529 and PXD101; the tetrapeptides, FK228, Apicidine and Trapoxin; the benzamides, MS-275 and CI-994 (N-acetyldinaline); and Ketones, Trifluoro-methyl ketone.

Although these HDAC inhibitors have their own structures, their inhibitory action is quite similar. The identified mechanism of the hydroxamic acid groups is to interact with the catalytic site of HDAC, and thereby block binding of substrates to active sites of HDACs[11]. Synthetic benzamides have a polar end to bind zinc ion within the HDAC catalytic pocket structure and inhibit their activity. Other HDAC inhibitors also bind to HDAC active site powerfully and inhibit HDAC activity.

Short-chain fatty acids are important, since they are abundantly found in feces and thus play major roles in anticancer activity of diet[12]. However, the action of these compounds is non-

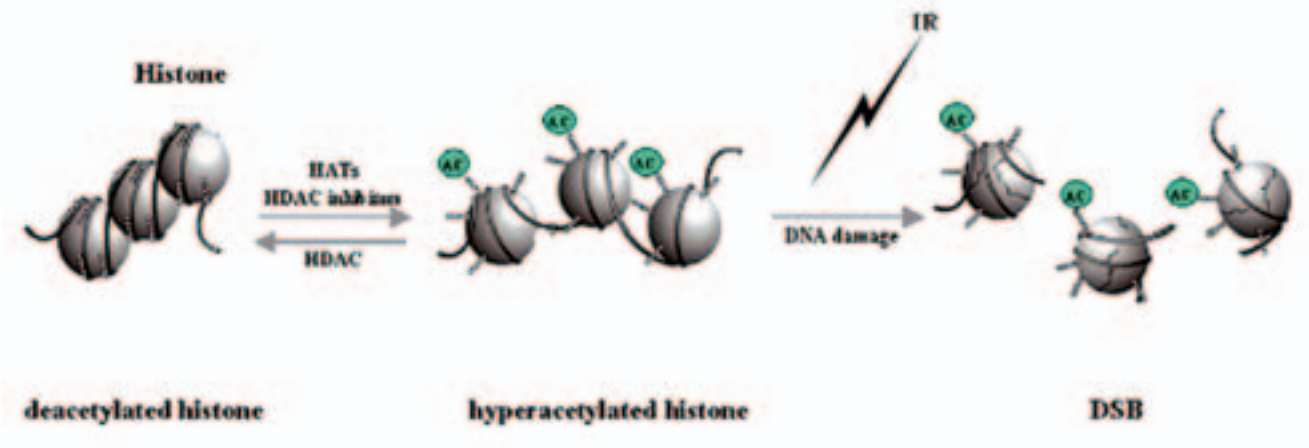

Fig. 1 Histone hyperacetylation induces relaxation of chromatin structure

Table 1

Group	Compounds
Short-chain fatty acids	Valproic acid (VA) Phenyl butyrate (PB) Phenyl acetate (PA) Sodium butyrate (SB)
Hydroxamic acid-derived compounds	Trichostatin (TSA) Suberoylanilide hydroxamic acid (SAHA) M-carboxycinnamic acid bis-hydroxamide (CBHA) Azelaic bis-hydroxamic acid (ABHA) NVP-LAQ824 LBH589 Oxamflatin PXD101 Scriptaid Pyroxamide
Cyclic tetrapeptides	Depsipeptide (FK228, FR901228) Apicidine Trapoxin HC-toxin Chlamydocin Depudesin CHAPS
Synthetic pyridyl carbamate derivative	AN-9 (Pivanex)
Synthetic benzamides	MS-275, CI-994 (N-acetyldinaline)
Ketones	Trifluoromethyl ketone a-ketomides

specific and weak. It is thus hard to achieve effective inhibiting concentrations in vivo. Among the members, VPA is a unique HDAC inhibitor. VPA is currently marketed as an anti-epilepsy agent and anti-migraine headaches. It has recently been discovered to inhibit HDAC activity at clinically applicable concentrations. Interestingly, VPA inhibits the class I HDAC activity better than class II HDAC[11], but its clinical trial shows that VPA alone has only a weak anticancer effect in patients with acute myeloblastic leukemia. When VPA was combined with the demethylating agent, 5-aza-2'deoxycitabine, a significant number of refractory leukemia patients achieved a complete remission[13]. More precise studies are required for evaluation of its clinical efficacy.

MOLECULAR MECHANISMS OF RADIOSENSITIZING EFFECT OF HDAC INHIBITORS

Some HDAC inhibitors have been shown to radiosensitize a broad array of cancer cells[14-17]. HDAC inhibitors are unique anticancer drugs, since they induce histone hyperacetylation and activate some transcription factors, but do not affect DNA itself[17-19], while many anticancer agents are genotoxic and interfere with DNA synthesis. This suggests that HDAC inhibitors

may not increase the occurrence of secondary malignancies after radiation therapy. In this context, development of HDAC inhibitors-mediated enhanced radiation therapy is important. To establish a combination therapy, the precise molecular mechanisms by which HDAC inhibitors enhance anticancer activity of radiation therapy must be discovered. Although the precise mechanisms remain uncertain, several possible explanations have been suggested as described below (Fig. 2).

First, K. Camphausen and et al[18] investigated the effects of MS-275 on the radiosensitivity of two human tumor cell lines, and found that MS-275-mediated enhanced radiosensitivity is correlated well with histone hyperacetylation and prolonged phosphorylation of histone H 2AX (H2AX) and its foci. This suggests a decrease in the repair of radiation-induced DNA double-strand breaks (DSBs). In addition, A. Munshi, et al[19] reported that NaB and TSA radiosensitized two melanoma cell lines, A375 and MeWo, and the radiosensitization correlated with functional impairment of DNA repair as determined by the host cell reactivation assay. Moreover, NaB significantly reduced the expression of the repair-related genes Ku70 and Ku86 and DNA-dependent protein kinase catalytic subunit in melanoma cells at the protein and mRNA levels. They also found that γ-H2AX foci persisted longer after ionizing exposure in the NaB-treated cells than in untreated cells. These accumulating data strongly suggest that HDAC inhibitors can inhibit DNA repair activity and

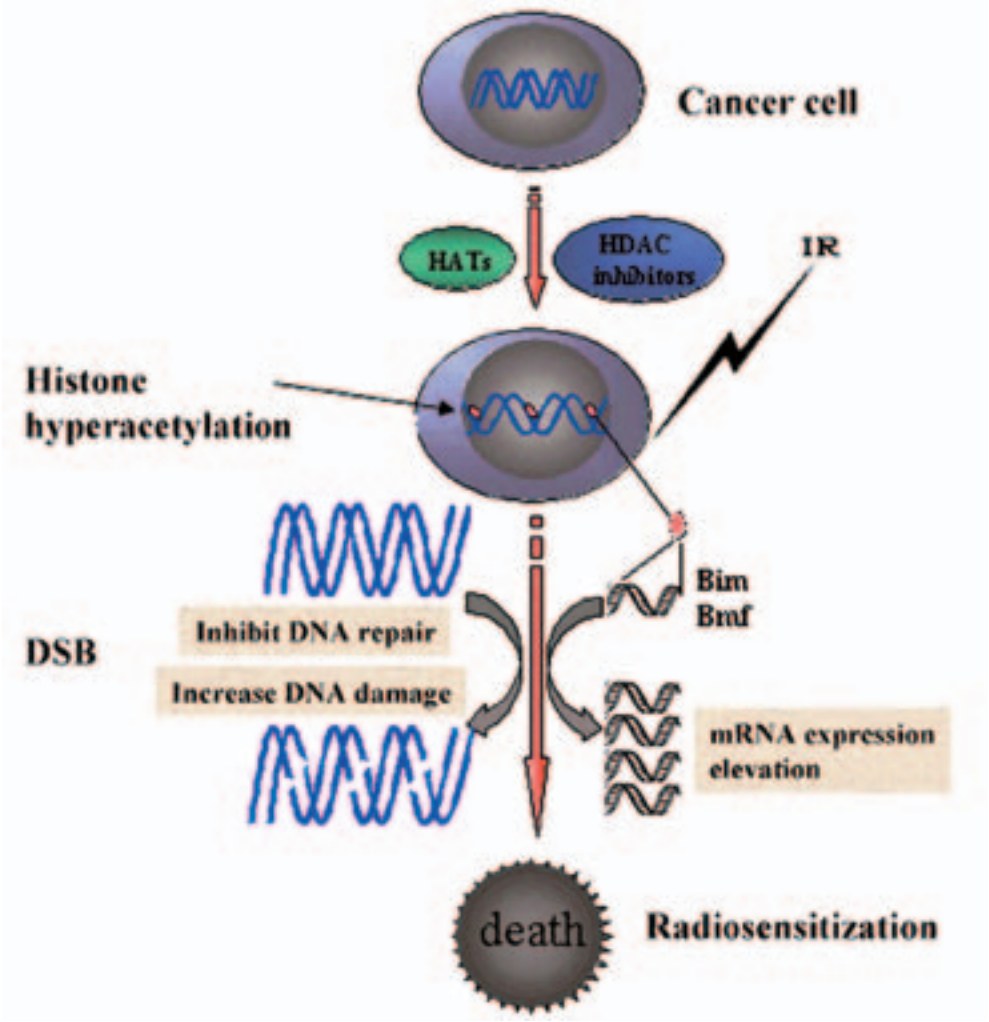

Fig 2 Possible mechanisms of HDAC inhibitor-mediated sensitization to radiation

enhance the anticancer effect of radiation.

Second, eukaryocytic DNAs are compacted with histones and other accessory proteins, while histone hyperacetylation may loosen compact DNA, allowing DNA to become more susceptible to damage [7, 8]. Consistent with this data, CBHA strongly increased γ-H2AX and its foci formation immediately after IR exposure[20]. Another HDAC inhibitor, FK228, similarly enhanced IR-induced γ-H2AX within 1 hour after IR. Importantly, the enhanced γ-H2AX was initially diminished and thereafter increased at 12 hours after IR. These data suggest that HDAC inhibitors may enhance IR-induced DNA damage itself.

Third, it is well known that histone hyperacetylation alters chromatin structure and increases the transcription activity of selected genes. To date, many apoptosis-related genes have been identified as target genes of HDAC inhibitor-mediated signals. Cyclin-dependent kinase inhibitor $p21^{WAF1}$ and gelsolin are clearly induced by HDAC inhibitors and their induction is suggested to be important for antiproliferative action or inhibition of tumor progression[21-23]. Interestingly, HDAC inhibitors can also activate the proapoptotic activity of BAX by acetylation of Ku70, which can abolish their physical interaction and allow mitochondrial targeting of BAX[24]. We and others found that several HDAC inhibitors increase expression of a pro-apoptotic BH3-only protein Bim[14, 25] and its related molecule Bmf[26], and suggestted their crucial role in their proapoptotic activity. Importantly, knockdown of Bmf expression strongly inhibits IR-induced apoptosis[27]. These accumulating data suggest that an increase in these BH3-only proteins may be one of the central mechanisms of IR-induced apoptosis.

CONCLUSION

We strongly suggest that HDAC inhibitors are promising radiosensitizers. The agents are not genotoxic and thus might not enhance secondary transformation after IR therapy. In addition, HDAC inhibitors cooperatively increase IR-induced DSB and induce apoptosis-related genes, especially Bim and Bmf. Our knowledge is obviously limited and the efficacy of combination therapy has been demonstrated only in mice models. However, these accumulating information may help us to develop a more potent HDAC inhibitors-combined IR therapy.

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