Histone Deacetylase Inhibitors
—Promising Agents for ‘Gene-Regulating Chemoprevention’ and ‘Molecular-Targeting Prevention’ of Cancer—

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Abstract

One of the best approaches against cancer is prevention. Inactivation of the p53 or p16INK4a genes has been extensively reported in most human cancer cells. Both p53 and p16INK4a function as tumor suppressors. Therefore, functional restoration of these molecules is considered to be one of the most useful methods for cancer prevention and therapy. We have proposed a concept termed ‘gene-regulating chemoprevention and chemotherapy’ regarding the above pathway. This concept assumes that transcriptional regulation by drugs on tumor-suppressor genes, downstream target genes or functionally similar genes (for example, family genes) of the tumor-suppressor genes would contribute to the prevention of human malignancies. Histone deacetylase (HDAC) inhibitors have been shown to be potent inducers of growth arrest, differentiation and apoptotic cell death. Previously, we demonstrated that HDAC inhibitors, such as sodium butyrate and trichostatin A (TSA), transcriptionally induce the cyclin-dependent kinase inhibitor p21WAF1/Cip1, a downstream target gene of p53, in a p53-independent manner. Furthermore, we have recently shown that HDAC inhibitors activate Gadd45, another downstream target gene of p53, and p19INK4d, a gene functionally similar to p16INK4a. Our results, taken together with previous findings, suggest that HDAC inhibitors may be one of the most attractive and promising agents for ‘gene-regulating chemoprevention’ and ‘molecular-targeting prevention’ of cancer.

Key words: histone deacetylase inhibitors, prevention, p53, p16INK4a, p21WAF1/Cip1, Gadd45, p19INK4d

Introduction

Recent progress in cancer research has clarified many of the molecular mechanisms involved in human carcinogenesis (1). However, overall mortality statistics are unlikely to change fundamentally unless cancer prevention is fully implemented (2). One of the best approaches against cancer is prevention. Chemoprevention, which uses pharmacological agents to preclude carcinogenesis, is one way of accomplishing cancer prevention. The success of chemoprevention depends on a mechanical understanding of carcinogenesis at the molecular level. Cancer arises through the accumulation of genetic abnormalities that enhance the growth or survival of developing tumor cells (1). Inactivation of p53 or p16INK4a is most prevalent in human cancer (3, 4). From further analysis, p53 and p16INK4a have been proven to be the most important molecules possessing tumor-suppressive function (3, 4). In other words, inactivation of these molecules is a critical event in human tumorigenesis.

Importance of inactivation of the p53 and p16INK4a genes in human carcinogenesis

The tumor-suppressor gene p53 is most frequently mutated in human malignant tumors (3). Furthermore, individuals with germ-line mutations of p53 are susceptible to many types of malignancies including brain cancer, leukemia, sarcomas and breast cancer, and the familial syndrome is called Li-Fraumeni syndrome (5). Mice with p53 gene deletions develop normally but are highly prone to tumor development. Indeed, p53 is not
required for normal cell growth, but acts to prevent cell proliferation under circumstances of cellular stresses (6). Hence, expressions of p53 increase following DNA damage, certain oncogenic stimuli, hypoxia and a variety of other cellular stresses. Activation of p53 prevents cell proliferation by inducing either cell-cycle arrest or apoptosis (6). The p53 protein is a sequence-specific DNA-binding transcription factor, and activates the transcription of genes implicated in cell cycle arrest and apoptosis, such as p21WAF1/Cip1, Bax, Gadd45, DR5, Siah-1, Noxa, AIP1 and PUMA. In particular, expression of the cyclin-dependent kinase inhibitor p21WAF1/Cip1 is transcriptionally activated by p53 and contributes to p53-dependent cell-cycle arrest by converting the retinoblastoma gene (RB) product to the active unphosphorylated form (6).

Inactivation of the p16INK4a gene, through gene deletions, point mutations or transcriptional silencing by methylation of the promoter is one of the most frequent defects contributing to human oncogenesis (4). Furthermore, p16INK4a-deficient mice are susceptible to several types of malignancies, and germ-line mutations in humans are associated with familial syndromes involving malignant melanoma and pancreatic cancer (7). p16INK4a negatively regulates the cyclin D-cyclin dependent kinase (CDK) 4/6 complexes, thereby suppressing phosphorylation of the RB tumor-suppressor gene product and inhibiting G1/S transition. The p16INK4a homologs, p15INK4b, p18INK4c and p19INK4d, have recently been identified (4). All of them bind directly to CDK4/6 and are therefore specific inhibitors of the cyclin D-CDKs. The four members, p15INK4b, p16INK4a, p18INK4c and p19INK4d, are called the INK4 family proteins.

**Gene-regulating chemoprevention**

Having understood the importance of p53 and p16INK4a in human carcinogenesis, functional restoration of p53 or p16INK4a is considered to be one of the most promising methods for cancer prevention and therapy. We have proposed that transcriptionally-regulating agents of tumor-suppressor genes, downstream target genes or functionally similar genes of the tumor-suppressor genes would be useful for the prevention of malignancies, which we term ‘gene-regulating chemoprevention’ (8). In the case of transcriptional silencing by methylation of the promoter (for example, p16INK4a), treatment with demethylating agents could restore the expression of the inactivated tumor-suppressor genes (9). If the tumor-suppressor genes themselves are abrogated by gene deletions or point mutations, stimulation of the expressions of downstream target genes of a tumor-suppressor gene (for example, p15INK4b, p18INK4c and p19INK4d as a family of p16INK4a) could compensate for the loss of function of the tumor-suppressor genes. On the basis of this concept, our extensive research has so far clarified that histone deacetylase (HDAC) inhibitors represent one class of transcriptionally-regulating agents of downstream target genes or functionally similar genes of tumor-suppressor genes (10). We describe these findings together with recent progress in this mini-review.

**Histone deacetylase (HDAC) inhibitors as promising agents for gene-regulating chemoprevention**

Histones are part of the core proteins of nucleosomes. Acetylation and deacetylation of these proteins play a role in the regulation of gene expression (11). Highly positively-charged hypoacetylated histones bind tightly to the phosphate backbone of DNA and suppress transcription, because transcriptional factors and RNA polymerase can not have access to the DNA. Acetylation neutralizes the charge of the histones and generates a more open DNA conformation, enabling transcriptional factors access to the DNA. Histones of the nucleosomes can be acetylated and deacetylated, and the amount of acetylation is controlled by the opposing activities of two types of enzymes, histone acetyltransferase (HAT) and HDAC. During the last decade, a number of HDAC inhibitors have been identified (11). HDAC inhibitors induce growth arrest, differentiation and apoptotic cell death in cancer cells. These agents also inhibit the growth of cancer cells in animal models.

Butyrate, a short-chain fatty acid, represents one class of HDAC inhibitors, and is considered to be an important molecule for preventing colorectal cancer (10, 12). Butyrate is one of the most abundant short-chain fatty acids in the large intestine and is generated by bacterial fermentation of dietary fibers (12). Previously, McIntyre et al. induced tumors with dimethylhydrazine in rats, and assessed the impact of different fiber-containing diets, such as wheat bran, guar or oat bran, on the number and size of tumors (13). Significantly fewer tumors were seen in the rats fed with wheat bran compared with those fed guar or oat bran, and the total tumor mass was lowest in rats fed with wheat bran. The concentration of butyrate in stools inversely correlated with tumor mass. Thus, the type of fiber, which is associated with high butyrate concentrations in the distal large bowel, is protective against colorectal cancer in an animal model. Moreover, high acetate and low butyrate ratios have been found in enema samples from patients with adenomatous polyps and colon cancer (14). These results suggest that