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Histone Deacetylase Inhibitors Repress Tumoral Expression of the Proinvasive Factor RUNX2

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Abstract

Aberrant reactivation of embryonic pathways occurs commonly in cancer. The transcription factor RUNX2 plays a fundamental role during embryogenesis and is aberrantly reactivated during progression and metastasization of different types of human tumors. In this study, we attempted to dissect the molecular mechanisms governing RUNX2 expression and its aberrant reactivation. We identified a new regulatory enhancer element, located within the *RUNX2* gene, which is responsible for the activation of the *RUNX2* promoter and for the regulation of its expression in cancer cells. Furthermore, we have shown that treatment with the anticancer compounds histone deacetylase inhibitor (HDACi) results in a profound inhibition of RUNX2 expression, which is determined by the disruption of the transcription-activating complex on the identified enhancer. These data envisage a possible targeting strategy to counteract the oncongenic function of RUNX2 in cancer cells and provide evidence that the cytotoxic activity of HDACi in cancer is not only dependent on the reactivation of silenced oncosuppressors but also on the repression of oncogenic factors that are necessary for survival and progression.
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Introduction

RUNX2, a transcription factor belonging to the Runt-related family, is necessary for osteoblast differentiation and skeletal morphogenesis (1, 2). Factors that are crucial during embryogenesis are often hijacked during cancer progression, and RUNX2 is not an exception. RUNX2 is increasingly recognized in cancer biology for its oncogenic properties and a large number of articles link the deregulation of RUNX2 expression with progression and metastasization of different types of epithelial tumors (1, 3–18). Regulation of *RUNX2* may occur at multiple levels and through multiple signaling pathways. However, the mechanisms that regulate the reactivation of this factor in cancer are still unknown. It has been reported that RUNX2 expression is higher in tumor than in normal tissue and that the level of expression of this protein has a negative prognostic value in a number of cancer types (16, 19–22). The *RUNX2* gene encodes for two isoforms starting from two separate promoters: the proximal P2 promoter expresses RUNX2 isoform I and the distal P1 promoter expresses isoform II (23, 24). The two isoforms differ for only a few aminoacids at the N-terminal regions, even though differences in their activity were reported. The existence of two alternative promoters suggests that the expression of the two isoforms is regulated through different mechanisms (24–27). Indeed, evidence exists that the use of the two promoters is context dependent. In particular, our group and others have shown that RUNX2 isoform I is the major RUNX2 isoform in tumor cells, while isoform II seems to be restricted to skeletal cells (16, 26). Noticeably, the majority of molecular signals known to control *RUNX2* act through the P1 promoter, while the regulation of the P2 promoter remains largely unknown (28–30). Dissecting the mechanisms controlling *RUNX2* P2 promoter is a necessary step to elucidate the complex network of molecular determinants that govern RUNX2 expression in tumor and it may help designing an appropriate therapeutic approach to counteract the prometastatic function of RUNX2.

Histone deacetylase inhibitors (HDACi) are a class of chemical compounds that block the activity of Zn-dependent HDAC, inducing the hyperacetylation of a number of proteins (31). Histone acetylation is associated with open chromatin structure and active transcription. Thus, it is believed that the major anticancer effect of HDACi is due to the reactivation of silent oncosuppressor genes. However, increasing evidence indicates that, besides histones, HDACi treatment affects acetylation and function of a number of nonhistonic proteins, opening new explanations to the mechanisms of action of these cancer drugs (32). Recent studies have shown that HDACi suppress the expression of a number of oncogenes that are highly expressed in cancer, supporting the hypothesis that besides reinducing suppressor genes, these drugs may impair cancer cell growth by blocking oncogenic signals that are necessary for tumor survival and progression (33–35).

In this study, we demonstrate for the first time that HDACi profoundly impairs the expression of RUNX2 isoform I in tumor cells. In the attempt to dissect the mechanism responsible for the HDACi-dependent RUNX2 inhibition, we identified a new enhancer within the *RUNX2* gene, which is responsible for the activation of the P2 promoter in tumor cells and contains the HDACi-responsive elements. We have shown that c-JUN binds to the ENH3 and is a positive regulator of RUNX2 expression.

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Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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