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Histone acetyltransferase p300 promotes the activation of human *WT1* promoter and intronic enhancer

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Abstract

The Wilms' tumor gene-1 (*WT1*) encodes a zinc finger protein involved in gene regulation during kidney, gonad, and heart development. In addition to its promoter, a 258 bp intronic enhancer is required for tissue-specific expression of *WT1* gene. p300 is a histone acetyltransferase (HAT) and exerts essential functions in gene regulation. Here, we show that p300 increased the expression of endogenous *WT1* mRNA and promoted the activation of the *WT1* promoter and intronic enhancer. The results also revealed that the adenovirus E1A repressed the p300 function, while the p300-binding defective E1A delta 2-36 did not, and p300 HAT activity was important for its function since p300 mutant with the HAT domain deleted partially abrogated its ability to activate the *WT1* promoter and intronic enhancer. Furthermore, p300 and c-Myb synergistically activated the expression of *WT1* gene. This study revealed that p300 and its HAT activity were involved in regulation of *WT1* transcription.

Keywords: Histone acetyltransferase; *WT1*; Transcription regulation; p300; c-Myb

Wilms' tumor or nephroblastoma is one of the commonest pediatric malignancies that occur in embryonic kidney and account for approximately 7.5% of all childhood tumors [1]. The Wilms' tumor gene, *WT1*,[2] was isolated by positional cloning in 1990 [2] and has been mapped to human chromosome 11p13. *WT1* is a tumor suppressor gene in that mutations are not only associated with Wilms' tumor but also with other tumors, including mesotheliomas, juvenile granulose cell tumor of the ovary, and secondary acute myelogenous leukemia. In addition, *WT1* plays a crucial role in early urogenital development [3]. *WT1* is expressed in normal fetal spleen and kidney cells, bone marrow, immature leukemic cells, and cells of the genitourinary system [2,4–6]. It has been reported that *WT1* gene is down-regulated during terminal differentiation of both K562 cells (a human erythroleukemia cell line derived from a patient with BC-CML) and HL60 cells (a human myelocytic leukemia cell line) [7,8]. Therefore, *WT1* gene is associated with the immature cells from which leukemic cells originate. It has been demonstrated that in addition to the promoter, a 258 bp enhancer in intron 3 is required for tissue-specific expression of *WT1* gene. Sequence analysis showed that this 258 bp fragment contains many potential binding sites for transcription factors, including Ets-1, GATA, c-Myb, and AP-2. Cotransfection experiments showed that c-Myb transactivated the enhancer [3], suggesting that it may play an important role in controlling *WT1* expression.

In eukaryotes, DNA is packaged into nucleosomes, which are further organized into chromatin fibers. Nucleosomes are thought to be barriers for the access of the transcription machinery to the promoter and enhancer of

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[2] *Abbreviations used:* CREB, cAMP-response element-binding protein; CBP, CREB-binding protein; HAT, histone acetyltransferase; *WT1*, Wilms' tumor gene-1; RT-PCR, reverse transcription-polymerase chain reaction.

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a gene. To overcome this repressive barrier, the cell contains numerous multisubunit chromatin-remodeling complexes that act by covalently modifying core histones, giving rise to a more accessible nucleosomal configuration. The best-studied histone modification is the acetylation of core histone tails, catalyzed by histone acetyltransferases (HATs) [9]. p300 and CBP are highly homologous coactivators that have acetyltransferase activity [10]. CBP was identified in 1993 as an interaction partner for the CREB transcription factor [11], and soon thereafter, the p300 cDNA encoding the 300 kDa protein known to be associated with the adenoviral protein E1A was cloned [12]. The p300/CBP cofactors promote gene transcription by bridging between DNA-binding transcription factors and the basal transcription machinery, or by providing a scaffold for integrating a variety of different proteins. They have also been shown to be able to acetylate histones and transcription factors through their HAT activity. p300 and CBP play essential roles in virtually all known cellular processes, including the decisions to grow, to differentiate or to undergo apoptosis [13,14]. A number of different signaling pathways can induce the binding of transcription factors to p300/CBP. Furthermore, some viral oncoproteins, such as adenovirus E1A and Simian virus (SV) 40 large T antigen, are capable of interacting with p300/CBP and thereby obstruct the function of cellular transcription factors by blocking p300/CBP functions [15]. p300/CBP can acetylate nucleosomal proteins [16,17] and the acetylation of histone tails has a significant impact in regulating transcription [18,19]. Although it has not been known whether the p300/CBP HAT activity regulates transcription by chromatin remodeling, a growing body of evidence suggests that other proteins, including transcription factors and the transcription apparatus [20–26], are also regulated by acetylation modification, thereby highlighting the potential importance of HAT activity in p300/CBP function. Numerous transcription factors, including Ets-1, c-Myb, and GATA family members [20,27,28], can interact, either directly or through coactivators, with p300/CBP and these interactions stimulate transcription of specific genes. These transcription factors can regulate *WT1* gene expression by transactivating the intronic enhancer of *WT1* [3]. This raises the question whether p300 and CBP may also be able to regulate *WT1* expression. To address this possibility, we performed transient transfection studies with 293T embryonic kidney cells, and the results are presented as follow.

Materials and methods

Reagents

Cell culture reagents were provided by Gibco-BRL, and cell culture plasticware was from Corning.

Constructs

The expression vectors containing the wild-type (wt) p300 (pCI-p300) and its HAT deletion derivative (pCI-p300 HAT delta 1472-1522) were kindly provided by Joan Boyes (Institute of Cancer Research, UK). The E1A 12S and E1A (12S) delta 2-36 constructs were generous gifts of Elizabeth Moran (Temple University, Pennsylvania). The plasmids expressing human c-Myb, mouse GATA-1, and pGL3-Basic vector were kind gifts from Odd S. Gabrielsen (University of Oslo, Norway), Alan Forster (Medical Research Council Laboratory of Molecular Biology, UK), and Maty Tzukerman (Technion-Israel Institute of Technology), respectively. For construction of the −480/+251 *WT1* promoter-driven luciferase plasmid containing the 258 bp *WT1* intronic enhancer (pGL3-P-E-Luc), the *WT1* promoter fragment was amplified by polymerase chain reaction (PCR) using the following primers: 5′-GGGTGCAAAGCAAGG GTA-3′ (sense) and 5′-CGGAGCGTGTGCTGAGAC-3′ (antisense). The fragment was cloned into pUCm-T vector (Sangon) and, after cutting with *Not*I and *Xho*I, inserted into pREP4-Luc vector (Promega). The *WT1* promoter fragment was then cut from the vector with *Nhe*I and *Xho*I, and inserted into pGL3-Basic, creating the *WT1* promoter/luciferase plasmid (pGL3-P-Luc). Meanwhile, the *WT1* intronic enhancer fragment was amplified with the primers 5′-GTCGACAAGCTTTTC CCCGCTCCG-3′ (sense) and 5′-GTCGACGGATCC CCGAGCACCAG-3′ (antisense). The fragment was also cloned into pUCm-T vector, and after cutting with *Sal*I, inserted into pGL3-P-Luc, creating the plasmid pGL3-P-E-Luc containing both the *WT1* promoter and intronic enhancer. The correct orientation of insertion was confirmed by restriction mapping. The plasmid pGL3-P-E-Luc was used in all the transfection experiments unless indicated otherwise.

Cell line and cell culture

293T human embryonic kidney epithelial cells were maintained in IMDM supplemented with 10% FBS, 100 μg /ml penicillin, and 100 μg/ml streptomycin.

Transient transfection and luciferase assay

293T cells were transiently transfected using a conventional calcium phosphate coprecipitation method with 2.5×10^5 cells and 1μg DNA unless indicated otherwise. Five to eight hours after transfection, cells were washed with fresh medium and incubated for additional 19–25 h at 37 °C before harvest. As a transfection control for the luciferase assays, the *Renilla* luciferase control plasmid pREP7-RLuc was cotransfected in all experiments. Cell lysates were collected and luciferase activities were measured with a Turner Designs TD-20/20 Lumi-