

Functional analysis of HBO1 in tumor development and inhibitor screening

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Abstract. The aim of the present study was to explore the functions of histone acetyltransferase binding to origin recognition complex (ORC) 1 (HBO1) during tumor development and to screen for HBO1 inhibitors. The chromatin immunoprecipitation sequencing (ChIP-seq) data of HBO1 in the RKO human colon cancer cell line (GSE33007) were downloaded from the Gene Expression Omnibus (GEO) database. The reads were then mapped back to a reference genome hg19. The PCR duplicate reads were removed by using SAMtools software and the shift was calculated using SPP and MASC software. The peak calling was carried out using MACS 1.4.0 software. Furthermore, the inhibitors of HBO1 were screened out from the Specs database using DOCK 6.6 software. The binding sites of HBO1 were mainly distributed in the intergenic, intronic and 3'-end regions. Further analysis revealed that a total of 9,467 target genes was identified around HBO1 binding sites in the RKO cell lines and those genes mainly participated in the cell cycle, biosynthetic process, as well as other processes. Finally, 5 inhibitors with best binding affinity in the positively charged cavity of HBO1 were screened out: i) 5-[(2-hydroxybenzylidene)amino]-2-(2-{4-[(2-hydroxybenzylidene)amino]-2-sulfonatophenyl}vinyl)benzenesulfonate, ii) 3-[4-(3-bromo-4-{2-[4-(ethoxycarbonyl)anilino]-2-oxoethoxy}-5-methoxybenzylidene)-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl]benzoic acid, iii) 4-(4-{3-iodo-5-methoxy-4-[2-(2-methoxyanilino)-2-oxoethoxy]benzylidene}-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)benzoic acid, iv) 5-chloro-1,3-bis{[3,5,6-trihydroxy-4-(octyloxy)tetrahydro-2H-pyran-2-yl]methyl}-1,3-dihydro-2H-benzimidazol-2-one and v) 4-{[4-(tetradecylamino)-1-naphthyl]diazenyl}benzoic acid. As a whole, in this study, we identified the possible binding sites and biological functions of HBO1. The potential inhibitors of HBO1 were also screened, which prove to be helpful for the inhibition of HBO1 during tumor development.

Introduction

Histone modifications, including acetylation, methylation and phosphorylation have been demonstrated to play critical roles in gene-specific transcriptional regulation, DNA replication, DNA damage response and repair (1,2). Acetylation, as the earliest identified histone modification, involves the transfer of an acetyl group from acetyl-CoA to the ϵ -amino group of lysine residue by histone acetyltransferases (HATs) (3).

Histone acetyltransferase binding to origin recognition complex (ORC) 1 (HBO1), belonging to the MYST family, was discovered in the context of DNA replication (3). HBO1, as an H4-specific histone acetylase, can interact with transcription factors (4), mRNA coding regions (5) and ORC (6). Researches have demonstrated that HBO1 is necessary for licensing and DNA replication (7) and that it is associated with replication origins specifically during the G1 phase of the cell cycle (8). Considering the critical role of HBO1 in DNA replication, it has been demonstrated that HBO1 mutations can lead to profound negative consequences, and even to oncogenesis (3). On the one hand, the HBO1 coding region was identified to be a common retroviral integration site (9). As infections with some viruses are considered to lead to oncogenesis, HBO1 may indirectly contribute to the induction of cancer (10). On the other hand, the binding partners of HBO1 are considered to be related to diseases. The interaction of HBO1 with androgen receptor was putatively linked to prostate cancer development (11) and the interaction of HBO1 with nuclear factor κ -light-chain-enhancer of activated B cells (NF- κ B) was connected with tumor suppression (12). Hence, HBO1 is critical in the development of tumors, although the mechanisms involved remain unclear.

In the present study, the possible binding sites of HBO1 on all chromosomes were explored based on chromatin immunoprecipitation sequencing (ChIP-seq) analysis. To explore the regulatory mechanisms of HBO1 during tumor development, the genes that can be regulated putatively by HBO1 were subjected to functional enrichment analysis. Furthermore, the structure and inhibitors of HBO1 were systematically explored, which may prove to be helpful for the discovery of novel anti-cancer drugs.

Data collection methods

ChIP-seq data of HBO1 in the RKO human colon cancer cell line. The ChIP-seq data [GSE33007 (13)] were downloaded

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Key words: histone acetyltransferase binding to origin recognition complex 1, tumor development, transcription factor, inhibitor

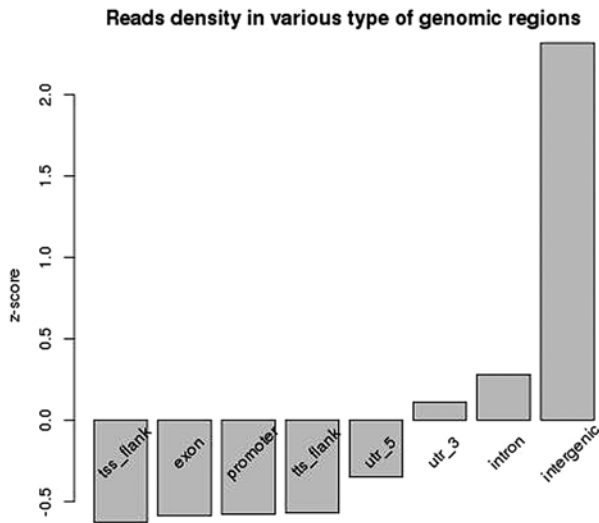


Figure 1. The distribution of histone acetyltransferase binding to origin recognition complex (ORC) 1 (HBO1) on all chromosomes.

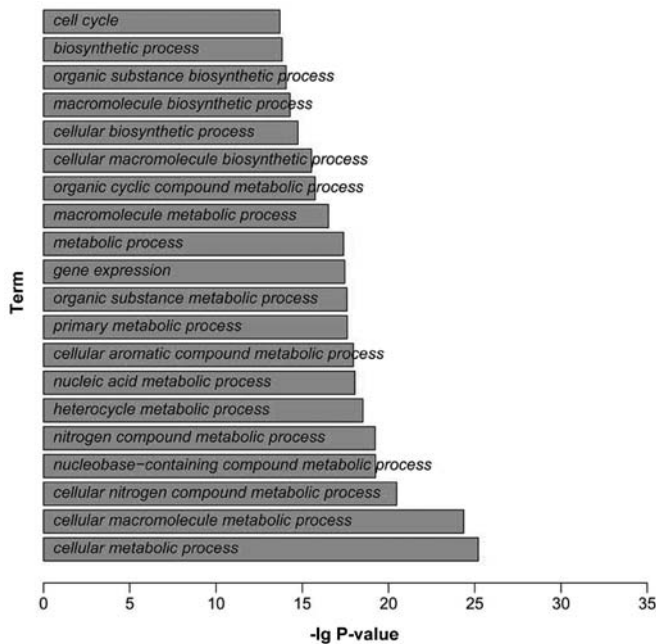


Figure 2. Results of functional enrichment analysis for the histone acetyltransferase binding to origin recognition complex (ORC) 1 (HBO1) target genes (top 20).

from the Gene Expression Omnibus (GEO) (14) database (<http://www.ncbi.nlm.nih.gov/geo/>). The data included two samples: one was obtained in the RKO colon cancer cell line and the other one was obtained in a normal cell line. The samples were sequenced on the Illumina Genome Analyzer IIx platform.

Analysis of ChIP-seq data. The reads were mapped back to a reference genome hg19 from the University of California, Santa Cruz (UCSC) Genome Browser (15) by using Bowtie software (16) (version 0.12.9). The reads were screened out for subsequent analysis with the criteria of unique alignment position and a mismatch number <2 . The PCR duplicate reads were then removed using SAMtools (17) to keep at most one read per

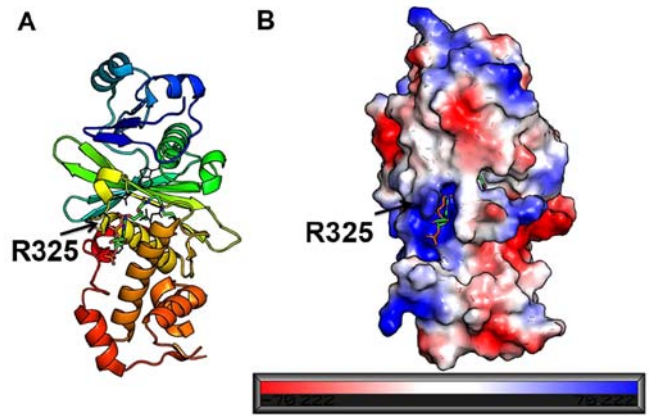


Figure 3. The 3D structure of histone acetyltransferase binding to origin recognition complex (ORC) 1 (HBO1). (A) The complex structure of HBO1 and acetyl-CoA. (B) The surface charge distribution of HBO1. Red represents a negative charge, blue represents a positive charge and white represents hydrophobic regions.

genomic position. The shifts of 5' and 3' are defined as a half of the average size of the ChIP-seq fragments for the experimental and control groups using SPP and MaSC software. The peak calling was carried out using MACS 1.4.0 software (18). The reads with a P-value >0.00001 were considered as the HBO1 binding sites.

Gene functional analysis. The target genes of HBO1 were identified using the ChIPpeakAnno (19) package within R. The functions of those genes were subjected to functional enrichment analysis based on the Gene Ontology (GO) database (<http://www.geneontology.org/>) using the getEnrichedGO module within the ChIPpeakAnno package (19). The criteria were set to a P-value $<2e-14$.

HBO1 structure analysis and inhibitor screening. The 3D structure of HBO1 (PDB ID: 2pq8) was downloaded from Protein Data Bank (PDB; <http://www.rcsb.org/pdb/home/home.do>) and the coenzyme binding site analysis and structure visualization were conducted using PyMOL software (20). The virtual screening of inhibitors was carried out using DOCK 6.6 software (21) following structure preprocessing with Chimera software (22). The force field was set to Amber99sb and the binding sites were set to be within 6 Å around the coenzyme. The Specs compound database was used for the screening and the top 5 compounds with best binding affinity were screened out and analyzed.

Results

HBO1 binding sites. Based on the ChIP-seq analysis, the enriched positions of HBO1 binding sites were mainly distributed in the intergenic, intron and 3'-end regions (Fig. 1).

Identification and functional analysis HBO1 target genes. To explore the regulatory mechanism of HBO1, the HBO1 target genes were identified and subjected to functional enrichment analysis. A total of 9,467 target genes was identified around HBO1 binding sites in the RKO cell line. Functional enrichment analysis revealed that the genes were mainly enriched

in the functions of cell cycle, biosynthetic process, organic substance biosynthetic process and macromolecule biosynthetic process (Fig. 2).

HBO1 structure analysis. HBO1 combined with an acetyl-CoA belongs to the HATs family. The surface of HBO1 was covered by charged residues and the acetyl-CoA was located at a cavity which was full of positively charged residues (Fig. 3). The entrance of the cavity was guarded by a positively charged side chain of R325, promoting the binding of negatively charged compounds.

HBO1 inhibitor screening. Based on the HBO1 3D structure and acetyl-CoA binding site, the inhibitors of HBO1 were screened out from the Specs database. As shown in Fig. 4, a total of 5 compounds with best binding affinity in the cavity were screened out: i) 5-[(2-hydroxybenzylidene)amino]-2-(2-{4-[(2-hydroxybenzylidene)amino]-2-sulfonatophenyl}vinyl)benzenesulfonate, ii) 3-[4-(3-bromo-4-{2-[4-(ethoxycarbonyl)anilino]-2-oxoethoxy}-5-methoxybenzylidene)-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl]benzoic acid, iii) 4-(4-{3-iodo-5-methoxy-4-[2-(2-methoxyanilino)-2-oxoethoxy]benzylidene}-3-methyl-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)benzoic acid, iv) 5-chloro-1,3-bis{[3,5,6-trihydroxy-4-(octyloxy)tetrahydro-2H-pyran-2-yl]methyl}-1,3-dihydro-2H-benzimidazol-2-one and v) 4-{[4-(tetradecylamino)-1-naphthyl]diazenyl}benzoic acid. The structures of the 5 compounds are depicted in Fig. 5 and the corresponding

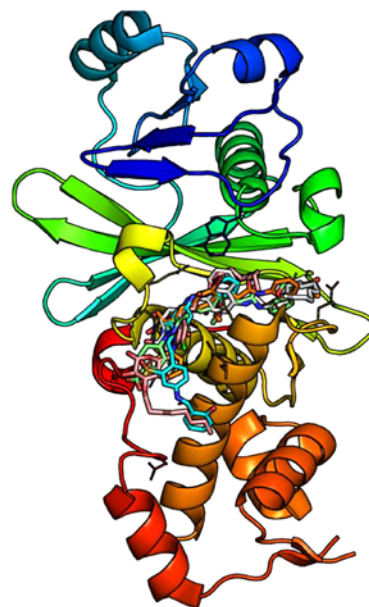


Figure 4. The inhibitors and histone acetyltransferase binding to origin recognition complex (ORC) 1 (HBO1) complex structure. The inhibitors are shown as sticks and the critical residues around the binding site are shown as lines and the rest of HBO1 are shown as squiggly-looking shapes.

Specs IDs are AO-861/15351016, AO-763/14815006, AA-516/33241036, AK-968/13031190 and AK-968/13031237, respectively.

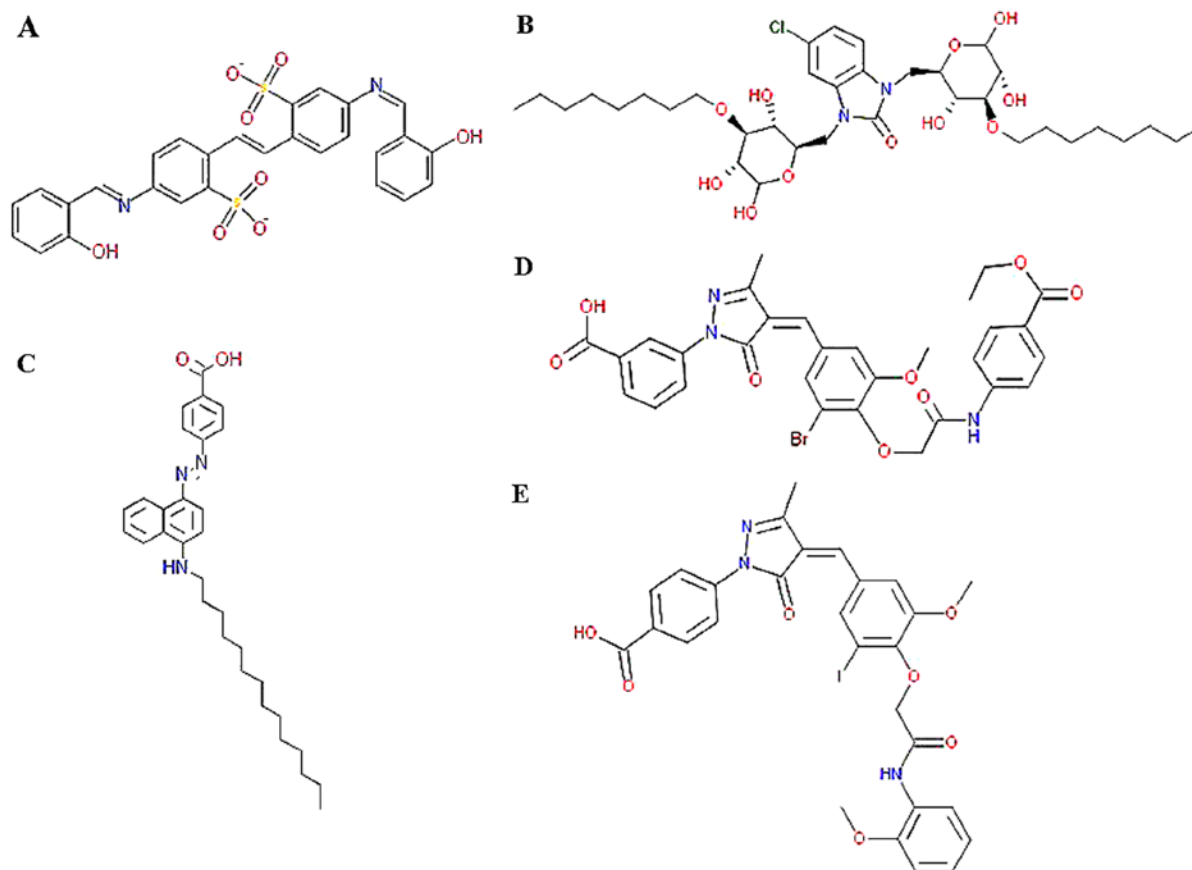


Figure 5. Structure of the 5 inhibitors. The SPECS IDs for the 5 inhibitors were (A) AO-861/15351016, (B) AO-763/14815006, (C) AA-516/33241036, (D) AK-968/13031190 and (E) AK-968/13031237.

Discussion

HBO1 has been demonstrated play critical roles in genome expression and maintenance (3). In this study, we explored the possible binding sites of HBO1 on all chromosomes. By focusing our efforts on the functions of HBO1, the genes that can be regulated by HBO1 were identified and subjected to functional analysis. Furthermore, the coenzyme binding site and surface charge distribution of HBO1 were explored and the potential inhibitors were screened out.

Based on the results of ChIP-seq analysis, HBO1 mainly binds upstream of gene transcription start sites and putatively enhances gene expression. Experiments have indicated that a strong enrichment of HBO1 near the homeobox A9 (HOXA9) and homeobox A10 (HOXA10) genes and the expression of HOXA10 is found to be downregulated upon the knockdown of HBO1 (13). ChIP analysis has also revealed that HBO1 is highly enriched throughout the coding regions of a large number of human genes, from the middle of the genes to the transcription end sites or centered around the transcription start sites (5). The critical role of HBO1 in gene transcriptional regulation implies that HBO1 probably participates in the regulation of tumor development.

The target genes of HBO1 were significantly related to the cell cycle process, based on functional enrichment analysis. The association of HBO1 with various inhibitor of growth (ING) tumor suppressor proteins suggests that it plays critical roles in gene regulation and DNA replication through histone H3 and H4 acetylation (13). Genomic analysis has indicated that the HBO1 and ING complexes are mainly involved in the regulation of the p53 pathways (13). Histone acetylase defective mutant experiments have also demonstrated that HBO1 acetylase activity is necessary for replication licensing. HBO1 can regulate the expression of cell division cycle 27 (CDC27), cyclin-dependent kinase inhibitor 2D (CDKN2D) and cell division cycle 20 (CDC20), which can participate in cell cycle progression. The protein encoded by CDC27 is a component of anaphase-promoting complex (APC) (23). Experiments have shown that the interaction between this protein and mitotic checkpoint proteins, such as mitotic arrest deficient 2 (Mad2), p55 cell-division cycle protein 20 (p55CDC) and BUB1 mitotic checkpoint serine/threonine kinase B (BUBR1) are involved in controlling the timing of mitosis (24). The protein encoded by CDKN2D can suppress the activation of the CDK kinases and regulate cell cycle G1 progression by forming a stable complex with CDK4 or CDK6 (25). Similarly, CDC20 was demonstrated to be a regulatory protein at multiple cell cycle points by interacting with several other proteins (26). CDC20 can bind to and promote the ubiquitin ligase activity of anaphase-promoting complex/cyclosome (APC/C) and enable the degradation of securin and cyclin B, thus promoting the onset of anaphase and mitotic exit (27). All these data demonstrate the importance of HBO1 in controlling cell proliferation and in the regulation of cell cycle.

Structure analysis indicated that the acetyl-CoA combined with HBO1 in the cavity was surrounded with positively charged residues. The acetyl-CoA binds in a bent conformation which facilitates an extensive set of protein interactions. In addition, the positively charged side chain of R325 in the cavity

entrance promotes the binding of negatively charged substrates. The potential inhibitors can combine with HBO1 perfectly in the cavity. The inhibitors possess various polar groups, such as carbonyl oxygen, hydroxyl oxygen and heterocyclic nitrogen, which can mediate interactions between HBO1 and inhibitors by protein backbone hydrogen bonds or protein side chain van der Waals interactions. Hence, the inhibition of HBO1 can interrupt the cell cycle or cell proliferation and putatively suppress tumor development.

In conclusion, in this study, the location and target gene function analysis of HBO1 indicated that HBO1 plays critical roles in the regulation of gene expression and the cell cycle. Furthermore, structure analysis and inhibitor identification provided the HBO1 regulatory mechanisms, which may prove to be helpful for the inhibition of tumor development.

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