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Histone acetyltransferase HBO1 inhibits NF-κB activity by coactivator sequestration

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Abstract

The MYST acetyltransferase HBO1 is implicated in the regulation of DNA replication and activities of transcription factors such as the androgen receptor. Since the androgen receptor and NF-κB transcription factors crossmodulate their transcriptional activity, we investigated whether HBO1 regulates NF-κB signaling. Here, we report that in 293T cells HBO1 reduced dose-dependently NF-κB activity stimulated by TNFα, or by overexpressing p65/RelA, RelB, or cRel. Mutational analysis showed that the N-terminal serine-rich region of HBO1 but not the acetyltransferase function was required for inhibition. Electrophoretic mobility-shift assays demonstrated that HBO1 was neither perturbing the formation of p65/RelA DNA complexes nor binding itself to the κB consensus sequence or to p65/RelA, suggesting that HBO1 reduced NF-κB activity by squelching a cofactor. These data establish a novel function for HBO1 showing that it reduced NF-κB activity by sequestrating an essential coactivator from the NF-κB transcriptional complex.

Keywords: Histone acetyltransferase; Gene regulation; Coactivators

NF-κB/Rel transcription factors control the expression of multiple genes involved in controlling immunity, inflammation, cellular proliferation, cancer, and apoptosis [1]. The members of this family (p65/RelA, p105/p50, p100/p52, RelB, and cRel) are characterized by a conserved N-terminal Rel-homology domain and C-terminal transactivation domains in p65/RelA, RelB, and c-Rel [1]. Extracellular signals activate the classical or alternative NF-κB signal transduction pathway resulting in the nuclear translocation of NF-κB transcription factors [1,2]. There, NF-κB transcription factors undergo posttranslational modifications regulating DNA binding, IκBα interaction, and interaction with coregulators, all of which modulate NF-κB activity [3].

HBO1 (histone acetyltransferase binding to ORC1; HUGO symbol MYST2), a member of the MYST family of histone acetyltransferases (HAT), is a ubiquitously expressed nuclear protein [4]. Human HBO1 interacts with ORC1 [4], MCM2 [5], androgen receptor (AR) [6], progesteron receptor [7], CDK11^{p58} [8], and ING4 and ING5 [9], and is involved in initiation of replication, progression through S phase of the cell cycle, and transcriptional regulation. Human HBO1 has no intrinsic HAT activity but acetylates histones as part of a multisubunit complex [4,9].

HBO1 downregulates the hormone-dependent AR activation [6]. Since AR has also been shown to negatively regulate NF-κB-dependent transcription [10,11] we investigated whether HBO1 is involved in regulation of NF-κB activity. In this report, we demonstrate that HBO1 suppressed NF-κB-induced transcription in an

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AR-independent manner most likely by competing with a coactivator regulating NF-κB activity.

Materials and methods

Plasmids. Expression vectors encoding human AR (pSG5−AR) [12] and human HA-tagged p65/RelA, RelB, and c-Rel were used. Luciferase reporter plasmids for NF-κB (pκB-luc, pNF-κB-luc (Stratagene)) and androgen receptor (pSLP−ARU-Luc), and the internal control plasmid pRL-TK (Promega) have been described [12,13]. HBO1 expression vectors pmyc-HBO1 (myc tag, CMV promoter) and pBud-HBO1 (no tag, EF1α promoter) were constructed by cloning the HBO1 coding sequence from pBSSKL74 [4] into pCMVmyc (Clontech) and pBudCE4 vectors (Invitrogen). All HBO1 deletion mutants were generated from pmyc-HBO1 by PCR and cloning into the pCMV-myc vector. Constructs were verified by sequencing (Synergene).

Cell culture and luciferase reporter assays. 293T cells were cultured in Dulbecco's modified Eagle's medium and 10% fetal calf serum (Sigma). Cells in 24-well plates (2×10^5 cells/well) were transfected with expression plasmids, 0.01 μg pRL-TK, and 0.2 μg luciferase reporter plasmids using Lipofectamine 2000 (Invitrogen). The total amount of DNA (0.8 μg) was kept constant by adding empty vector DNA. Cells were treated with 10 ng/ml TNF-α (R&D Systems) for 20 h. Luciferase activities were measured 24 h posttransfection using the Dual-Luciferase-Reporter Assay System (Promega). Induction was calculated as the ratio of relative luciferase activity from stimulated and non-stimulated samples. The results (means ± SEM) of three independent experiments are shown. For AR reporter experiments, dextran/charcoal-treated fetal bovine serum (HyClone) was used. Dihydroxytestosterone (DHT, Fluka) was used at 10 nM.

Electrophoretic mobility-shift assay. Nuclear extracts from 293T cells were prepared and analyzed by EMSA as described previously [14]. For competition experiments, an excess of either cold or non-specific oligonucleotides was incubated with nuclear extract for 10–15 min on ice prior to addition of the probe.

Site-directed mutagenesis. Point mutations in HB01 were introduced using the QuikChangeII kit (Stratagene). Sequencing was used to verify that only the desired mutation was present.

Results

Repression of NF-κB transactivation by HBO1

Reports showing a repressive effect of HBO1 on hormone-stimulated AR transactivation and mutual transcriptional interference between p65/RelA and AR [10,11] suggested that AR and HBO1 may regulate cooperatively NF-κB. Therefore, we examined in 293T cells the effect of HBO1 on NF-κB activation using luciferase reporter assays. Cells were transiently cotransfected with increasing amounts of HBO1 expression vector (pmyc-HBO1) and then treated with TNFα. The results showed that HBO1 decreased the TNFα-mediated transactivation of NF-κB in a dose-dependent manner (Fig. 1A). Comparable results were obtained when expressing HBO1 without tag (pBud-HBO1) or using a firefly luciferase reporter (pNF-κB-luc) whose promoter contained five repeats of the NF-κB binding site (data not shown). These data indicated that the repressive effect of HBO1 on TNFα-mediated NF-κB transactivation was an intrinsic property of the protein and was independent of the tag or the promoter upstream of the firefly luciferase reporter gene.

Since HBO1 is almost exclusively localized to the nucleus [6], the effect of HBO1 on NF-κB activation by p65/RelA, RelB, and cRel was investigated. NF-κB reporter assays in 293T cells demonstrated that HBO1 inhibited NF-κB transactivation by p65/RelA (Fig. 1B), RelB (Fig. 1C), and cRel (Fig. 1D). Similar results were obtained with the luciferase reporter (pNF-κB-luc) indicating that the effect of HBO1 was independent of the promoter used to express firefly luciferase. Cotransfecting 293T cells with increasing amounts of p65/RelA and constant quantities

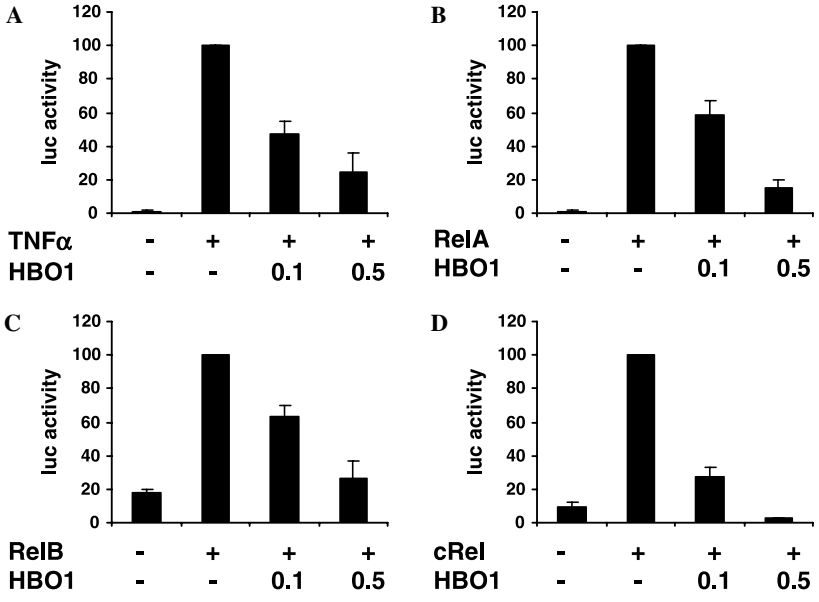

Fig. 1. HBO1 attenuates NF-κB activity induced by TNFα (A), p65/RelA (B), RelB (C), and cRel (D). Fifty namograms of p65/RelA, RelB, and cRel expression vectors and indicated amounts (in μg) of HBO1 expression vector were transfected. Results are expressed relative to the sample without HBO1 (= 100%).

of HBO1 demonstrated that HBO1 repression of NF-κB activity could be overcome by excess p65/RelA. These results suggested that either HBO1 and p65/RelA competed for coactivator(s) required for full transcriptional activity of p65/RelA, or HBO1 interacted with p65/RelA thereby reducing p65/RelA DNA binding and/or its transcriptional activity.

Repressive activity of HBO1 on NF-κB signaling does not require androgen receptor

Mutual transcriptional interference between p65/RelA and AR has been described in COS-1 and CV-1 cells [11]. Transient transfection experiments in 293T cells confirmed that AR was able to repress TNFα-mediated activation of NF-κB in the presence of DHT (Fig. 2A). Since HBO1 has been shown to interact with AR and to repress hormone-dependent AR transactivation in CV-1 and PC-3 cells [6], we asked whether suppression of NF-κB by HBO1 was mediated by hormone-activated AR. The inhibitory activity of HBO1 on DHT-dependent AR signaling was confirmed in 293T cells using pSLP−ARU-Luc reporter (Fig. 2B). Then, 293T cells were cotransfected with expression constructs for cRel, HBO1, and/or AR. The degree of downregulation of cRel transactivation by HBO1 was not changed by the addition of DHT to the culture medium (Fig. 2C). In contrast, repression of cRel transactivation by AR was only observed with DHT (Fig. 2C), corroborating that the assay system was capable of measuring hormone-dependent effects. These results strongly indicated that NF-κB repression by HBO1 did not require AR.

HBO1 repression occurs by competing for NF-κB coactivators

To repress NF-κB transactivation, HBO1 could directly bind to the κB consensus sequence, interact with NF-κB to prevent either DNA binding of NF-κB or formation of a fully active transcriptional complex, or compete for NF-κB coactivators. To examine these possibilities, 293T cells were cotransfected with different combination of HBO1 and/or p65/RelA, or treated with TNFα at 10 ng/ml for 10 min, 30 min, or 24 h. Nuclear extracts were analyzed by electrophoretic mobility-shift assays with an oligonucleotide corresponding to the κB site (Fig. 3). No slower migrating probe was observed in cells expressing HBO1 only (lane 2) showing that HBO1 does not interact with the κB probe. Moreover, transfection of HBO1 into cells overexpressing p65/RelA or stimulated by TNFα cells did not affect the migration pattern or the signal strength of the p65/RelA DNA complex (lanes 4, 6, 8, 10, and 12). These data underline that HBO1 repression is not caused by reducing p65/RelA DNA binding or by direct interaction of HBO1 with p65/RelA. The latter was confirmed by co-immunoprecipitation experiments (data not shown). However, they are consistent with a mechanism in which HBO1 interferes with coactivator binding to the NF-κB transcriptional complex.

The N-terminal serine-rich domain of HBO1 is required for NF-κB inhibition

To map HBO1 domains responsible for repression of NF-κB transcriptional activity, HBO1 deletion mutants (Fig. 4A) were tested in p65/RelA-dependent NF-κB reporter assays in 293T cells. Mutants with C-terminal deletions (HBO1/1−473, HBO1/1−362, HBO1/1–245, and HBO1/1–184; Fig. 4A) were able to repress NF-κB activity to a similar level as observed with the full-length HBO1 protein (Fig. 4B). The HAT domain mutant $HBO1_{G485D}$ downregulated p65/RelA-mediated NF-κB activity as the normal protein (data not shown). In contrast, the N-terminal deletion mutant HBO1/246−611 failed to show any inhibitory activity. These results strongly argued that it is mainly the N-terminal serine-rich domain of HBO1 (amino acids 1–184) which is required for inhibition of NF-κB transactivation.

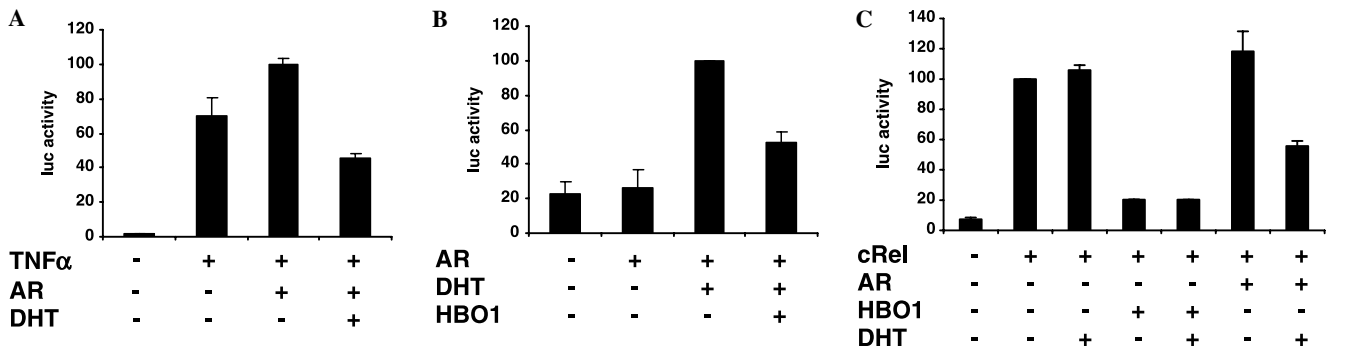

Fig. 2. HBO1 suppression of cRel transactivation is independent of androgen receptor. 293T cells were transfected as indicated with luciferase reporter plasmids for NF-κB (A,C) or AR (B) and 100 ng pSG5-AR (A,C), 100 ng pmyc-HBO1 (B,C), and 50 ng cRel expression vector (C). Results are expressed relative to TNFα and AR (A), AR with DHT (B), and cRel alone (C) set as 100%.

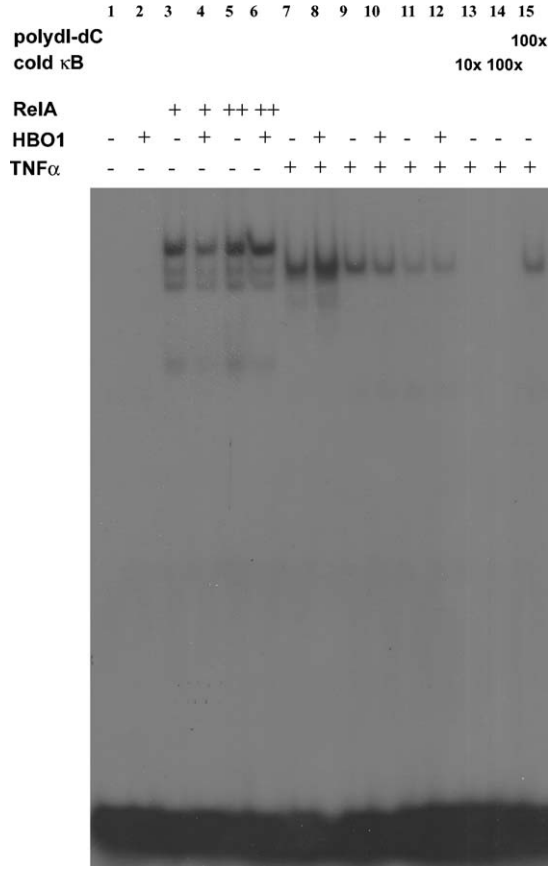

Fig. 3. HBO1 does not compete with NF-κB for binding to cognate DNA. EMSA was performed with 293T cells in 6-well plates treated as follows: Transfection with 1.25 μg pmyc-HBO1 (lanes 2,4, 6, 8, 10, and 12), with RelA expression vector (250 ng in lanes 3–4; or 500 ng in lanes 5–6), or incubation with 10 ng/ml TNFα for 24 h (lanes 7–8), for 30 min (lanes 9–10 and 13–15), and for 10 min (lanes 11–12). As controls, nuclear extracts were preincubated with 10× (lane 13), 100× (lane 14) molar excess of cold probe, or with 100× (lane 15) molar excess of non-specific probe.

Discussion

The acetyltransferase HBO1, characterized by the MYST domain providing acetyltransferase function and a unique but essential C2HC zinc finger, is involved in DNA replication, cell cycle progression, and transcriptional regulation [15]. Here, we demonstrated that HBO1 inhibits in a dose-dependent manner NF-κB activity stimulated either by TNFα or by overexpression of p65/RelA, RelB, and cRel. These findings support a role of HBO1 in the regulation of nuclear events of the NF-κB signal transduction cascade, which is consistent with its nuclear localization. Analysis of HBO1 deletion mutants indicated that the serine-rich N-terminal region was required for NF-κB inhibition while the acetyltransferase activity was dispensable. This domain revealed no significant sequence homologies to other nonorthologous proteins with the exception of the region between amino acids 180 and 212 which was related to CCHHC zinc-binding domains found in the neural zinc finger factor/myelin transcription factor family [16]. Two tandem repeats of this motif are required for high-affinity interaction of these transcription factors with their cognate binding sequence which is different from the NF-κB site [16]. This explains why HBO1 despite the presence of this motif is not binding to the κB sequence (see EMSA data, Fig. 3). Furthermore, HBO1 was identified to repress DHT-mediated AR transactivation which required the region of HBO1 comprising amino acids 1–360 but not the C-terminal domain (amino acids 230–611) [6]. Although an intrinsically disordered structure is predicted for the HBO1 N-terminal domain using DisEMBL [17], this does not necessarily preclude function because it has been suggested that absence of folded globular structure may be required for specific molecular function [18]. Since HBO1 plays a role in different biochemical reactions the disordered structure of the HBO1 N-terminal region may be required to get the flexibility necessary for allowing interaction with structurally different proteins. In summary, these results highlight the importance of the serine-rich N-terminal region of HBO1 in repression of several transcription factors.

The results of EMSA experiments showed that HBO1 was not binding to the κB sequence since DNA binding of p65/RelA was not prevented by overexpression of HBO1. Furthermore, HBO1 was not interacting with p65/RelA since the migration pattern of the p65/RelA DNA complex was identical in the absence and presence of HBO1 and co-immunoprecipitation experiments were negative. Thus, we propose that HBO1 suppresses NF-κB by squelching the interaction of NF-κB transcription factors with coactivator(s) present in limiting quantities and which have equal or higher affinity to HBO1 than to NF-κB. There are numerous transcriptional coregulators of NF-κB activity including CBP, SRC-1, PCAF, TBP, TAF, TFIIB, Mediator, PC4, EIA 13S, TLS/FUS, RAC3, 53BP2, and BRCA1 (see references in [19]). The NF-κB coactivator SRC-1 is a good candidate since co-immunoprecipitation and functional studies in 293T cells have shown that it interacts with the N-terminus (amino acids 1–340) of HBO1 [7]. To explain the repression of NF-κB signaling by HBO1, we propose a model in which the N-terminal region of HBO1 binds to and prevents the coactivator SRC-1 from interacting with the NF-κB transcriptional complex, thereby decreasing NF-κB activity.

In conclusion, we have identified HBO1 as a novel negative regulator of NF-κB transactivation. The inhibitory function can be ascribed to the serine-rich N-terminal region of HBO1 which interacts with NF-κB coactivators and interferes, when overexpressed, with the formation of a fully active transcriptional complex. Interestingly, higher levels of HBO1 mRNA have been reported in breast cancer cell lines [20] suggesting that aberrant HBO1 expression may be relevant in pathological situations.

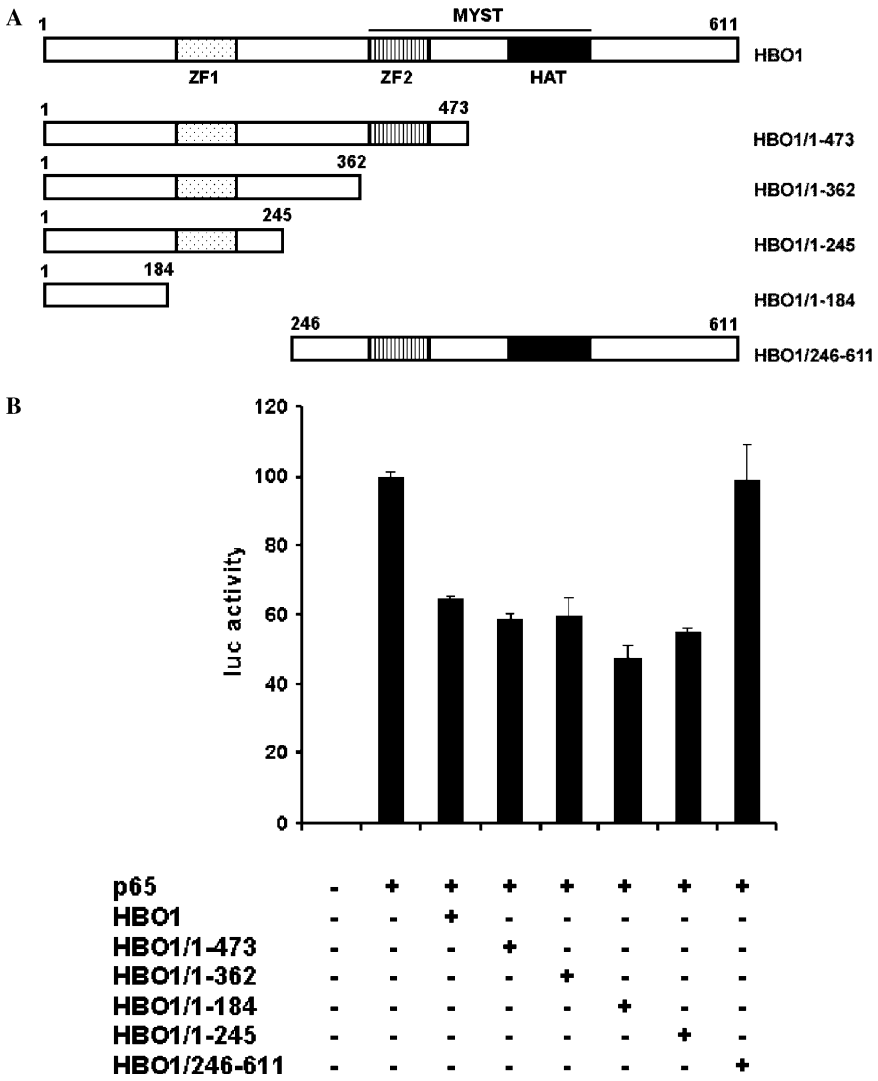

Fig. 4. The N-terminal region of HBO1 is involved in repression of p65/RelA-mediated NF-κB transactivation. (A) Schematic overview of the deletion mutants of HBO1 depicting the first and second putative zinc finger (ZnF1, ZnF2), and the histone acetyltransferase domain (HAT). (B) 293T cells were transfected with NF-κB luciferase reporter plasmids, 50 ng p65/RelA expression vector and equimolar amounts of each HBO1 mutant expression vector. Results are expressed relative to p65/RelA (=100%).

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