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HIC1 interacts with a specific subunit of SWI/SNF complexes, ARID1A/BAF250A

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ABSTRACT

HIC1, a tumor suppressor gene epigenetically silenced in many human cancers encodes a transcriptional repressor involved in regulatory loops modulating p53-dependent and E2F1-dependent cell survival and stress responses. HIC1 is also implicated in growth control since it recruits BRG1, one of the two alternative ATPases (BRM or BRG1) of SWI/SNF chromatin-remodeling complexes to repress transcription of *E2F1* in quiescent fibroblasts. Here, through yeast two-hybrid screening, we identify ARID1A/BAF250A, as a new HIC1 partner. ARID1A/BAF250A is one of the two mutually exclusive ARID1-containing subunits of SWI/SNF complexes which define subsets of complexes endowed with anti-proliferative properties. Co-immunoprecipitation assays in WI38 fibroblasts and in BRG1−/− SW13 cells showed that endogenous HIC1 and ARID1A proteins interact in a BRG1-dependent manner. Furthermore, we demonstrate that HIC1 does not interact with BRM. Finally, sequential chromatin immunoprecipitation (ChIP-reChIP) experiments demonstrated that HIC1 represses *E2F1* through the recruitment of anti-proliferative SWI/SNF complexes containing ARID1A.

Introduction

SWI/SNF-type complexes are evolutionarily conserved multisubunit chromatin-remodeling complexes which use the energy of ATP hydrolysis to mobilize nucleosomes and remodel chromatin [1]. They play a crucial role in proper differentiation, development and tumor suppression [1]. The term SWI/SNF complex refers actually to a heterogenous small series of related complexes of variable composition associating either of the two related but distinct core ATPases BRG1 and BRM to seven to ten non-catalytic subunits [1–7]. These non-catalytic subunits referred to as BRM- or BRG1-associated factors or BAFs contain various DNA-binding and protein-binding motifs which modulate the targeting and activity of the ATPase [1]. One of these stably associated non-catalytic BAF components is a member of the ARID (A-T rich interaction domain) family of DNA-binding proteins. Similar to the BRG1 and BRM ATPases, two ARID-containing proteins the ARID1A/BAF250A and its paralog ARID1B/BAF250B are also alternative, mutually exclusive subunits of the complexes but can associate with both ATPases [3–5].

Distinct subsets of SWI/SNF complexes endowed with anti-proliferative properties and containing ARID1A or endowed with pro-proliferative properties and containing ARID1B have been characterized through siRNA-knockdowns of each specific ARID1 subunit [5]. Similarly, antagonistic roles have been demonstrated for SWI/SNF complexes containing either of two related ATPases BRM and BRG1 in a model of osteoblast differentiation [6]. Together, these results have revealed an unanticipated degree of specialization of function for SWI/SNF complexes [1,5].

BRG1 has been implicated in the activation and repression of multiple genes involved in cell cycle control and growth regulation through the modulation of chromatin structure in various tissues and physiological conditions [7]. Recently, we have shown that BRG1 is required for the transcriptional repression of *E2F1* in quiescent fibroblasts through its interaction with the transcriptional repressor HIC1 [8]. This HIC1-mediated repression is strictly dependent of BRG1 and required for the serum deprivation-induced growth arrest [8].

HIC1 (*hypermethylated in cancer 1*) is a tumor suppressor gene located at 17p13.3, a region frequently deleted and epigenetically silenced in human cancers [9,10]. *HIC1* encodes a sequence-specific transcriptional repressor with five C_2H_2 zinc fingers mediating DNA binding to a consensus binding site (HiRE) consisting of a 5′-$^{C}/_{G}$NG$^{C}/_{G}$G**GGCA**$^{C}/_{A}$CC-3′ sequence centered on a GGCA motif [11]. It also contains a central region that recruits CtBP co-repressor complexes [12] as well as a N-terminal BTB/POZ domain capable of autonomous transcriptional repression [13]. *HIC1* is a direct target gene of P53 transactivation through a P53-responsive element [9,14,15]. In fact, a regulatory feed-back loop between HIC1 and P53 has been deciphered in which HIC1 directly represses transcription of *SIRT1* in response to nutrient deprivation [16] or to modulate P53-dependent DNA-damage responses [17]. Indeed, SIRT1 is a NAD^+-dependent Class III HDAC which deacetylates

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and thereby inactivates P53 [17]. Recently, we have shown that SIRT1 also deacetylates HIC1. However, in striking contrast with P53, this deacetylation strengthens the transcriptional repression potential of HIC1 [18].

HIC1, SIRT1, and E2F1 are also implicated in a regulatory feedback loop since HIC1 represses the *E2F1* promoter [8] and E2F1 activates *HIC1* [22]. Furthermore, E2F1 is a crucial activator of *SIRT1* transcription in response to DNA-damage but SIRT1 binds E2F1 and deacetylates it thus inhibiting E2F1-mediated gene activation [19,20].

Herein, we identified, by yeast two-hybrid screening, ARID1A/BAF250A as a novel partner of HIC1. This interaction occurs between the two HIC1 repression domains and the central 1355–1451 residues of ARID1A, located downstream of the ARID domain. By co-immunoprecipitation analyses, we show that endogenous HIC1 and ARID1A proteins interact in human fibroblasts WI38 but not in the BRG1−/− human adrenal carcinoma cell line, SW13. Consistent with these results, we demonstrate that HIC1 does not interact with BRM, the alternative ATPase of SWI/SNF complexes. Finally, by sequential chromatin immunoprecipitation (ChIP-reCHIP), we demonstrate that in serum-starved WI38 cells HIC1 and ARID1A co-occupy the *E2F1* promoter and *ATOH1*, another HIC1 target gene but not the *SIRT1* promoter. Thus, HIC1 is involved in the repression of some target genes in part through the recruitment of the anti-proliferative SWI/SNF complexes defined by the presence of the specific subunits, BRG1 and ARID1A.

Materials and methods

Yeast two-hybrid screen. Bait cloning and Y2H screening were performed by Hybrigenics, S.A., Paris, France. For bait cloning, the BTB-central region of HIC1 (1–422) was PCR-amplified and cloned in frame with a C-terminal LexA DNA-binding domain. The bait construct was checked by sequencing the entire insert, and was subsequently transformed in the L40ΔGAL4 yeast strain. A human breast tissue random-primed cDNA library, transformed into the Y187 yeast strain and containing ten million independent fragments, was used for mating. The screen was performed in conditions ensuring a minimum of 50 million interactions tested, in order to cover five times the primary complexity of the yeast-transformed cDNA library. Seventy-nine millions of interactions were actually tested with HIC1. After selection on medium lacking leucine, tryptophane, and histidine, positive clones were picked, and the corresponding prey fragments were amplified by PCR and sequenced at their 5′ and 3′ junctions.

Constructs. The pcDNA3-FLAG-HIC1 expression vector has been previously described [12]. The HA-tagged hBRM cDNA cloned in the CMV expression vector pCGT has been obtained through the courtesy of Dr. Christian MUCHARDT (Pasteur Institute, Paris).

Cell culture, transfection, and co-immunoprecipitation analyses. HEK 293T and BRG1−/− SW13 cells were maintained in Dulbecco medium supplemented with 10% fetal calf serum. WI38 were purchased from ATCC (14 passages) and cultured in MEM (Gibco) supplemented with 10% fetal calf serum, non-essential amino acids, and sodium pyruvate. HEK293T were transfected for 6 h in Opti-MEM (Gibco) by the PEI (Euromedex) method in 100 mm diameter dishes with 2.5 μg of DNA and then were incubated in fresh complete medium, as previously described [18]. Forty-eight hours after transfection, cells were rinsed two times in cold PBS and lysed in cold IPH buffer (50 mM Tris, pH 8, 150 mM NaCl, 5 mM EDTA, 0.5% NP-40, protease inhibitor cocktail (Roche)).

For co-immunoprecipitation experiments, cell lysates were cleared by centrifugation (14,000 rpm, 30 min). The supernatants were incubated overnight with 4 μl of antibody. Then, protein A-G Sepharose beads (Amersham Biosciences) were added for 30 min. The beads were washed three times with IPH buffer. Proteins were eluted by boiling in Laemmli loading buffer and separated by SDS/PAGE before Western blotting.

Western blot and antibodies. Western blots were performed with the anti-HIC1 2563 and anti-HIC1 325 polyclonal antibodies as previously described [12]. Anti-FLAG M2 is a monoclonal antibody (F3165; Sigma). The mouse monoclonal anti-BAF250A/ARID1A antibodies (sc-32761X) and rabbit polyclonal anti-BRG1 antibodies (BRG1 H-88, sc10768) were purchased from Santa Cruz. The mouse monoclonal anti-HA antibodies (MMS 101-P) and anti-CtBP2 (612044) were obtained from Babco and BD Biosciences, respectively. The secondary antibodies were horseradish peroxidase-linked antibodies raised against rabbit or mouse immunoglobulins (Amersham).

Sequential chromatin immunoprecipitation (ChIP-reChIP). For ChIP-reChIP experiments, we used eight non-confluent WI38 100 mm dishes per second ChIP. The cells were maintained in MEM without serum for 72 h. The first round of chromatin immunoprecipitation ChIP was performed according to published protocols with slight modifications. Briefly, formaldehyde was added directly to the cultured cells to a final concentration of 1% for 10 min at 37 °C. The cross-linking was stopped by adding glycine to a final concentration of 0.125 M. After 5 min at 37 °C, cells were lysed directly in the plates by resuspension in cell lysis buffer for 5 min. Then, the samples were pelleted, resuspended in nuclei lysis buffer and sonicated to chromatins with an average size of 250 bp using a BioRuptor (Diagenode, Liege, Belgium). After preclearing with a 50% slurry of protein A-G beads pre-incubated with salmon sperm DNA and BSA for 4 h at +4 °C, the chromatins were incubated with the anti-HIC1 antibodies, normal rabbit IgG or with no antibodies overnight. The antibody-bound chromatin was then pooled down for 30 min with protein A-G beads and washed extensively. After this first round of immunoprecipitation, the beads were pooled by centrifugation in TE buffer and incubated in 100 μl of elution buffer for 10 min at 65 °C. After centrifugation, the supernatant was diluted in 900 μl of IP buffer and incubated with the second antibody and was proceeded as described above. The chromatin bound after this second antibody round was pooled with protein A-G beads and washed extensively as described above. Then, it was eluted two times by 250 μl of elution buffer. After addition of 20 μl of 5 M NaCl, the cross-linking was reversed by overnight incubation at 65 °C. The immunoprecipitated DNAs as well as whole cell extract DNAs (input) were purified by treatment with RNAse A and then proteinase K followed by purification on Nucleobond Extract II (Macherey-Nagel).

The purified DNAs were used for PCR analyses using relevant primers for *E2F1*, *ATOH1*, *SIRT1*, and *GAPDH*.

Results and discussion

To further characterize the repression mechanisms brought about by HIC1 on its target genes, we conducted a yeast two-hybrid screen using the two autonomous repression domains of HIC1, the BTB/POZ domain and the central region, as bait to screen a human mammary gland library (Fig. 1A). Seventy-nine millions of interactions were actually tested with HIC1 and after growth on selection medium, positive clones were obtained. One clone and three clones contained the complete coding sequences of human CtBP1 and CtBP2, respectively, in close agreement with our previous work [12].

Three clones corresponded to nucleotides 4437–4807 of the ARID1A/BAF250A mRNA (NM_006015) whereas two other clones corresponded to nucleotides 4418–4726 thus defining a 289 nucleotides contig. The ARID1A/BAF250A protein is a huge 2285 aa protein defining a specific subset of SWI/SNF chromatin remod-