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Bag-1M inhibits the transactivation of the glucocorticoid receptor via recruitment of corepressors

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ABSTRACT

The Bcl-2 associated athanogene 1M (Bag-1M) is known to repress the transactivation of the glucocorticoid receptor (GR). We report here that Bag-1M inhibits the action of GR via recruitment of corepressors, including nuclear receptor corepressor (NcoR) and silencing mediator for retinoic acid and thyroid hormone receptor (SMRT), and histone deacetylase (HDAC)3 to the genomic response element of a glucocorticoid-regulated human metallothionein IIa (hMTIIa) gene. A mutant GR lacking the interaction with BAG-1M fails to recruit the corepressors NcoR and SMRT. RNAi-mediated knock down of corepressors and the use of HDAC inhibitor relieved Bag-1M-induced repression on the transactivation of the GR. In addition, Bag-1M is not involved in the degradation of the receptor. These findings indicate a novel mechanism by which Bag-1M acts as a corepressor and downregulates the activity of the GR.

Structured summary:
MINT-7216164: *HDAC3* (uniprotkb:O15379) *physically interacts* (MI:0914) with *Bag1* (uniprotkb:Q99933) by *anti bait coimmunoprecipitation* (MI:0006)
MINT-7216183: *NCOR* (uniprotkb:O75376) *physically interacts* (MI:0914) with *Bag1* (uniprotkb:Q99933) by *anti bait coimmunoprecipitation* (MI:0006)
MINT-7216175: *SMRT* (uniprotkb:Q9Y618) *physically interacts* (MI:0914) with *Bag1* (uniprotkb:Q99933) by *anti bait coimmunoprecipitation* (MI:0006)

1. Introduction

The Bcl-2 associated athanogene 1 (Bag-1) proteins are cochaperones that are involved in the regulation of nuclear receptor action [1–5]. In human, the Bag-1 gene encodes four isoforms of the Bag-1 proteins (Bag-1L, Bag-1M, Bag-1S and p29), all of which are expressed through alternative translation initiation sites from the same mRNA [6]. Although it was initially identified as a Bcl-2 binding protein to suppress apoptosis [7], Bag-1 has been known to interact with and regulate the activity of other proteins. For example, Bag-1 interacts with and inhibits the function of the tumor suppressor p73 [8]. Furthermore, the medium isoform, Bag-1M is increased in the hippocampus of Alzheimer's disease patients and binds to Tau protein and amyloid precursor protein (APP), and overexpressed Bag-1M induces increased level of Tau and APP that are related with the pathology and treatment of Alzheimer disease [9,10].

The different Bag-1 proteins exert varying effects on the transactivation function of nuclear receptors. The largest isoform, Bag-1L, enhances the transactivation of androgen receptor (AR) [11] but inhibits the action of glucocorticoid receptor (GR) [12], whereas, Bag-1M, downregulates the transactivation of GR [13]. Recent study revealed that Bag-1M localized to the glucocorticoid response element (GRE) in a hormone sensitive manner and repressed the DNA binding by the GR [14]. This provides another evidence that molecular chaperones and cochaperones can modulate the nuclear receptor action at the genomic response element apart from the previous finding that molecular chaperone p23 was present at the response element in the presence of hormone and disrupted receptor-mediated transcriptional activation [15].

Abbreviations: Bag-1, Bcl-2 associated athanogene 1; GR, glucocorticoid receptor; GRmt, glucocorticoid receptor K496L/I497G mutant; Hsp, heat shock protein; hMTIIa, human metallothionein IIa; ChIP, chromatin immunoprecipitation; siRNA, small interfering RNA; IB, immunoblot; GRE, glucocorticoid response element; AR, androgen receptor; NcoR, nuclear receptor corepressor; SMRT, silencing mediator for retinoic acid and thyroid hormone receptor; HDAC, histone deacetylase

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Moreover, the Bag-1 cochaperone also affects the protein level of the GR through its ubiquitin like domain that is involved in the degradation of GR in a hormone dependent manner in the presence of another cochaperone chromatin C-terminus of heat shock protein (Hsp) 70 interacting protein (CHIP) [16].

Glucocorticoid regulates the expression of target genes involved in many pathophysiological processes. A human methallothionein IIa (hMTIIa) gene has been identified and used as a model to investigate the action of GR [17]. Although heavy metal cations are the most potent inducers of MT in mammals, other agents also initiate increases in MT expression [18]. For example, glucocorticoid transactivates the expression of hMTIIa gene through the GR that binds to the GRE located upstream of the gene [17].

Here, we report that Bag-1M is not involved in the degradation of GR through the ubiquitin–proteosome pathway as a potential mechanism for down-regulation of GR activity. Rather, our data suggest that Bag-1M recruits corepressors and histone deacetylase (HDAC), in a hormone dependent manner, to the GR genomic response element of the hMTIIa gene. In line with this, knock-down of corepressors and inhibition of HDAC activity reversed Bag-1M-mediated negative effect.

2. Materials and methods

2.1. Cell culture

COS-7 and HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% fetal calf serum at 37 °C and in an atmosphere of 5% CO_2. All culture media contained 100 units/ml penicillin and 100 μg/ml streptomycin.

2.2. Plasmid constructs

The plasmids expressing the wild-type human GR, the mutant GR, Bag-1M, the indicator plasmid pGL3MMTV, control plasmid pRenilla-luc and the hMTIIa reporter plasmid H1S CAT have previously been reported [14]. pEGFP-C2 was commercial available from Clontech, Palo Alto, CA.

2.3. Antibodies

For ChIP assay, GR (P-20), Bag-1 (FL-274), nuclear receptor corepressor (NcoR) (H-303), silencing mediator for retinoic acid and thyroid hormone receptor (SMRT) (C-19) and HDAC3 (H-99) antibodies and rabbit IgG and goat IgG (all from Santa Cruz Biotechnology, Santa Cruz, CA) were used. The following antibodies were used for immunoprecipitation: Bag-1 (FL-274), NcoR (H-303), SMRT (C-19), HDAC3 (H-99), all from Santa Cruz. For immunoblot (IB), Bag-1 (C-16, Santa Cruz), Actin (I-19, Santa Cruz), SMRT (N-20, Santa Cruz) and NcoR (06-892, Upstate) antibodies were employed.

2.4. Transfection assay and luciferase reporter gene assay

COS-7 cells were transiently transfected with plasmid constructs using Fugene 6 reagent (Roche Diagnostics, Basel, Switzerland) according to the manufacturer's instructions. The efficiency of transfection was determined as the percentage of green fluorescent cells following cotransfection of pEGFP-C2, a mammalian expression plasmid encoding for enhanced green fluorescent protein. It remained stable throughout the reported experiments at a level of 75–80%. The reporter gene assay has previously been described [14]. In this assay, three independent experiments were performed and the data were analyzed, by a software for the student's *t*-test, to calculate the probability value (*P*-value) to check, whether or not, a significant difference exists between two groups of data obtained under conditions of different treatments.

2.5. Immunoprecipitation and IB

The transiently transfected and dexamethasone treated COS-7 cells were further treated with 1 mM dimethyl 3,3′-dithiobispropionimidate (Pierce Biotechnology, Rockford, IL) for 30 min to crosslink the protein. Cells were lysed in buffer (0.5% NP-40, 20 mM Tris, pH 8.0, 100 mM NaCl, 1 mM EDTA, 10% glycerol) and preincubated with protein A sepharose (Amersham Bioscience, Shanghai, China). Bag-1, NcoR, SMRT or HDAC3 antibodies were used for immunoprecipitation and IB was followed with Bag-1 antibody.

2.6. ChIP and re-ChIP

ChIP and re-ChIP were performed as previously described [19]. For ChIP in transfected COS-7 cells, 10% of the precleared supernatant was taken out as input. The PCR reactions of input and immunoprecipitated material were run with 28 cycles. The primers used for the PCR amplification are (in 5′–3′ direction): CCG GTT ACT GTG ATG CTG CA (hMTIIa for), GCG GGA GGA CAC AGT GTA CC (hMTIIa rev). For Re-ChIP, 20% of the elute after the first precipitation but before the second precipitation was obtained as input. The PCR reactions of input and immunoprecipitated material were run with 32 cycles. For ChIP in HeLa cells, 20% of the precleared supernatant was used as input and the PCR reactions were run with 28 cycles.

2.7. RNA interference

NcoR small interfering RNA (siRNA) (M-003518-01), SMRT siRNA (M-020145-02) and the luciferase GL3 siRNA used as control, were all purchased from Dharmacon. Transfection of COS-7 and HeLa cells with the siRNA was performed using an Oligofectamine reagent (Invitrogen, Shanghai, China) at a final concentration of 150 nM. The cells were finally collected for measurement of luciferase activity, real-time PCR and immunoblot.

2.8. Real-time PCR

The primers (in 5′–3′ direction): TCG GAT ACG TCA TCA GCA CC (for) and TCC CTC CTG TCC TGT ACT CGA (rev) were used for the detection of hMTIIa gene expression. Human β-actin gene expression was detected as a control by the primers: TCA CCC ACA CTG TGC CCA T (for) and CTC TTG CTC GAA GTC CAG GG (rev). The ABI PRISM 7000 sequence detection system and the SYBR Green PCR Master Mix (Applied Biosystem, Shanghai, China) and 40 cycles were used for the amplification. Three independent real-time PCRs were run and the data were analyzed, by a software for the Student's *t*-test, to calculate the *P*-value to check, whether or not, a significant difference exists between two groups of data obtained under conditions of different treatments.

3. Results and discussion

3.1. Bag-1M recruits corepressors and HDAC

Since we have previously shown that Bag-1M and GR are present at the GRE of a glucocorticoid-regulated human metallothionein IIa (hMTIIa) gene [14], it is worthwhile to investigate how Bag-1M alters the action of the GR complex at the GRE. ChIP was therefore carried out to identify the possible factors that are involved in the GR complex. COS-7 cells that are devoid of GR and Bag-1M [12] were transfected with an hMTIIa reporter gene. In addition, Bag-1M, the wild-type GR or a mutant GR (glucocorticoid