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C-terminal binding proteins (CtBPs) attenuate KLF4-mediated transcriptional activation

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ABSTRACT

We aimed to examine the physical interaction between CtBPs and KLF4 and the potential importance of this interaction. Co-immunoprecipitation indicated that CtBP1 indeed interacted with KLF4. This was supported by the co-localization of both KLF4 and CtBP1 in the promoter regions of KLF4 downstream target genes. In addition, overexpression of CtBP1 significantly decreased KLF4-mediated transcriptional activation in both an artificial (pGL5) and genuine (IAP and Keratin-4) reporter system. Mutations in the potential CtBP binding motif in KLF4 were accompanied by loss of the inhibitory effect of CtBP1 in the reporter assay and of the physical interaction with CtBP1. Overall, our results suggest that CtBPs attenuate KLF4-mediated transcriptional activation through the physical interaction with KLF4.

Structured summary:
MINT-7261981, MINT-7261995: *KLF4* (uniprotkb:O43474) *physically interacts* (MI:0915) with *CTBP1* (uniprotkb:Q13363) by *anti tag coimmunoprecipitation* (MI:0007)
MINT-7262008, MINT-7262023: *CTBP1* (uniprotkb:Q13363) *physically interacts* (MI:0915) with *KLF4* (uniprotkb:O43474) by *anti bait coimmunoprecipitation* (MI:0006)

1. Introduction

Regulation of gene expression is a fundamental cellular process. Critical to the control of gene expression are transcription factors that bind to specific DNA sequences and subsequently modulate gene transcription. A family of the C2H2-zinc finger proteins exhibits homology to the *Drosophila melanogaster* segmentation gene product, Krüppel 3, hence the name Krüppel-like factors (KLFs). KLFs play important roles in many fundamental biologic processes, including development, proliferation, differentiation and apoptosis [1]. One member of this family, Krüppel-like factor 4 (KLF4), also known as gut-enriched Krüppel-like factor (GKLF), was found to be highly expressed in epithelial cells of many tissues, including small intestine and skin [2]. In these tissues, by directly binding to CACCC motif in the promoter region of its target genes, KLF4 transcriptionally regulates its target genes resulting in inhibition of proliferation and promotion of terminal differentiation [3,4].

C-terminal binding proteins (CtBPs) family proteins are modulators of several essential cellular processes [5]. Vertebrate genomes code for two related proteins, CtBP1 and CtBP2. CtBP family proteins are highly conserved. The founding member of this family, CtBP1 was discovered as a cellular protein that interacted with the C-terminus of adenovirus E1A proteins [6]. CtBPs function as transcriptional co-repressors. A large number of sequence-specific DNA-binding transcriptional repressors of Drosophila and mammalian species mediate their activity in a CtBP-dependent manner [7]. At present, more than thirty different vertebrate transcriptional regulators have been reported to modulate their activity through recruitment of CtBPs.

The various DNA-binding repressors recruit CtBP through a conserved CtBP binding motif, a Pro-X-Asp-Leu-Ser (PXDLS) CtBP interaction domain (Fig. 1A) originally identified in adenovirus E1A protein [8]. A PYDLA motif was also found in the N-terminal region of human KLF4 protein (aa 53–57, Fig. 1A). In the present study, we aimed to examine the physical interaction between CtBPs and KLF4 and the potential importance of this interaction. Our findings suggest that CtBPs attenuate KLF4-mediated transcriptional activation, and this attenuation is most likely fulfilled through directly interacting with KLF4 via PYDLA motif.

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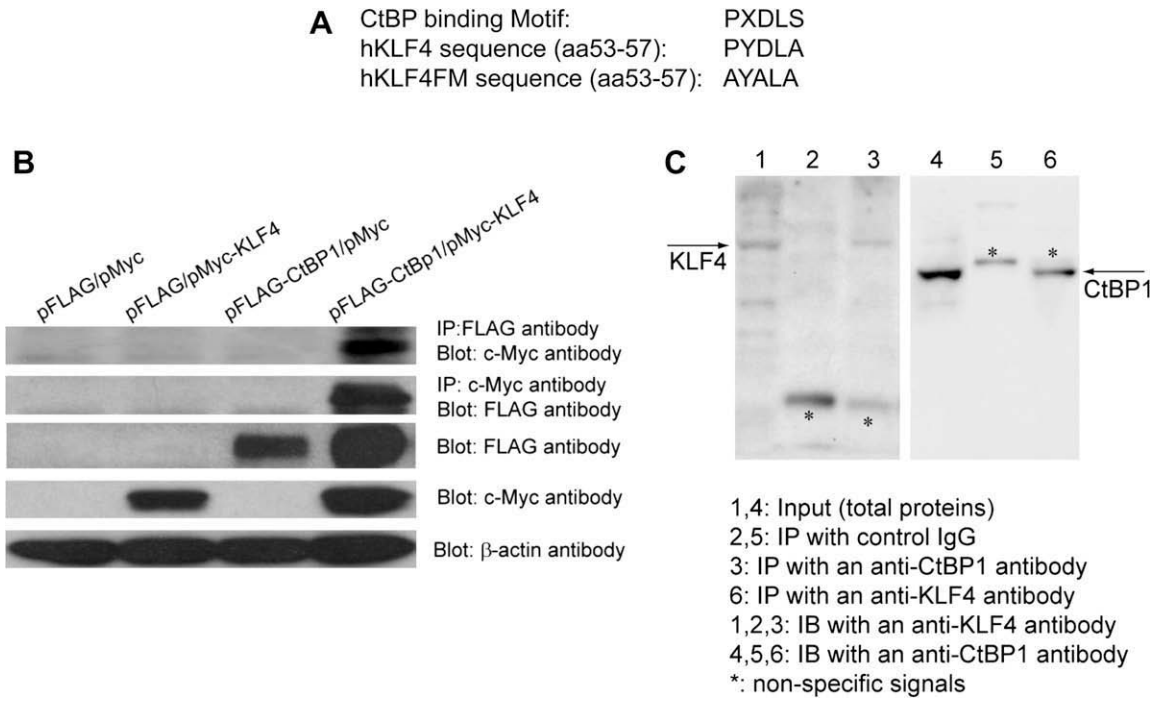

Fig. 1. CtBP1 associated with KLF4. (A) The sequences of CtBP consensus binding motif, potential hKLF4 CtBP binding motif and the mutant hKLF4 binding motif. (B) Co-IP and Western blot analysis to examine the physical interaction between pMyc-KLF4 and pFLAG-CtBP1. Transient transfections using AGS cells, Co-IP, and Western blotting were performed as described in Section 2. (C) Endogenous Co-IP and Western blot analysis. Protein extracts from AGS cells and anti-KLF4 and anti-CtBP1 antibodies were used for the assay.

2. Materials and methods

2.1. Cell culture

AGS cells and KLF4 Mouse embryo fibroblasts (MEFs) ($KLF4^{+/-}$, $KLF4^{-/-}$) cells or CtBP MEFs ($CtBP^{+/-}$, $CtBP^{-/-}$) cells were cultured in complete Dulbecco's modified Eagle's medium (DMEM) similarly as described [9]. Human colon cancer HCT 116 cells were grown in complete McCoy's medium.

2.2. Plasmids

Plasmids used for artificial promoter reporter assay were as follows: pGL5-Luc reporter plasmid, pM vector, pM/KLF4, pFLAG vector, pFLAG-CtBP1, pFLAG-CtBP2 (from Dr. G. Chinnadurai), and pM/KLF4FM, a mutated version of KLF4 that harbors point mutations in potential CtBP binding motif (Fig. 1A), IAP and KRT luciferase reporter constructs (from Drs C. Liu and A. Rustgi, respectively), pcDNA 3/Myc-His B vector (pMyc), pMyc-KLF4, and pMyc-KLF4FM. All the cloned plasmids have been sequence confirmed.

2.3. Transient transfection and co-immunoprecipitation assays

Plasmids were transfected into indicated cells using Lipofectamine 2000 (Invitrogen). Proteins were extracted, followed by Western blotting analysis using mouse anti-FLAG tag (SIGMA), rabbit anti-Myc tag (ABR), rabbit anti-human β-actin (SIGMA) antibodies. Endogenous Co-IP experiments were similarly performed using an anti-KLF4 antibody (Santa Cruz) and an anti-CtBP1 antibody (Santa Cruz) together with IgG controls.

2.4. Chromatin immunoprecipitation (ChIP) assays

CHIP assays using AGS or KLF4 MEFs cells were performed as previously described [9]. Extracted DNA samples from transfected AGS cells were used to amplify promoter fragments of KLF4 target genes. The promoter fragments of target human genes were amplified and the primer pairs were as follows: CCND1 (Upper: 5′-TCTACACCCCCAACAAAACCAA-3′; Lower: 5′-ACTCTTCGGGCTGC CTTCCTAC-3′), p21 (Upper: 5′-GACCGGCTGGCCTGCTGGAACT-3′; Lower: 5′-GCACGCTTGGCTCGGCTCGGCTCTGG-3′), IAP (Upper: 5′-GGG CCC ATG GAA AAC AGA CTC A-3′; Lower: 5′-AGACGCGTTG CCACTCCTTCAT-3′), Laminin α1 (Upper: 5′-GGCAAACAAAGTCGG GAACAAG-3′; Lower: 5′-TAGGAGGTGGGC AGAGAAGGTG-3′), HDC (Upper: 5′-GAACTGAGGGCTCTTTTACG-3′; Lower: 5′-CAGTGTGG GCCCTTTATTTA-3′) and Keratin-4 (Upper: 5′-GATCGCCACCTA CCGCAAACTG-3′; Lower: 5′-GAGCCGGAGCCAAAGCCACTAC-3′). Extracted DNA samples from transfected KLF4 MEFs cells were used to amplify promoter fragments of KLF4 targets mouse genes. The primer pairs used were as follows: CCND1 (Upper: 5′-CCCCC AGCGAGGAGGAA-3′; Lower: 5′-AGGCGGACCCATTGCTTAGA-3′), IAP (Upper: 5′-CACCCAACCTGCAGGAGACAT A-3′; Lower: 5′-GCT AGGGAACCAAGGCACCAG-3′), p21 (Upper: 5′-CTG CCT CCC GAG TGC TGT G-3′; Lower: 5′-GGG GCC CCG ATG GTA CCG-3′), HDC (Upper: 5′-TGGCAATTCTTCCCCCTTACG-3′; Lower: 5′-GCTCCTGCC CTGGCTTCTCTAT-3′) and Keratin-4 (Upper: 5′-CTTGGCCTGGG TAGCGGTTTTT-3′; Lower: 5′-TGATGGTGGCGGAAGAGGTGAT-3′). PCR products were analyzed on 2.0% agarose gels containing ethidium bromide (0.25 μg/ml).

2.5. Transient cotransfection and dual luciferase assays

HCT 116 cells were used for transient co-transfection and dual luciferase assay using a previous published protocol [9].

2.6. Total RNA isolation and quantitative real-time PCR

CtBP MEFs cells were grown to 80% confluence in six-well plates and then the culture medium was replaced by DMEM without FBS for another 1 h, 2 h, 4 h and 8 h, respectively. RNA samples were