CtBP2 Promotes Human Cancer Cell Migration by Transcriptional Activation of Tiam1

Genes & Cancer
3(7-8) 481–490
© The Author(s) 2012
Reprints and permission:
sagepub.com/journalsPermissions.nav
DOI: 10.1177/1947601912463695
http://ganc.sagepub.com

Seema Paliwal^{1,2}, Ngoc Ho¹, Daniel Parker¹, and Steven R. Grossman³

Submitted 23-June-2012; accepted 13-Sep-2012

Abstract

The mammalian COOH-terminal binding proteins (CtBPs) CtBP1 and CtBP2 are metabolically regulated transcriptional co-repressors that are degraded upon acute exposure to the alternative reading frame (ARF) tumor suppressor. We reported previously that CtBP stimulates cell migration in certain contexts via repression of PTEN transcription and activation of the phosphatidylinositol 3-kinase (PI3K) pathway. We have now identified an additional and direct mechanism for CtBP stimulation of cell migration via regulation of T-cell lymphoma invasion and metastasis 1 (Tiam1) protein. Tiam1 is a guanine nucleotide exchange factor (GEF) for Rac GTPase that plays a critical role in regulating cell adhesion, invasion, and migration and has been directly implicated in the promotion of cancer progression and metastasis. We noted a strict positive correlation between CtBP2 and Tiam1 expression levels and that CtBP promotion of cell migration required CtBP-dependent transcriptional activation of Tiam1. RNA interference (RNAi)-mediated knockdown of CtBP2 in human colon or lung carcinoma cells led to decreased Tiam1 protein and mRNA expression, while overexpression of CtBP2 increased Tiam1 expression levels. RNAi and overexpression studies also demonstrated that Tiam1 is a key downstream mediator of CtBP2-mediated cell migration. An analysis of the Tiam1 promoter revealed binding sites for the CtBP-interacting Kruppel-like factor 8 (KLF8), and a Tiam1 promoter luciferase reporter was induced in the presence of both KLF8 and CtBP2, consistent with KLF8-dependent CtBP transactivation of Tiam1. Chromatin immunoprecipitation analyses demonstrated CtBP2 occupancy of the Tiam1 promoter that was dependent on the presence of KLF8. Our results indicate that Tiam1 is a transcriptional activation target of CtBP2 and that this interaction promotes the pro-oncogenic function of CtBP2 leading to cancer cell migration. Transcriptional activation thus plays a role in CtBP pro-oncogenic functions along with the previously characterized CtBP co-repressor function.

Keywords:

CtBP, TIAM, migration, invasion, transactivation

Introduction

COOH-terminal binding proteins (CtBPs) promote cancer cell migration, invasion, and survival via transcriptional co-repression of multiple tumor suppressor genes involved in cell motility, adhesion, and the apoptotic program.¹⁻³ The conserved CtBP1 and CtBP2 paralogs encode a conserved central dehydrogenase homology domain with an NAD⁺/NADH binding region within this domain that acts as a regulatory switch, with the repressor activity induced by conformational changes caused by NADH binding.⁴⁻⁸ Due to the metabolic impact of hypoxia on NADH levels, CtBP has been proposed as a mechanistic link between hypoxic tumor environments and tumor progression.^{1,2} CtBP1 and CtBP2 are overexpressed in colon, breast, and prostate cancer,⁹ and increased levels have been linked to the loss of tumor suppressors such as APC, Hipk2, and alternative reading frame (ARF).¹⁰

CtBP2's ability to direct cell migration has been previously linked to its ability to repress PTEN expression and thereby stimulate phosphatidylinositol 3-kinase (PI3K) activity, resulting in Rac-dependent migration.² The ability of the ARF tumor suppressor to inhibit migration/invasion

and metastasis independent of p53 activation in animal models¹¹ has also been linked to its ability to inactivate CtBP through direct binding and targeting of CtBP to the proteasome.¹² Thus, a complete understanding of the mechanism by which CtBP activity is linked to the induction of cancer cell migration is critical to understanding mechanisms of ARF tumor suppression and also will likely contribute to our understanding of invasion/metastasis in human cancer.

The control of cell migration by ARF has been linked to the modulation of Rac GTPase activity, at least in part due to CtBP-mediated repression of PTEN and activation of

¹Department of Cancer Biology, University of Massachusetts Medical School, Worcester, MA, USA.

²Department of Surgery, University of Massachusetts Medical School, Worcester, MA, USA.

³Division of Hematology, Oncology, and Palliative Care, Massey Cancer Center, Virginia Commonwealth University, Richmond, VA, USA.

Corresponding Author:

Steven R. Grossman, Division of Hematology, Oncology, and Palliative Care, Massey Cancer Center, Virginia Commonwealth University, 1101 East Marshall Street, Sanger 6030, Richmond, VA 23298, USA (Email: srgrossman@vcu.edu).

PI3K.² Indeed, deregulated activity of Rac/Rho GTPases results in enhanced cancer cell migratory, invasive, and metastatic properties.^{13,14} In human cancers, there is increasing evidence of deregulation of GTPases such as Rac and Rho caused by deregulated expression and/or activity of associated GTP exchange factors (GEFs).¹⁵ GEFs promote formation of the active GTP-bound state of GTPases, and GTPase-activating proteins (GAPs) return GTPases to the GDP-bound inactive state.

T-cell lymphoma invasion and metastasis 1 (Tiam1) is a GEF for Rho-like GTPases and specifically activates Rac1 *in vivo* and Rac, cdc42, and Rho *in vitro*.¹⁶⁻¹⁸ Tiam1 and its paralog Tiam2 are conserved among vertebrates, and Tiam1 is ubiquitously expressed in adult tissues.¹⁹ The Tiam1-Rac1 pathway regulates numerous biological properties, especially cell adhesion and migration,^{20,21} and its activation is associated with an increased invasiveness of many tumors, such as nasopharyngeal carcinoma,²² breast cancer,²³ and retinoblastoma.²⁴ Tiam1 expression is also transcriptionally regulated during the epithelial to mesenchymal transition.²⁵ Tiam1-deficient mice develop fewer and smaller Ras-induced skin tumors than wild-type mice,²⁶ and cells with endogenously high levels of Tiam1, or with ectopic overexpression of Tiam1, demonstrate increased cellular migration and metastatic potential in mouse models.²⁷ In colon tumor cells, Tiam1 affects multiple properties associated with acquisition of the metastatic phenotype and may represent a marker of colon tumor progression and metastasis in a subset of tumors.²⁸

In this work, we found that the CtBP-driven migration of colon cancer cells² was markedly accelerated by Tiam1 and that Tiam1 expression rescued migration after CtBP2 depletion. CtBP2 and Tiam1 were linked in a transcriptional pathway, where CtBP2 co-activated, not repressed, Tiam1 expression. Chromatin immunoprecipitation (ChIP) analysis demonstrated that CtBP2 was recruited to the Tiam1 promoter by the basic Kruppel-like factor 8 (KLF8) transcription factor. Tiam1 is thus a heretofore unknown downstream activation gene target of CtBP2 required for CtBP's ability to promote the migration of colon carcinoma cells and is likely an important downstream mediator of CtBP's pro-oncogenic functions.

Results and Discussion

CtBP2 positively regulates Tiam1 expression. Given the critical role for Tiam1 in cellular invasion and migration²⁸ and the correlation of CtBP induction of migration with the activation of Rac GTPase activity,² we examined whether the Rac/Rho GEF Tiam1 was a direct or indirect downstream target for CtBP transcriptional regulation. HCT116 (p53^{-/-}) cells were transfected with control or CtBP2 siRNA and then analyzed for Tiam1 protein and mRNA expression levels. Tiam1 protein abundance was reduced by approximately 40% (Suppl. Table S1) in CtBP2 siRNA-treated

cells as compared with control siRNA-treated cells (Fig. 1A, left panel). To assess whether CtBP2 was regulating Tiam1 expression at the transcriptional level, Tiam1 mRNA levels were analyzed in HCT116 (p53^{-/-}) cells 24 hours after treatment of the cells with CtBP2 siRNA (Fig. 1A, right panel). qRT-PCR analysis confirmed that Tiam1 mRNA expression was also downregulated approximately 40% ($P < 0.05$) upon CtBP2 depletion in HCT116 (p53^{-/-}) cells (Fig. 1A), most likely accounting for the observed decrease in protein abundance upon CtBP2 depletion.

If CtBP2 depletion decreased Tiam1 protein and mRNA levels, then raising the level of cellular CtBP2 might be expected to increase Tiam1 protein and mRNA levels. To test the effect of CtBP2 overexpression on Tiam1 levels, control, wild-type, or G189A NADH binding mutant (transcriptional repression defective⁵) V5-tagged CtBP2 cDNA expression vector was transfected into HCT116 (p53^{-/-}) cells. CtBP2-expressing HCT116 (p53^{-/-}) cells showed substantially increased (2.3-fold) Tiam1 protein levels as compared to control cells (Fig. 1B, left panel, and Suppl. Table S1). qRT-PCR also revealed a markedly increased abundance of Tiam1 mRNA after HCT116 (p53^{-/-}) cells were transfected with CtBP2 (2.75-fold over control) (Fig. 1B, right panel), consistent with the effect of CtBP2 on Tiam1 protein levels acting at the level of mRNA abundance. Compared with wild-type CtBP2, NADH binding defective CtBP2 was significantly defective in activating Tiam1 mRNA expression (1.5- v. 2.75-fold induction; $P < 0.05$), with no detectable protein induction (Fig. 1B), suggesting that the effect of CtBP2 on Tiam1 mRNA levels was linked to CtBP2/NADH interaction similar to the linkage of CtBP2 transcriptional repression to NADH binding. Thus, CtBP2 appears to control Tiam1 expression through control of Tiam1 mRNA expression.

To determine if CtBP acts to increase Tiam1 mRNA and protein levels in cells other than HCT116 (p53^{-/-}) colon cancer cells, H1299 human lung cancer cells were also tested in the same manner for CtBP2 regulation of Tiam1. Tiam1 protein and mRNA expression levels were determined by immunoblots and qRT-PCR, respectively, in H1299 cells transfected with CtBP2 shRNA. As seen in HCT116 (p53^{-/-}) cells, CtBP2 depletion resulted in a 50% decrease in the Tiam1 protein level (Fig. 1C, top left panel and bottom left panel, and Suppl. Table S1) as well as a more modest but statistically significant ($P < 0.05$) 20% decrease in the Tiam1 mRNA level (Fig. 1C). Furthermore, as also seen in the colon cancer cells, CtBP2 overexpression in H1299 cells resulted in increased Tiam1 protein (3-fold) and mRNA (2-fold) levels and to a similar extent as observed in HCT116 (p53^{-/-}) cells (Fig. 1D).

Tiam1 overexpression rescues cell migration after CtBP2 knockdown. CtBP2 expression has been previously reported to promote HCT116 cell migration, and CtBP2's depletion completely abrogates cell migration activity.² To test if

Figure 1. CtBP2 regulates Tiam1 protein and RNA levels in HCT116 ($p53^{-/-}$) and HI299 cells. **(A)** HCT116 ($p53^{-/-}$) cells were transfected with control siRNA or CtBP2-specific siRNA, and protein expression levels of Tiam1, CtBP2, and GAPDH in the cell lysates (24 hours) were determined by Western blotting (left). mRNA isolated after CtBP2 or control siRNA treatment of HCT116 ($p53^{-/-}$) cells was subjected to qRT-PCR using GAPDH- and Tiam1-specific primers and fold change of Tiam1 levels normalized to control GAPDH determined (right). **(B)** HCT116 ($p53^{-/-}$) cells were transfected with vector alone, Pc-V5CtBP2, or Pc-V5CtBP2-G189A. Subsequently, protein expression levels of Tiam1, CtBP2, and GAPDH in the cell lysates (24 hours) were determined by Western blotting (left). mRNA isolated after V5CtBP2, CtBP2-G189A, or control vector transfection of HCT116 ($p53^{-/-}$) cells was subjected to qRT-PCR using GAPDH- and Tiam1-specific primers and fold change of Tiam1 levels normalized to control GAPDH determined (right). **(C)** HI299 cells were transfected with control GFP or CtBP2 shRNA, and protein expression levels of Tiam1, CtBP2, and GAPDH in the cell lysates (24 hours) were determined by Western blotting (top left). Quantitation of unsaturated blot from **C** above with normalization to loading control (bottom left). mRNA isolated after CtBP2 or control GFP shRNA treatment of HI299 cells was subjected to qRT-PCR using GAPDH- and Tiam1-specific primers and fold change of Tiam1 levels normalized to control GAPDH determined (right). **(D)** HI299 cells were transfected with vector alone (Pc) or Pc-V5CtBP2, and protein expression levels of Tiam1, CtBP2, and GAPDH in the cell lysates (24 hours) were determined by Western blotting (left). mRNA isolated after Pc-V5CtBP2 or control vector treatment of HI299 cells was subjected to qRT-PCR using GAPDH- and Tiam1-specific primers and fold change of Tiam1 levels normalized to control GAPDH determined (right). All experiments (**A-D**) were done in triplicate. Error bars indicate ± 1 standard deviation. When compared to respective control siRNA or shRNA (**A, C**) or empty expression vector (**B, D**), all experimental values differed from the control with $P < 0.05$, including the induction of Tiam1 mRNA by CtBP2-G189A in **B**. In addition to statistical comparisons to the control, the asterisk in **B** indicates that the respective experimental values differed from each other with $P < 0.05$. Immunoblots (**A-D**) were each representative of 3 independent experiments.

Tiam1 overexpression could rescue the inhibition of cell migration caused by CtBP2 knockdown, HCT116 ($p53^{-/-}$) cells were first transfected with control, CtBP2 siRNA, or CtBP2 siRNA combined with Tiam1 cDNA expression vector, followed by wound migration assay (Fig. 2). While HCT116 ($p53^{-/-}$) cells transfected with CtBP2 siRNA showed the expected drastic reduction in basal cell migration activity as compared to the control, cells transfected

with CtBP2 siRNA followed by Tiam1 overexpression demonstrated a partial reversal of the block in cell migration caused by CtBP2 depletion, with significant restoration of nearly 50% of the normal migratory activity compared with CtBP2 knockdown alone ($P < 0.05$) (Fig. 2A). Both CtBP2 knockdown and overexpression of the Tiam1 protein were confirmed by immunoblots (Fig. 2A, right panel). This experiment also reinforces the finding that CtBP2

Figure 2. Functional interplay of CtBP2 and Tiam1 in regulating colon cancer cell migration. **(A)** Block of cell migration by CtBP2 knockdown was rescued by Tiam1 overexpression. HCT116 cells were transfected with control siRNA (control), CtBP2 siRNA, or CtBP2 siRNA + Pc-Tiam1 and cultured to confluency. Cells were scraped to create a wound and incubated for 24 hours. Percentage of wound closure was determined as described in Materials and Methods and plotted for each condition (**left**). Levels of CtBP2, Tiam1, and GAPDH were determined by Western blot analysis (**right**). **(B)** Synergistic effect of CtBP2 and Tiam1 depletion on migration in HCT116 (p53^{-/-}) cells. HCT116 (p53^{-/-}) cells were transfected with shGFP (control), shTiam1, CtBP2 siRNA, or shTiam1 + CtBP2 siRNA as indicated. Twenty-four hours later, a monolayer wound was introduced, and cells were incubated a further 24 hours. Percentage of wound closure was determined (**left**). Levels of CtBP2, Tiam1, and GAPDH were determined by Western blot analysis (**right**). **(C)** Cell migration induced by CtBP2 was further induced by overexpression of Tiam1. Monolayers of HCT116 (p53^{-/-}) cells expressing empty vector (control), Pc-V5CtBP2, Pc-Tiam1, or Pc-V5CtBP2 + Pc-Tiam1 were wounded and incubated for 12 hours. Percentage of wound closure was calculated (**left**). V5, CtBP2, Tiam1, and GAPDH protein levels were determined by immunoblots (**right**). Each experiment (**A-C**) was conducted in triplicate and mean percentage of wound closure plotted. Error bars indicate ± 1 standard deviation. When compared to the respective control siRNA (**A**), shRNA (**B**), or empty expression vector (**C**), all experimental values (**A-C**) differed from the control with $P < 0.05$. In addition to statistical comparisons to the control, the asterisk (**A-C**) or + (**C**) indicates that the respective experimental values differed from each other with $P < 0.05$.

depletion leads to a reduction in Tiam1 levels (Fig. 2A, right panel).

Functional interplay of CtBP2 and Tiam1 in the regulation of cell migration. To determine if CtBP2 and Tiam1 regulate cell migration through the same functional pathway, CtBP2 and Tiam1 were depleted individually or in combination

using siRNA and shRNA, respectively, or overexpressed individually or in combination, following which cell migration was analyzed. The effect of CtBP2 and Tiam1 depletion individually resulted in the inhibition of HCT116 (p53^{-/-}) cell migration, with CtBP2 knockdown exerting a more profound inhibition (70% reduction) than Tiam1 knockdown (25% reduction; $P < 0.05$) when either was compared to control siRNA (Fig. 2B, left panel). Notably, further abrogation of cell migration was observed with the simultaneous knockdown of both CtBP2 and Tiam1 (~90% reduction; $P < 0.05$ for comparison of shTiam1 + siCtBP2 v. siCtBP2) (Fig. 2B, left panel). Immunoblots confirmed the appropriate individual effect of Tiam1 and CtBP2 siRNA in each of the cell lysates, and the combination of CtBP2 siRNA with Tiam1 shRNA resulted in the additional depletion of Tiam1, consistent with an activator function for CtBP2 in Tiam1 expression (Fig. 2B, right panel). Thus, both CtBP2 and Tiam1 contribute directly to HCT116 (p53^{-/-}) cell migration, although the difference in the biological effect of CtBP2 versus Tiam1 knockdown, which both result in similar Tiam1 levels, indicates that CtBP2 likely exerts its effects on migration through both Tiam1-dependent and -independent pathways.²

To further investigate the hypothesis that direct regulation of Tiam1 expression by CtBP2 results in migratory phenotypes, CtBP2 or Tiam1 was overexpressed individually, or in combination, in HCT116 (p53^{-/-}) colon cancer cells, followed by migration assay. Cell migration increased 10- to 15-fold in colon

cancer cells transfected individually with CtBP2 or Tiam1 expression vectors (Fig. 2C). However, cells co-transfected with CtBP2 and Tiam1 together showed a significant additive (2- to 3-fold) increase in cell migration as compared to the expression of each factor alone ($P < 0.05$ for comparison of CtBP2 + Tiam1 expression v. either expressed alone), suggesting that CtBP2 and Tiam1 cooperate in regulating

cell migration (Fig. 2C). Immunoblotting verified the overexpression of CtBP2 and Tiam1, and interestingly, the levels of Tiam1 achieved were equivalent after Tiam1 or CtBP2 overexpression. With both factors overexpressed, there was a significant additional increase in Tiam1 expression correlating with the additional increase in migration seen when both were overexpressed (Fig. 2C, right panel).

CtBP2 regulation of the Tiam1 promoter through KLF8 recognition elements. Given the possibility that CtBP2 was acting as a transcriptional activator of the Tiam1 gene, the Tiam1 promoter region was analyzed for recognition sites relevant to CtBP binding transcription factors. Three sites with an exact match to KLF8 (ZNF741/BKLF3) recognition elements²⁹ were identified within the -1 to -2,200 upstream promoter region of the Tiam1 locus (Fig. 3A). We therefore hypothesized that KLF8 recruits CtBP2 to the Tiam1 promoter and that this recruitment in turn regulates Tiam1 promoter activity. To test this hypothesis, a luciferase reporter containing the Tiam1 promoter was co-transfected into HCT116 (p53^{-/-}) cells with CtBP2, or CtBP2 and KLF8 expression vectors, as indicated (Fig. 3B). Co-expression of CtBP2/KLF8 activated the Tiam1 promoter by approximately 1.5-fold ($P < 0.05$) (Fig. 3B), whereas there was no significant activation of the Tiam1 promoter after the expression of CtBP2 alone. Neither CtBP2 alone nor CtBP2 in combination with KLF8 expression vectors enhanced the expression of a control reporter lacking KLF8 recognition elements (Fig. 3B).

To confirm that intact KLF8 sites were necessary for CtBP2 regulation of Tiam1, Tiam1 promoter luciferase reporters containing either all wild-type KLF8 binding sites or with the KLF8 binding site nearest to the transcription start site mutated were transfected into HCT116 (p53^{-/-}) cells along with expression vectors for 1) KLF8, 2) CtBP2 and KLF8, or 3) mutant CtBP2 (defective in NADH binding) and KLF8 (Fig. 3C). Co-expression of CtBP2 with KLF8 resulted in an approximately 1.5-fold increase in wild-type Tiam1 promoter activity ($P < 0.05$), while the mutant Tiam1 promoter was not activated by combined CtBP2/KLF8 expression (Fig. 3C). This suggests that the KLF8 binding site is required for CtBP2/KLF8 activation of the Tiam1 promoter. Further, when a mutant CtBP2 defective for NADH binding and co-repressor activity² was co-transfected with KLF8, the combination had no effect on Tiam1 promoter activity, suggesting that CtBP2 and its NADH binding activity, in concert with KLF8, is required for co-activation of Tiam1 (Fig. 3C). Thus, KLF8 elements and the CtBP2 NADH binding domain are crucial for CtBP/KLF8-mediated regulation of the Tiam1 promoter.

CtBP2 is recruited to the Tiam1 promoter. Previous studies have shown that CtBP2 binds to KLF8 and regulates the

expression of genes downstream of KLF8 recognition elements.^{29,30} To address whether CtBP2 is directly recruited to the Tiam1 promoter, CtBP2 ChIP was performed using chromatin from HCT116 (p53^{-/-}) colon carcinoma cells and either control IgG or anti-CtBP2 antibody (Fig. 3D). The Tiam1 PCR primer set amplified a fragment encompassing the proximal KLF8 binding site at -975bp relative to the transcription start site of the Tiam1 promoter that is critical for CtBP regulation of the promoter (Fig. 3A). A control primer set amplified a fragment located 10 kb upstream of the Tiam1 promoter, whereas the E-cadherin promoter primer set was used as a positive control for a promoter occupied by CtBP2 but not via KLF8 sites³¹ (Fig. 3D). To also test the hypothesis that KLF8 was directly targeting CtBP2 to the Tiam1 promoter, the chromatin for these analyses was prepared from HCT116 (p53^{-/-}) cells expressing either a control (shGFP) or KLF8-specific shRNA (sh-KLF8). The efficacy of KLF8 knockdown was determined by RT-PCR (Fig. 3E). Equally robust CtBP2 binding was observed with both the Tiam1 and E-cadherin promoter regions in chromatin from shGFP cells interrogated by the respective primer sets (Fig. 3D). Adding specificity to these data, CtBP2 did not localize to the control fragment, and the control IgG ChIP with the Tiam1 primer set also did not yield any signal (Fig. 3D). Consistent with a CtBP2-targeting role for KLF8 at the Tiam1 promoter, CtBP2 recruitment to the Tiam1 promoter was reduced in the absence of KLF8 (consistent with the level of its knockdown) (Fig. 3E). KLF8 depletion had no effect on CtBP2 recruitment to the E-cadherin promoter, as CtBP is recruited to the E-cadherin promoter by E-box factors (Twist, Snail^{1,32,33}) rather than KLF8 (Fig. 3D).

Tiam1 as a transactivation target and downstream effector of CtBP-induced cell migration. CtBP acts by transcriptionally regulating several genes involved in major pathways promoting cancer, as identified in a wide range of studies in humans, mice, and *Drosophila*.^{6,10,34} CtBPs have a direct role in stimulating cell migration and invasion, a characteristic that is linked to its prometastatic function in cancer.^{2,3} In this report, we demonstrated that CtBP2 transcriptionally activates Tiam1 to promote colon cancer cell migration. Tiam1 was found to be invariably upregulated in cells overexpressing CtBP2 and downregulated with CtBP depletion. This effect was observed both at the protein and mRNA expression levels, and a transcriptional analysis identified Tiam1 as a transcription activation target of CtBP2.

We found CtBP2 to be recruited to the Tiam1 promoter largely through KLF8, as is the case for CtBP recruitment to the Bik promoter.³¹ This suggests that Tiam1 is a transcriptional activation target of CtBP via KLF8 binding and that the KLF8 elements are crucial for CtBP/KLF8-mediated activation of the Tiam1 promoter. Tiam1 is therefore a

Figure 3. Tiam1 is a transcriptional activation target of CtBP via KLF8 binding. **(A)** Diagram of KLF8 recognition elements in the Tiam1 promoter. The mutation in the Tiam1 promoter at the KLF8 recognition site used in **B** is shown. **(B)** CtBP2 activates the Tiam1 promoter in the presence of KLF8. A Tiam1 promoter luciferase reporter plasmid (pGL3-Tiam1 luc ["Tiam1"]) or empty reporter plasmid (pGL3-luc ["vector"]) was co-transfected with or without Pc-V5CtBP2 or Pc-V5KLF8 into HCT116 (p53^{-/-}) cells along with a control reporter plasmid expressing *Renilla* luciferase (pRL-TK). Normalized mean firefly luciferase activity from 3 independent experiments is shown. Error bars indicate ± 1 standard deviation. The asterisk or + indicates that the respective experimental values differed from each other with $P < 0.05$. **(C)** CtBP2 regulates the Tiam1 promoter through KLF8 recognition elements. (i) Luciferase reporter alone ("vector"), (ii) Tiam1 promoter luciferase reporter ("Tiam1"), and (iii) mutant Tiam1 promoter luciferase reporter ("mTiam1") were co-transfected with vector alone, Pc-V5CtBP2, or Pc-V5CtBP2-G189A in the presence of Pc-V5KLF8 into HCT116 (p53^{-/-}) cells along with a control reporter plasmid expressing *Renilla* luciferase (pRL-TK). Normalized mean firefly luciferase activity from 3 independent experiments is shown. Error bars indicate ± 1 standard deviation. The asterisk indicates that the respective experimental values differed from each other with $P < 0.05$. **(D)** **(Left)** KLF8 mediated recruitment of CtBP2 to the Tiam1 promoter. ChIP assay of the Tiam1 promoter was performed using chromatin obtained from shGFP or shKLF8-expressing HCT116 (p53^{-/-}) cells. After immunoprecipitation with anti-CtBP2 or control IgG antibodies, immunoprecipitated and input DNA was amplified by PCR using (i) a Tiam1 primer specific for the Tiam1 promoter, (ii) a negative control primer set that amplifies a fragment 10 kb upstream of the Tiam1 promoter, or (iii) positive control E-cadherin promoter primers. PCR products were electrophoresed in an agarose gel and stained with ethidium bromide. **(Right)** Quantitation of the ChIP results from **D** above. Experiment shown is representative of 3 independent experiments. **(E)** Efficacy of KLF8 shRNA used in the ChIP experiment in **D** above. shRNA targeting KLF8 or GFP was expressed in HCT116 cells. RT-PCR was performed to determine the knockdown of KLF8 mRNA with GAPDH mRNA level as an internal control. RT-PCR products were electrophoresed in an agarose gel and stained with ethidium bromide.

novel target for CtBP2 transcriptional regulation, but unlike its previously well-understood co-repression role, CtBP2 here is playing a paradoxical but previously described role

as a transcription activator.³⁴⁻³⁸ Notably, in H1299 cells but not HCT116 cells, the changes in the Tiam1 protein were consistently greater (positive or negative) than the changes

in mRNA expression, suggesting that in these cells, additional posttranscriptional mechanisms may also be at play in the CtBP regulation of Tiam1 protein abundance.

CtBP2 transcriptional activation of Tiam1 resulted in increased colon cancer cell migration, as HCT116 (p53^{-/-}) colon cancer cells transfected with CtBP2 siRNA followed by Tiam1 overexpression demonstrated rescue of the block in cell migration caused by CtBP2 depletion. Thus, CtBP2 depletion reduced cell migration by reducing Tiam1 levels, and overexpression of Tiam1 in these cells rescued migration. Furthermore, HCT116 (p53^{-/-}) colon carcinoma cells underwent increased migration in response to CtBP2, and exogenous Tiam1 markedly accelerated this process.

CtBP engages multiple pathways to induce cell migration. We have previously reported that CtBP2 potently promotes cell migration by decreasing PTEN levels. Lower PTEN abundance correlates with activation of the PI3K pathway, a major regulator of cell migration via generation of PIP₃, which can activate the GEF P-Rex as well as modulate other GEFs and GTPase-activating proteins (GAPs) to activate Rho family GTPases.^{2,39} In this report, Tiam1 activation by CtBP2 was identified to also play a critical and indispensable role in CtBP2-induced cell migration, although the impact of CtBP2 depletion was more potent on migration than that of Tiam1 depletion despite equal levels of Tiam1 depletion in both cases (Fig. 2B). This would be consistent with CtBP regulating migration by multiple pathways, as it can activate the promigratory PI3K pathway (via PTEN repression) simultaneously with upregulating Tiam1 (Fig. 4). This model raises the interesting possibility that migration is quantitatively regulated by the summed activity of multiple GEFs and GAPs that act on Rho family GTPases. Additionally, CtBP2 depletion's more potent impact on migration than Tiam1 depletion could also be due, in part, to the CtBP2 transcriptional activation of other Tiam1-related proteins, most importantly its paralog Tiam2, which encodes a similar GEF activity.^{40,41}

Role of Tiam1 in migration/invasion and metastasis. Tiam1 associates with activated, oncogenic Ras-GTP to stimulate Rac activity, and CtBP potently induces activated Rac and migratory/invasive behavior in cancer cell lines.² Tiam1 has been directly implicated in cell adhesion and migration, and its activation is associated with increased tumor invasiveness.²⁸ Specifically, Tiam1 overexpression contributes to colon carcinoma growth at metastatic sites in an orthotopic nude mouse model,²⁷ while Tiam1 knockdown in a colorectal cancer cell line resulted in the effective inhibition of *in vitro* growth and invasive ability of colorectal cancer cells and inhibition of *in vivo* growth of lung and liver metastases.⁴² Thus, the promigration function of CtBP2 could contribute to colon cancer metastasis through transcriptional

Figure 4. Proposed model for the role of CtBP in cell migration. CtBP coordinately regulates cancer cell migration via repression of PTEN, activating the PI3K pathway,^{2,39} and also by direct transcriptional activation of the GEF Tiam1 (this work). Both PI3K and Tiam1 positively regulate Rac and other Rho family small GTPases via GEF activity (intrinsic to Tiam; induced by PI3K via PIP₃ regulation of P-Rex and other GEFs) and/or modulation of GAP activities.

regulation of Tiam1, and Tiam1 may mediate CtBP's proinvasive and prometastatic functions.^{3,11}

CtBP as a transcriptional activator. CtBP family proteins have been well characterized for their role as transcriptional co-repressors for many transcription factors.³⁴ This study expands the transcriptional role of CtBP2 as an activator in addition to its well-defined role as a transcriptional co-repressor. Previous reports have suggested that in addition to its canonical repressor activity, CtBP can also mediate transcription activation in certain contexts.³⁴ In mice, the expression of the Wnt3A target gene *Brachyury* was abnormally low in E10.5 CtBP2^{-/-} embryos compared to E9.5 embryos,⁴³ suggesting that mCtBP2 could function as a direct or indirect transcriptional activator of *Brachyury*. In *Drosophila*, dCtBP co-activates the transcription of certain Wnt target genes after stimulation with Wnt, even while repressing other Wnt target genes in the absence of Wnt.³⁶ hCtBP1 was also recently identified as an activator of MDR1 gene transcription in human multidrug-resistant cancer cells.³⁸ Our results support a model where CtBP is a gene-specific regulator of cell migration, with some targets such as PTEN and Bik undergoing CtBP repression,² while

other targets such as Tiam utilize CtBP for activation of their expression (Fig. 4).

As CtBP-based therapeutics are developed,⁴⁴ and with increasing evidence for aberrant Tiam activity in cancer, Tiam as a GEF enzyme appears to be an attractive target for anticancer drug discovery. The simultaneous inhibition of both Tiam and CtBP may lead to potent effects, especially in the realm of inhibiting cancer cell invasion and metastasis.

Materials and Methods

Cell cultures and transfections. DMEM and McCoy's medium (Invitrogen, Carlsbad, CA) were supplemented with 10% fetal bovine serum and 100 U/mL of penicillin. Human H1299 (lung carcinoma; p53 null) cells were cultured in DMEM, and HCT116 human colon cancer cells (ARF silenced) with a targeted deletion of p53 (p53^{-/-})⁴⁵ were grown in McCoy's medium (Invitrogen). Cells were incubated at 37°C in a humidified, 5% CO₂ environment. Expression plasmids were transfected using Fugene (Roche, Basel, Switzerland), and small interfering RNA (siRNA) duplexes were transfected using Oligofectamine (Invitrogen). The siRNA concentration used for transfection was 40 nmol/L, except where noted. The siRNA sequence for human CtBP2 (hCtBP2) was 5'-AAGCGCCUUGGUCAGUAAUAG-3' and for shKLF8 was 5'-CTGGTCGATATGGATAAACTCA-3'. A human Tiam1 shRNA clone was obtained from Open Biosystems (Huntsville, AL). Nontargeting control siRNA (IX) was obtained from Dharmacon (Lafayette, CO). Vector alone, Tiam1, CtBP2, or CtBP2 mutant-expressing stable H1299 cells were generated using geneticin selection. shGFP and shTiam1-expressing stable H1299 cells were generated using puromycin selection.

Plasmids. Pc-V5CtBP2, Pc-V5CtBP2-G189A, and Pc-V5KLF8 have been described.^{2,12,31} Pc-Tiam1 expression plasmid was the generous gift of A. Mercurio.

Antibodies and Western blotting. Cells were lysed in lysis buffer (20 mM HEPES, pH 7.4, 0.5% Triton X-100, 2 mM MgCl₂, 10 μM ZnCl₂, 2 mM N-ethylmaleimide, 1 mM phenylmethylsulfonyl fluoride, and 240 mM NaCl) containing complete Mini Protease Inhibitor Cocktail Tablets (Roche). Antibodies used were as follows: CtBP2 (BD Transduction Laboratories, Franklin Lakes, NJ), hARF (Abcam, Cambridge, UK), V5 tag (Invitrogen), Tiam1 (Santa Cruz Biotechnology, Santa Cruz, CA), and GAPDH (Advanced ImmunoChemical, Long Beach, CA). Anti-rabbit IgG-horseradish peroxidase and anti-mouse IgG-horseradish peroxidase conjugates (Amersham, Amersham, UK) were used with enhanced chemiluminescence detection (Amersham) for Western blotting.

qRT-PCR. mRNA transcripts for human Tiam1 and GAPDH were analyzed by qRT-PCR using SYBR green (Applied Biosystems, Foster City, CA) and an ABI 7300 (Applied Biosystems). Relative amounts of the mRNA transcripts were calculated using the $\Delta\Delta CT$ method with GAPDH and β -actin mRNA as internal references. qRT-PCR for Tiam1 and GAPDH was performed using the following primers:

Tiam1

sense: 5'-AAGACGTACTCAGGCCATGTCC-3'
antisense: 5'-GACCCAAATGTCGCAGTCAG-3'

GAPDH

sense: 5'-ATCACCATCTTCCAGGAGCGA-3'
antisense: 5'-GCCAGTGAGCTTCCCGTTCA-3'.

Cell motility and migration assays. For wound assays, equal numbers of cells were plated at 50% confluency in 6-well plates, and 24 hours after plating, the cells were transfected with either siRNA or expression plasmids. Twenty-four hours after transfection, the confluent monolayer of cells was scraped to introduce a wound, and the cells were incubated for another 12 or 24 hours. Captured images were used to compare and quantify the percentage of wound closure by measuring the migration distance of cells found in the cleared area from the point the cells were scraped to the migration front. Five independent 100× fields were measured for each experiment as described,² and the values were normalized to the negative control set, which was set at 100% for each experiment. The percentage of wound closure and standard deviation were calculated for 3 experiments.

Tiam promoter luciferase reporter assay. A 2.0-kb region of the human Tiam1 promoter (−2,200 to −267) was amplified by PCR from human genomic DNA and inserted into the firefly luciferase reporter, pGL3 (Promega, Madison, WI). A KLF8 binding defective mutant Tiam1 promoter plasmid was generated from the pGL3-Tiam1 wild-type luciferase reporter plasmid using the QuikChange PCR protocol (Stratagene, La Jolla, CA). HCT116 cells were transfected with 1) pGL3-Tiam1, 2) pGL3-mTiam1, and 3) a control plasmid expressing the *Renilla* luciferase (pRL-TK), with vector alone, CtBP2, or an NADH binding defective allele (G189A) of CtBP2 together with KLF8. The expression of reporter genes was determined using a Dual Luciferase assay kit (Promega) after 24 hours of transfection.

ChIP assay. Cells were plated for 24 hours, and approximately 10⁸ cells were used for each ChIP assay. Cells were washed once in PBS and were treated with 1% formaldehyde in cold PBS for 10 minutes at 4°C with continuous shaking. Glycine (final concentration of 125 mM) was

added to quench the formaldehyde for 5 minutes at 4°C with continuous shaking. Cells were then harvested and washed twice with ice-cold PBS. Nuclei were isolated by incubating the cells in nucleus isolation buffer (5 mM PIPES, pH 8.0, 85 mM KCl, and 0.5% NP-40) for 20 to 30 minutes on ice. The nuclei were harvested at 4°C by centrifuging the cell suspension at 7,000g for 5 minutes and then resuspending the pellet in 2 mL of RIPA buffer (150 mM NaCl, 1% v/v NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris, pH 8.0, and 5 mM EDTA) containing complete Mini Protease Inhibitor Cocktail Tablets (Roche). Chromatin was fragmented to approximately 200 to 700 bp by sonication. Nuclear debris was removed by centrifuging the lysates at 4°C for 15 minutes at 14,000 rpm. The lysate was precleared by incubation with protein G Sepharose beads (Sigma-Aldrich, St. Louis, MO) for 30 minutes at 4°C. Immunoprecipitation was then performed overnight at 4°C with control (mouse IgG) and CtBP2 antibody, protein G Sepharose beads (Sigma-Aldrich) were added, and the immunocomplexes were then allowed to bind to the beads for 2 hours at 4°C. The beads were washed once each with RIPA buffer, RIPA buffer with 500 mM NaCl, immunoprecipitation wash buffer (10 mM Tris-HCl, pH 8.0, 250 mM LiCl, 0.5% NP-40, 0.5% sodium deoxycholate, and 1 mM EDTA), and finally with Tris-EDTA. Beads were resuspended in 200 µL of elution buffer (50 mM Tris-HCl, pH 8.0, 10 mM EDTA, and 1% SDS) with Proteinase K and incubated overnight at 55°C. DNA was extracted using phenol chloroform, precipitated in the presence of glycogen by ethanol, allowed to air dry, and dissolved in 10 mM Tris-HCl (pH 8.0), 1 mM EDTA. The immunoprecipitated DNA was diluted 10-fold to ensure that the amplified product was in the linear range following amplification by PCR. The following sets of primers were used to amplify different regions of the indicated genes:

Tiam1, primer set 1

sense: 5'-AATTAGGCAGCCTCACTCCA-3'

antisense: 5'-CCCCTGAAATCTGTTCTCCA-3'

Tiam1, primer set 2

sense: 5'-CGTGTCTCTGGGGAAAGAAAA-3'

antisense: 5'-AGGACTCCCAGGGTTCACTT-3'

nonspecific irrelevant primers 20 kb upstream of KLF8 binding sites

sense: 5'-ACCTTGCCTGGCCTTATTTT-3'

antisense: 5'-CCTGAGGTCAGGAGTTCGAG-3'

E-cadherin

sense: 5'-TAGCCTGGCGTGTTGGTGTG CACCTG-3'

antisense: 5'-GTGCGTGGCTGCAGCCAGGT GAGCC-3'.

RT-PCR analysis was done to confirm KLF8 knockdown from HCT116 cells expressing either a control (shGFP) or KLF8-specific shRNA (sh-KLF8). RT-PCR was performed using the following primers:

KLF8

sense: 5'-AGGTGGCTCAATGCAGGTAT-3'

antisense: 5'-CATGGGCAGAGACTGCACTA-3'

GAPDH

sense: 5'-ATCACCATCTTCCAGGAGCGA-3'

antisense: 5'-GCCAGTGAGCTTCCCGTTCA-3'.

Acknowledgments

The authors thank A. Mercurio and members of the Mercurio laboratory for assistance with reagents and for helpful discussions. The authors also thank Dr. Kevin Hogan for his comments and editorial assistance with the article.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the authorship and/or publication of this article.

Funding

The author(s) received the following financial support for the research, authorship, and/or publication of this article: S.R.G. was supported by an ACS Research Scholar Award, and S.P. was supported by the Pancreatic Cancer Alliance.

References

1. Zhang Q, Wang SY, Nottke AC, Rocheleau JV, Piston DW, Goodman RH. Redox sensor CtBP mediates hypoxia-induced tumor cell migration. *Proc Natl Acad Sci U S A*. 2006;103:9029-33.
2. Paliwal S, Kovi RC, Nath B, Chen YW, Lewis BC, Grossman SR. The alternative reading frame tumor suppressor antagonizes hypoxia-induced cancer cell migration via interaction with the COOH-terminal binding protein corepressor. *Cancer Res*. 2007;67:9322-9.
3. Chen YW, Paliwal S, Draheim K, Grossman SR, Lewis BC. p19Arf inhibits the invasion of hepatocellular carcinoma cells by binding to C-terminal binding protein. *Cancer Res*. 2008;68:476-82.
4. Chinnadurai G. CtBP family proteins: more than transcriptional corepressors. *Bioessays*. 2003;25:9-12.
5. Kumar V, Carlson JE, Ohgi KA, et al. Transcription corepressor CtBP is an NAD(+)-regulated dehydrogenase. *Mol Cell*. 2002;10:857-69.
6. Chinnadurai G. CtBP, an unconventional transcriptional corepressor in development and oncogenesis. *Mol Cell*. 2002;9:213-24.
7. Fjeld CC, Birdsong WT, Goodman RH. Differential binding of NAD+ and NADH allows the transcriptional corepressor carboxyl-terminal binding protein to serve as a metabolic sensor. *Proc Natl Acad Sci U S A*. 2003;100:9202-7.
8. Mani-Telang P, Sutrias-Grau M, Williams G, Arnosti DN. Role of NAD binding and catalytic residues in the C-terminal binding protein corepressor. *FEBS Lett*. 2007;581:5241-6.

9. Thomas G, Jacobs KB, Yeager M, *et al.* Multiple loci identified in a genome-wide association study of prostate cancer. *Nat Genet.* 2008;40:310-5.
10. Chinnadurai G. The transcriptional corepressor CtBP: a foe of multiple tumor suppressors. *Cancer Res.* 2009;69:731-4.
11. Chen YW, Klimstra DS, Mongeau ME, Tatem JL, Boyartchuk V, Lewis BC. Loss of p53 and Ink4a/Arf cooperate in a cell autonomous fashion to induce metastasis of hepatocellular carcinoma cells. *Cancer Res.* 2007;67:7589-96.
12. Paliwal S, Pande S, Kovi RC, Sharpless NE, Bardeesy N, Grossman SR. Targeting of C-terminal binding protein (CtBP) by ARF results in p53-independent apoptosis. *Mol Cell Biol.* 2006;26:2360-72.
13. Heasman SJ, Ridley AJ. Mammalian Rho GTPases: new insights into their functions from in vivo studies. *Nat Rev Mol Cell Biol.* 2008;9:690-701.
14. Parri M, Chiarugi P. Rac and Rho GTPases in cancer cell motility control. *Cell Commun Signal.* 2010;8:23.
15. Vigil D, Cherfils J, Rossman KL, Der CJ. Ras superfamily GEFs and GAPs: validated and tractable targets for cancer therapy? *Nat Rev Cancer.* 2010;10:842-57.
16. Strumane K, Rygiel TP, Collard JG. The Rac activator Tiam1 and Ras-induced oncogenesis. *Methods Enzymol.* 2006;407:269-81.
17. Mertens AE, Roovers RC, Collard JG. Regulation of Tiam1-Rac signalling. *FEBS Lett.* 2003;546:11-6.
18. Collard JG, Habets GG, Michiels F, Stam J, van der Kammen RA, van Leeuwen F. Role of Tiam 1 in Rac-mediated signal transduction pathways. *Curr Top Microbiol Immunol.* 1996;213(Pt 2):253-65.
19. Habets GG, van der Kammen RA, Stam JC, Michiels F, Collard JG. Sequence of the human invasion-inducing TIAM1 gene, its conservation in evolution and its expression in tumor cell lines of different tissue origin. *Oncogene.* 1995;10:1371-6.
20. Sander EE, van Delft S, ten Klooster JP, *et al.* Matrix-dependent Tiam1/Rac signaling in epithelial cells promotes either cell-cell adhesion or cell migration and is regulated by phosphatidylinositol 3-kinase. *J Cell Biol.* 1998;143:1385-98.
21. Minard ME, Kim LS, Price JE, Gallick GE. The role of the guanine nucleotide exchange factor Tiam1 in cellular migration, invasion, adhesion and tumor progression. *Breast Cancer Res Treat.* 2004;84:21-32.
22. Zhang XM, Jin H, Chen JZ, Yu LN, Li YF, Ding YQ. [Relationship between Tiam-1 expression and the biological behaviors of nasopharyngeal carcinoma]. *Nan Fang Yi Ke Da Xue Xue Bao.* 2009;29:26-8.
23. Lane J, Martin TA, Mansel RE, Jiang WG. The expression and prognostic value of the guanine nucleotide exchange factors (GEFs) Trio, Vav1 and TIAM-1 in human breast cancer. *Int Semin Surg Oncol.* 2008;5:23.
24. Adithi M, Venkatesan N, Kandam M, Biswas J, Krishnakumar S. Expressions of Rac1, Tiam1 and Cdc42 in retinoblastoma. *Exp Eye Res.* 2006;83:1446-52.
25. Hu J, Wang YD, Li YF, Wang YJ, Han HX. [Correlation between T lymphoma invasion and metastasis 1 expression and epithelial-mesenchymal transition in human colorectal carcinomas]. *Nan Fang Yi Ke Da Xue Xue Bao.* 2009;29:232-5.
26. Malliri A, van der Kammen RA, Clark K, van der Valk M, Michiels F, Collard JG. Mice deficient in the Rac activator Tiam1 are resistant to Ras-induced skin tumours. *Nature.* 2002;417:867-71.
27. Minard ME, Herynk MH, Collard JG, Gallick GE. The guanine nucleotide exchange factor Tiam1 increases colon carcinoma growth at metastatic sites in an orthotopic nude mouse model. *Oncogene.* 2005;24:2568-73.
28. Minard ME, Ellis LM, Gallick GE. Tiam1 regulates cell adhesion, migration and apoptosis in colon tumor cells. *Clin Exp Metastasis.* 2006;23:301-13.
29. Turner J, Crossley M. Cloning and characterization of mCtBP2, a co-repressor that associates with basic Kruppel-like factor and other mammalian transcriptional regulators. *EMBO J.* 1998;17:5129-40.
30. van Vliet J, Turner J, Crossley M. Human Kruppel-like factor 8: a CACCC-box binding protein that associates with CtBP and represses transcription. *Nucleic Acids Res.* 2000;28:1955-62.
31. Kovi RC, Paliwal S, Pande S, Grossman SR. An ARF/CtBP2 complex regulates BH3-only gene expression and p53-independent apoptosis. *Cell Death Differ.* 2010;17:513-21.
32. Grooteclaes M, Deveraux Q, Hildebrand J, Zhang Q, Goodman RH, Frisch SM. C-terminal-binding protein corepresses epithelial and proapoptotic gene expression programs. *Proc Natl Acad Sci U S A.* 2003;100:4568-73.
33. Grooteclaes ML, Frisch SM. Evidence for a function of CtBP in epithelial gene regulation and anoikis. *Oncogene.* 2000;19:3823-8.
34. Chinnadurai G. Transcriptional regulation by C-terminal binding proteins. *Int J Biochem Cell Biol.* 2007;39:1593-607.
35. Phippen TM, Sweigart AL, Moniwa M, Krumm A, Davie JR, Parkhurst SM. Drosophila C-terminal binding protein functions as a context-dependent transcriptional co-factor and interferes with both mad and groucho transcriptional repression. *J Biol Chem.* 2000;275:37628-37.
36. Fang M, Li J, Blauwkamp T, Bhambhani C, Campbell N, Cadigan KM. C-terminal-binding protein directly activates and represses Wnt transcriptional targets in Drosophila. *EMBO J.* 2006;25:2735-45.
37. Bhambhani C, Chang JL, Akey DL, Cadigan KM. The oligomeric state of CtBP determines its role as a transcriptional co-activator and co-repressor of Wingless targets. *EMBO J.* 2011;30:2031-43.
38. Jin W, Scotto KW, Hait WN, Yang JM. Involvement of CtBP1 in the transcriptional activation of the MDR1 gene in human multidrug resistant cancer cells. *Biochem Pharmacol.* 2007;74:851-9.
39. Cain RJ, Ridley AJ. Phosphoinositide 3-kinases in cell migration. *Biol Cell.* 2009;101:13-29.
40. Rooney C, White G, Nazgiewicz A, *et al.* The Rac activator STEF (Tiam2) regulates cell migration by microtubule-mediated focal adhesion disassembly. *EMBO Rep.* 2010;11:292-8.
41. Chiu CY, Leng S, Martin KA, Kim E, Gorman S, Duhl DM. Cloning and characterization of T-cell lymphoma invasion and metastasis 2 (TIAM2), a novel guanine nucleotide exchange factor related to TIAM1. *Genomics.* 1999;61:66-73.
42. Liu L, Zhang Q, Zhang Y, Wang S, Ding Y. Lentivirus-mediated silencing of Tiam1 gene influences multiple functions of a human colorectal cancer cell line. *Neoplasia.* 2006;8:917-24.
43. Hildebrand JD, Soriano P. Overlapping and unique roles for C-terminal binding protein 1 (CtBP1) and CtBP2 during mouse development. *Mol Cell Biol.* 2002;22:5296-307.
44. Straza MW, Paliwal S, Kovi RC, *et al.* Therapeutic targeting of C-terminal binding protein in human cancer. *Cell Cycle.* 2011;9:3740-50.
45. Bunz F, Dutriaux A, Lengauer C, *et al.* Requirement for p53 and p21 to sustain G2 arrest after DNA damage. *Science.* 1998;282:1497-501.