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C-Terminal Binding Protein 1 Modulates Cellular Redox via Feedback Regulation of MPC1 and MPC2 in Melanoma Cells

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Background: Recent studies have illustrated that the transcription co-repressor, C-terminal binding protein 1 (CtBP1), links the metabolic alterations to transcription controls in proliferation, EMT, genome stability, metabolism, and lifespan, but whether CtBP1 affects the cellular redox homeostasis is unexplored. This study was designed to investigate the mechanism of CtBP1-mediated transcription repression that contributes to the metabolic reprogramming.

Material/Methods: Knockdown of CtBP1 in both mouse MEF cells and human melanoma cells changed cell redox homeostasis. Further, chromatin immunoprecipitation (ChIP) and luciferase reporter assay were performed for identification of CtBP1 downstream targets, pyruvate carrier 1 and 2 genes (MPC1 and MPC2), which contribute to redox homeostasis and are transcriptionally regulated by CtBP1. Moreover, blockage of the cellular NADH level with the glycolysis inhibitor 2-Deoxy-D-Glucose (2-DG) rescued MPC1 and MPC2 expression. MTT assay and scratch assay were performed to investigate the effect of MPC1 and MPC2 expression on malignant properties of melanoma cells.

Results: The data demonstrated that CtBP1 directly bound to the promoters of MPC1 and MPC2 and transcriptionally repressed them, leading to increased levels of free NADH in the cytosol and nucleus, thus positively feeding back CtBP1's functions. Consequently, restoring MPC1 and MPC2 in human tumor cells decreases free NADH and inhibits melanoma cell proliferation and migration.

Conclusions: Our data indicate that MPC1 and MPC2 are principal mediators that link CtBP1-mediated transcription regulation to NADH production. The discovery of CtBP1 as an NADH regulator in addition to being an NADH sensor shows that CtBP1 is at the center of tumor metabolism and transcription control.

MeSH Keywords: **CtBP1 • Melanoma • NADH**

Abbreviations: **MEFs** – mouse embryonic fibroblast; **CtBP1** – C-terminal binding protein 1; **MPC1** – mitochondrial pyruvate carrier 1; **MPC2** – mitochondrial pyruvate carrier 2; **EMT** – epithelial-mesenchymal transition; **2-DG** – 2-Deoxy-D-glucose; **NADH** – nicotinamide adenine dinucleotide; **Ct** – control; **SC** – scramble; **OE** – overexpression; **KD** – knockdown

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Background

Pyruvate participates in carbon metabolism regulation as a product of glycolysis and is a major substrate for the tricarboxylic acid cycle in mitochondria. Hypoxia, which is low oxygen tension, is a common feature of malignant tumors [1]. The initial lack of oxygen impairs mitochondrial function, and pyruvate is reduced to lactate by lactate dehydrogenase, representing the first wave of free NADH in the cytosol and nucleus [2,3].

Carboxyl-terminal binding protein 1 (CtBP1) is a transcriptional co-repressor and a NADH sensor that activates multiple oncogenic pathways [4]. Although CtBP1 is mainly expressed during early embryonic development, its reactivation has been reported in colorectal cancer, melanoma, breast cancer, and head and neck cancers [5–10]. CtBP1 activation has been shown to promote proliferation and genomic instability, suppress apoptosis, and induce epithelial-mesenchymal transition [5–10].

The increase in CtBP1, like increasing levels of cellular NADH under hypoxia conditions, affects proliferation [11], EMT [12], and genome stability [10], but whether it directly affects metabolism, especially the redox balance between lactate and pyruvate, is unexplored.

The mitochondrial pyruvate transporters MPC1 and MPC2 are essential for mitochondrial pyruvate transport from the cytosol to the mitochondrial following glycolysis in yeast, *Drosophila*, and humans, by forming a multimeric complex embedded in the mitochondrial inner membrane [13]. Lost expression of MPC1 and MPC2 is found in a variety of cancers, and low MPC1 expression levels in colon cancer, kidney cancer, and lung cancer are associated with poor survival [14]. However, how MPC1 and MPC2 are regulated in cancer cells still remains unknown. Here, we explored whether CtBP1-mediated transcription repression contributes to metabolic reprogramming by suppressing the mitochondrial influx of pyruvate.

Material and Methods

Cell culture and transfections

Mouse MEFs and human melanoma cells, A375 and SK-MEL-28, were cultured in DMEM with 10% FBS. The mouse MEFs cells were described previously [15]. For hypoxia treatment, cells were exposed to 1% O_2 and 5% CO_2 balanced with N_2. For 2-DG treatment, melanoma cells were cultured with an additional 10 mM 2-DG (Sigma, St. Louis, MO, USA) for specified times.

In knockdown experiments, cells were treated with siRNA against CtBP1, MPC1, and MPC2 from Dharmacon using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Knockdown of MPC1 and MPC2 was performed by treating cells with 20 nM siRNA using the Lipofectamine RNAiMax transfection reagent (Thermo Scientific, Rockford, IL, USA), according to the manufacturer's instructions. The All-Stars non-targeting siRNA (Qiagen, MD, USA) was used as the control for siRNAs targeting mouse MPC1 and MPC2. SiRNAs targeting CtBP1 were described previously [6,15]. Sequences of the sense strands of targeting siRNAs, which include a 3' tt DNA overhang, were as follows. MPC1: (1) UCAACUACGAGAUGAGUAAtt, and (2) GGAAAACACAGAAUGCUAtt, and MPC2: (1) CCGAUA AGGUGAUGCUAAAtt, and (2) UGGAUAAAGUGGAGUUGUUtt.

Cloning

Human MPC1 and MPC2 were cloned from 293T cell cDNA. 2×flag tag was fused at the N-terminal of MPC1 and MPC2. Then, the flag-tagged fragment was cloned into PCDNA3 vector for additional expression experiments.

The sequences between –300 and 0 bp region of the MPC-1 and MPC-2 promoters was constructed on pGL4.26 by using the following primers. For MPC1 promoter, forward: 5'-CGCGCTAGC ACCCGGCCACGCCTTACGGCC-3', reverse: 5'-GATCTCGAGCCACTGCAGGTCGCCCCAAG-3'. For MPC2 promoter, forward: 5'-CGC GCTAGCGAGGCTGCCGACTGCCAGCCC-3', forward: 5'-GATAAGCTT CCCATTTTAACTACGGGCCTG-3'.

Western blotting and quantification

RIPA150 lysis buffer with protease inhibitor (Sigma, USA) was used for cell lysate preparation. Lysate samples were separated by SDS-PAGE and transferred to PVDF membranes (Bio-Rad, USA). The membranes were then blotted with primary antibodies to CtBP1 (EMD Millipore, Billerica MA, USA), FLAG (Sigma, St. Louis, MO, USA), MPC1 (Cell Signaling Technology, USA), MPC2 (Cell Signaling Technology, USA), and actin (Sigma, St. Louis, MO, USA) overnight at 4°C followed by incubation with secondary antibodies (Cell Signaling Technology, USA) for 1 h at room temperature. Signals were detected using enhanced chemiluminescence reagent (Thermo Scientific, USA). For blot bands quantification, ImageJ software was used for quantifying all bands, and targeted protein expression levels were normalized to β-actin band values.

qRT-PCR

Total RNA was isolated using TRIzol (Invitrogen, Carlsbad, CA, USA) as previously described [12]. One hundred nanograms of RNA was reverse-transcribed to cDNA from each sample using cDNA Synthesis kits (Thermo Scientific, Rockford, IL, USA). An 18S probe was used as an internal control. Each sample was examined in triplicate. The relative RNA expression levels were determined by normalizing with internal controls, the values

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of which were calculated using the comparative Ct method. Primers used for qRT-PCR are as follows.
Mouse CtBP1forward: 5'-CGAGGAACGCAAAGGACACAGG-3',
reverse: 5'-TAGGCGGGGCAAGAGGAAGC-3'.
Human CtBP1 forwad: 5'-GGACCTGCTCTTCCACAGCGACT-3'
reverse: 5'-CCTTGTCTCATCTGCTTGACGGTGA-3'.
18S forward: 5'-TGACGGAAGGGCACCACCAG-3',
reverse: 5'-GCACCACCACCCACGGAATC-3'.
Human MPC1 forward: 5'-TGCCTACAAGGTACAGCCTCGGAAC-3',
reverse: 5'-GATAAGCCGCCCTCCCTGGAT-3'.
Mouse MPC1 forward: 5'-TCGTGCTGAAGGGAAAACACAGAA-3',
reverse: 5'-GGGTTTAGGGACTCTCGGCTATTCAA-3'
Human MPC2 forward: 5'-CCCGCCTCGTCCTGTCAAAG-3',
reverse: 5'-AACGGAGCCAAAGGTCACAAACA-3'.
Mouse MPC2 forward: 5'-CTTTGCGGGACTCGGCCTCT-3',
reverse: 5'-GGGGCGGCTCGTCACTTTCT-3'.

Chromatin immunoprecipitation (ChIP) and luciferase reporter assay

A375 cells, with or without hypoxia or 2-DG (Sigma, St. Louis, MO, USA) treatment, were used for ChIP assay with an anti-CtBP1 antibody and normal rabbit IgG, as described previously [12]. Cells were cross-linked with 1% formaldehyde for 15 min, then stopped by 0.125 M glycine. Cell pellets were collected and sonicated in lysis buffer. Fragmented DNA was precipitated with CtBP1 antibody (EMD Millipore, Billerica MA, USA) and protein A beads (RepliGen, Waltham, MA, USA). Precipitated protein/DNA complexes were reverse cross-linked with additional 350 mM NaCl at 65°C for 6 h. The DNA fragments were then purified and used for PCR analysis. Primer sets spanning the MPC1 and MPC-2 promoters were used to q-PCR-amplify the ChIP samples are as follow.
MPC1 forward: 5'-CGGTTGCTAGGCTCCAG-3',
reverse: 5'-ACAGTCCTGTGGGTCAG-3'.
MPC2 forward: 5'-GAGAAGGGAAAGTGAAGCTG-3',
reverse: 5'-CGGGCCTGCTTAATCAAAG-3'.

An empty Renilla luciferase vector (pGL4.79) was used for normalization. The reporters were co-transfected with CtBP1-expressing plasmids [16] and the luciferase activity was measured. Empty plasmid was used for control.

NAD+/NADH ratio measurement

The NADH/NAD+ ratio was measured as described previously [12,17]. Cells at 80% confluence were cultured in a 6-cm culture dish and homogenized in 100 µL 1M HClO3 and neutralized with 50 µL of 2M KHCO3. The concentrations of pyruvate and lactate were measured fluorimetrically after an enzymatic cycling reaction using 5 µL of sample. Values for both pyruvate and lactate were detected within the linear range.

MTT assay

We plated 1000 A375 and SK-MEL-28 cells in 96-well plates. Cells were used for MTT assay the next day. MTT assay kits were purchased from Sigma (St. Louis, MO, USA) and all steps were performed according to the manufacturer´s recommendations. Experiments were repeated 3 times.

Scratch assay

A375 and SK-MEL-28 cells were individually transfected with scramble or empty vector, or PCDNA3 vector containing MPC1 and MPC2, or siRNAs against to MPC1 and MPC2 or treated with 10 mM 2-DG, respectively. Cells were cultured in 6-well plates until complete confluence. A pipette was used for generating uniform wounds in the confluent monolayer of cells. After 16 h, the percentage of wound closure area was measured using ImageJ software. Three independent experiments were performed for the assay.

Invasion assay

A375 or SK-MEL-28 cells were transfected with scramble or empty vector, or PCDNA3 vector containing MPC1 and MPC2, or siRNAs against to MPC1 and MPC2 for 48 h. Then, the transfected 1×10^5 cells seeded on the upper side of a Matrigel-coated 6.5 mm polycarbonic Transwell chamber (Corning Costar, USA) with serum-free DMEM culture medium, and the lower side of the Transwell chamber was filled with DMEM culture medium with 10% FBS. For the 2-DG treatment group, 10 mM 2-DG was added to the upper side. After incubation for 24 h, the cells that stayed on the upper surface of the chamber were removed with cotton swabs. We washed the chamber with 1XPBS 2 times and stained the penetrated cells on the lower surface of the chamber using a Hema 3 kit (Thermo Scientific, Rockford, IL, USA) following the manufacturer's instruction. Images were taken with a NikonTS-100 microscope.

Statistical analysis

The data are presented as mean ±SD for 3 independent experiments and analyzed by *t* test or one-way ANOVA. Multiple comparisons between the groups were performed using S-N-K method. $p<0.05$ was considered statistically significant.

Results

MPC1 and MPC2 are required for cellular redox homeostasis

First, we examined the lactate and pyruvate contents of the cells following perturbation of MPC1- and MPC2-mediated

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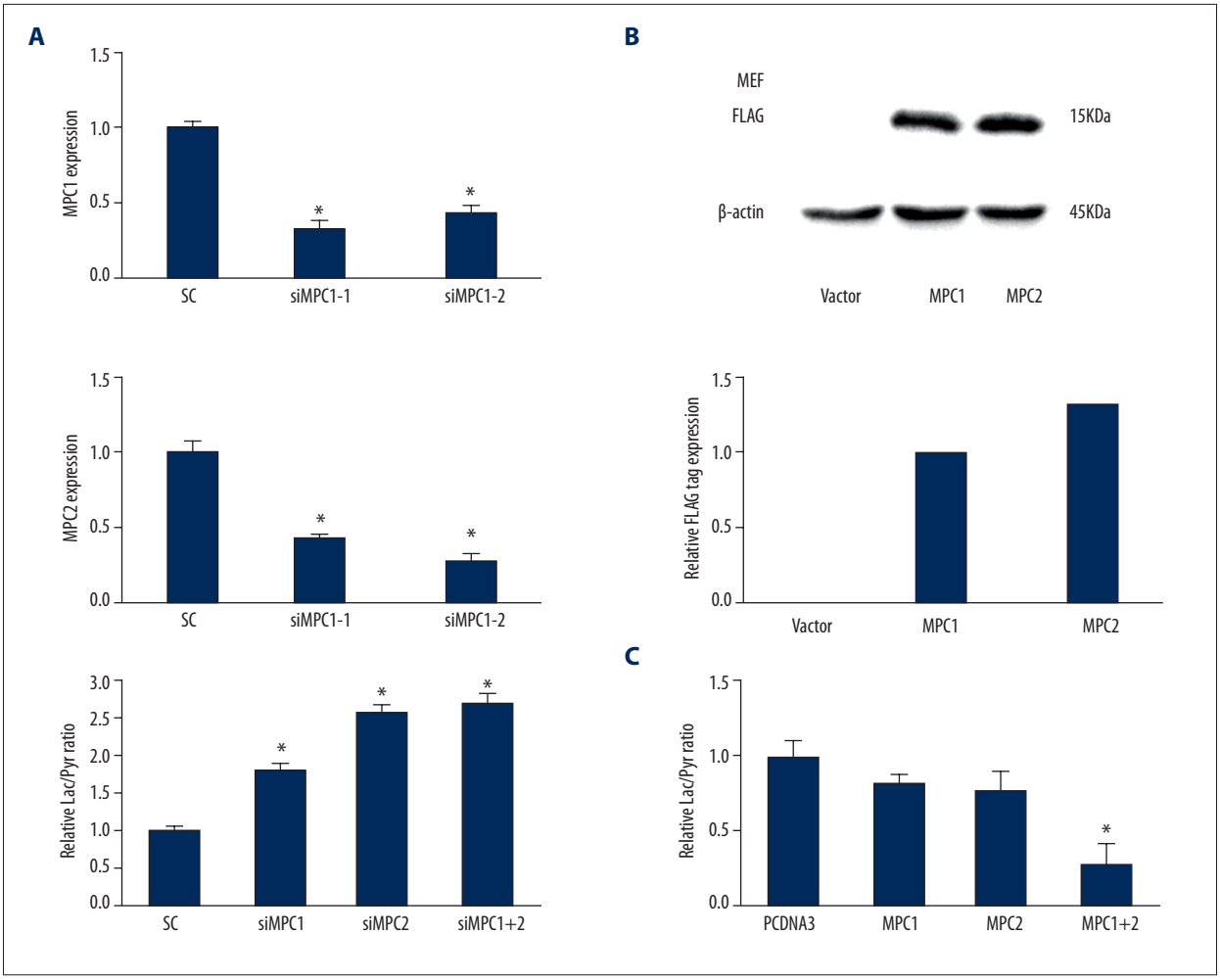

Figure 1. MPCs regulated cellular NADH level. (**A**) Knocking down MPC1 or MPC2 increased the lactate/pyruvate ratio in MEFs. Mouse MEFs transfected with siRNAs against MPC1 or MPC2 increased lactate/pyruvate ratios. (**B**) Western blot assay for flag-tagged human MPC1 and MPC2 overexpression in MEFs cells. The relative protein expression levels were quantified and are shown in the bar diagram. (**C**) Transfection of MPC1 and MPC2 to MEFs cells decreased lactate/pyruvate ratio. * Indicates P<0.05.

function. MPC1 and MPC2 knockdown in MEFs upregulated the ratio of lactate to pyruvate, indicating an increased free NADH level in the cytosol and nucleus [15]. Simultaneous ablation of both MPC1 and MPC2 did not further increase the lactate-to-pyruvate ratio, suggesting that MPC1 and MPC2 work in a functional unit (Figure 1A).

Next, we transfected MPC1 and MPC2 to the MEFs cells and found that additional expression of MPC1 and MPC2 decreased their lactate-to-pyruvate ratio (Figure1B, 1C), suggesting that the mitochondrial pyruvate transport is a key step in metabolic reprogramming that affects NADH in the cytosol and nucleus. Note that even though MPC1 or MPC2 expression alone localized to the mitochondrial [13], both MPC1 and MPC2 are required for restoring the normal lactate-to-pyruvate ratio, reflecting the obligated oligomerization of the 2 components of the mitochondrial pyruvate carrier in redox maintenance.

CtBP1 transcriptionally represses MPC1 and MPC2 expression

We next explored whether CtBP1 plays a role in MPC1 and MPC2 transcription control. Overexpression of CtBP1 in MEFs decreased the MPC1 mRNA level as well as the MPC2 mRNA level (Figure 2A). Using ChIP assay, we found that CtBP1 directly bound to the promoter regions of the MPC1 and the MPC2 genes (Figure 2B). CtBP1's repression of these regulatory regions was confirmed by luciferase reporter assays (Figure 2C), suggesting that CtBP1 functions as a transcriptional co-repressor for MPC1 and the MPC2 transcription.

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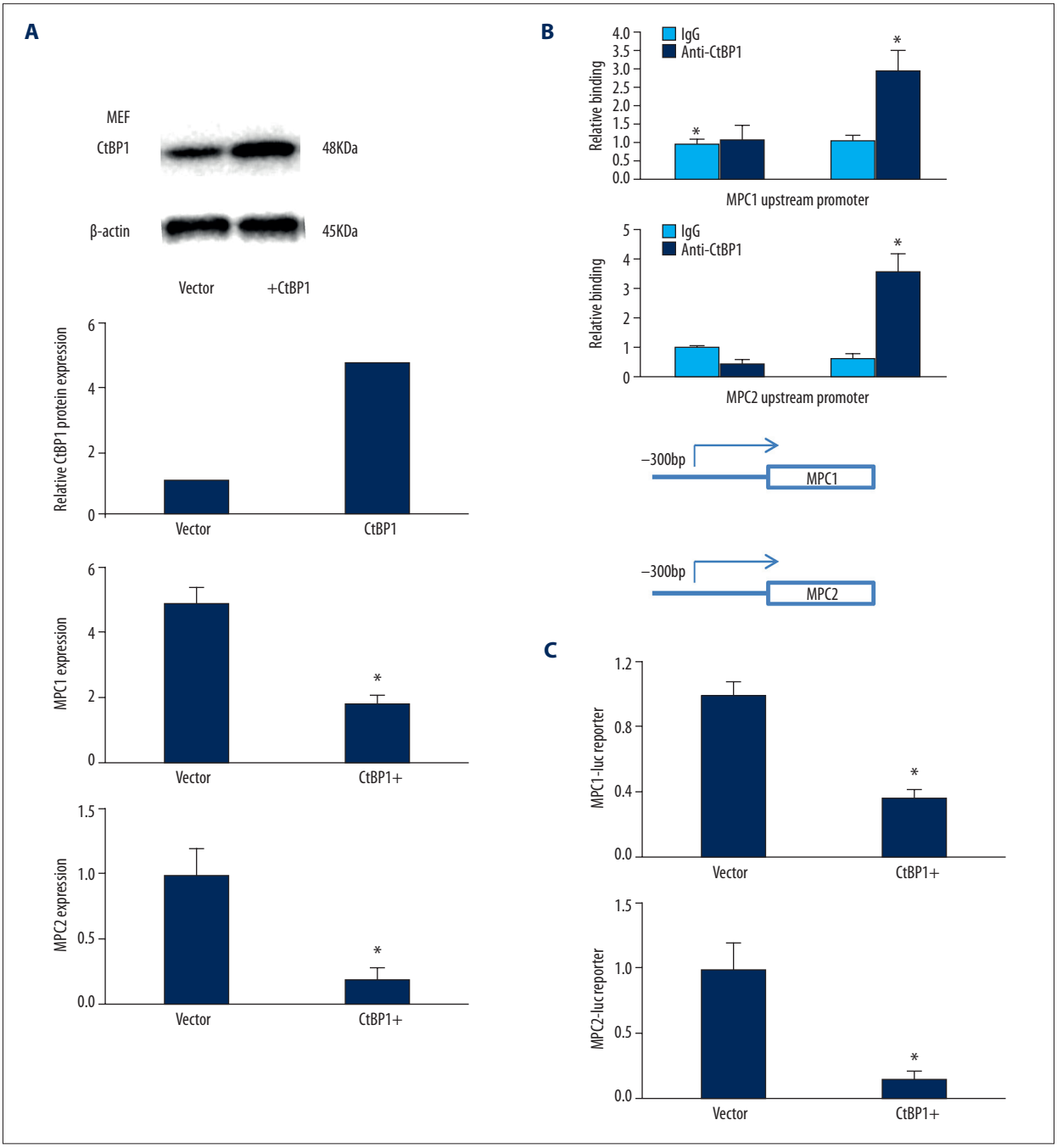

Figure 2. CtBP1 transcriptionally repressed MPC1 and MPC2. (**A**) CtBP1 overexpression downregulated MPC1 and MPC2 mRNA expression. The relative protein expression levels were quantified and are shown in the bar diagram. (**B**) Upper panel: CtBP1 bound to the regulatory region of MPC1 and MPC2 promoter. Lower panel: The sketch represents the regulatory region. (**C**) The luciferase reporter assay in MEFs cells showing that CtBP1 repressed transcription of MPC1 and MPC2 genes through the regulatory regions on their gene. * Indicates P<0.05.

NADH modulates CtBP1-mediated MPC1 and MPC2 repression in melanoma cells

In a spectrum of tumor cells, the expression of CtBP1 is high and is correlated with poor survival [18–20]. Here, we investigated the CtBP1-mediated regulation of the MPC1 and the MPC2 genes in melanoma cells. CtBP1 was knocked down using siRNAs in A375 cells, and both the MPC1 and the MPC2 mRNA levels and protein levels were increased (Figure 3A), confirming the key role of CtBP1 in regulating MPC1 and MPC2

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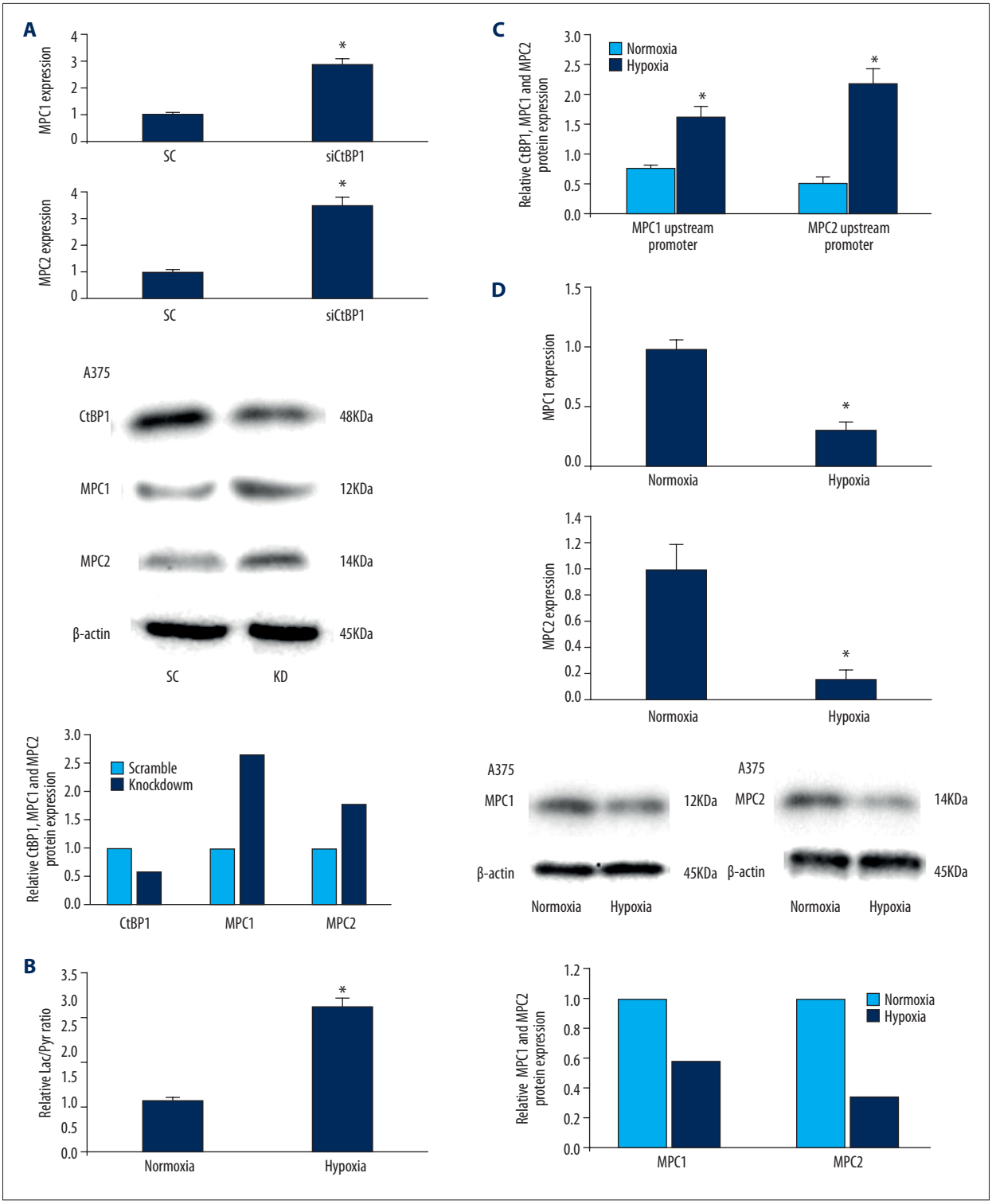

Figure 3. Hypoxia induced MPC1 and MPC2 repression mediated by CtBP1. (**A**) SiRNA-mediated CtBP1 knockdown in A375 cells increased MPC1 and MPC2 mRNA and protein expression. The relative protein expression levels were quantified and are shown in the bar diagram. (**B**) A375 cells cultured under hypoxia for 3 h had increased lactate/pyruvate ratio. (**C**) Hypoxia (3 h) increased CtBP1 recruitment to the MPC1 and MPC2 promoters in A375 cells. (**D**) Hypoxia (3 h) decreased MPC1 and MPC2 mRNA and protein expression. The relative protein expression levels were quantified and are shown in the bar diagram. * Indicates P<0.05.

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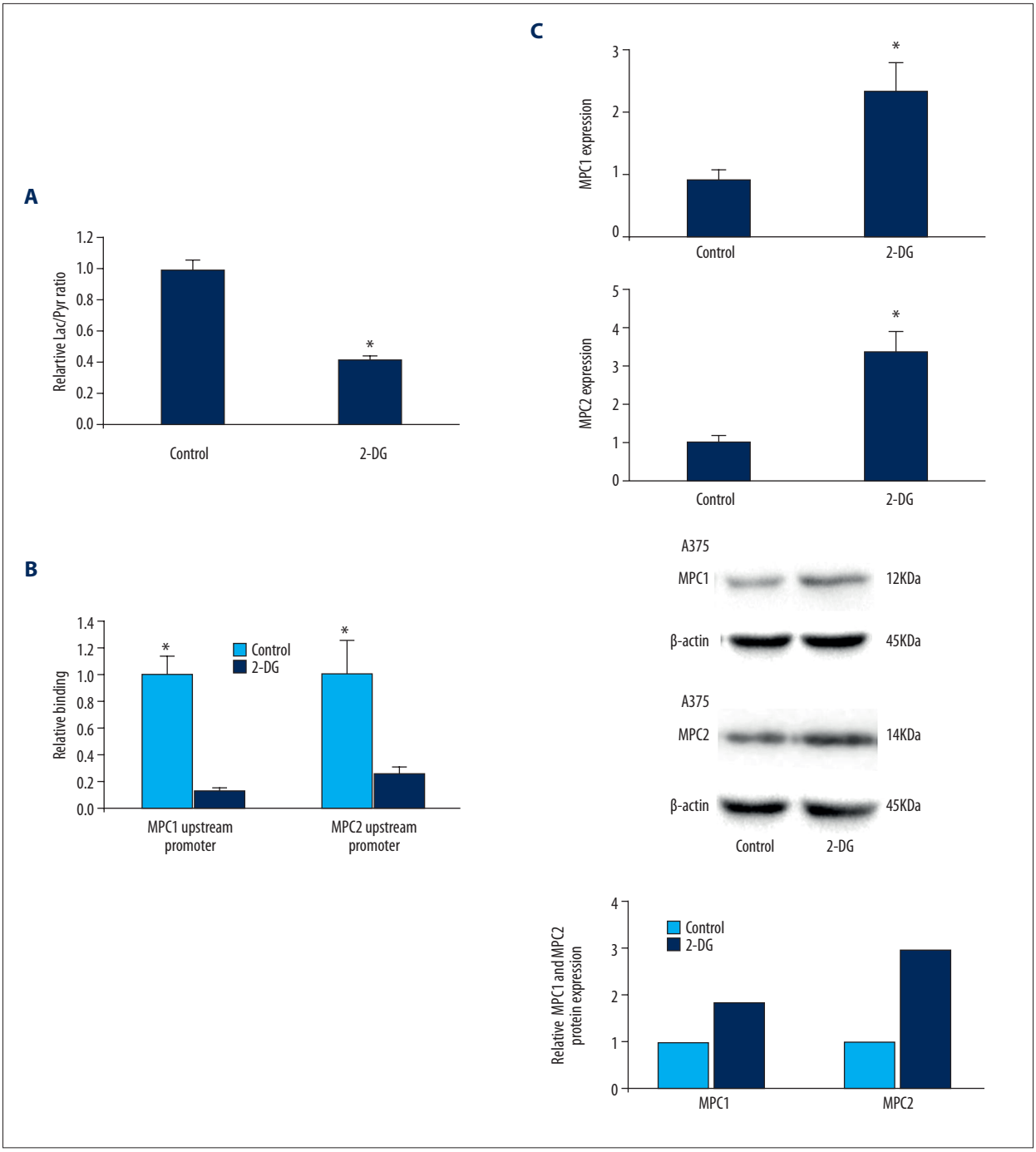

Figure 4. NADH blockage rescues the MPC1 and MPC2 expression in human melanoma cells. (**A**) 10 mM 2-DG treatment for 24 h decreased A375 cell lactate/pyruvate ratio. (**B**) 2-DG treatment decreased the CtBP1 recruitment to the MPC1 and MPC2 promoters. (**C**) 2-DG treatment increased the mRNA and protein levels of the MPC1 and MPC2 genes. The relative protein expression levels were quantified and are shown in the bar diagram. * Indicates P<0.05.

transcription. As the first NADH-sensitive transcriptional co-repressor identified, CtBP1 conveys its redox-sensitive transcriptional control via its NADH-dependent recruitment to the CtBP1 target genes. Therefore, we explored whether CtBP1's regulation of the MPC1 and the MPC2 genes is subject to NADH control. As expected, CtBP1's recruitment to the MPC1 and the MPC2 promoters was increased by hypoxia treatment, which elevated the NADH level in A375 cells (Figure 3B, 3C), suggesting that the MPC1 and the MPC2 genes are suppressed in human tumor cells by the increased CtBP1 protein as well as

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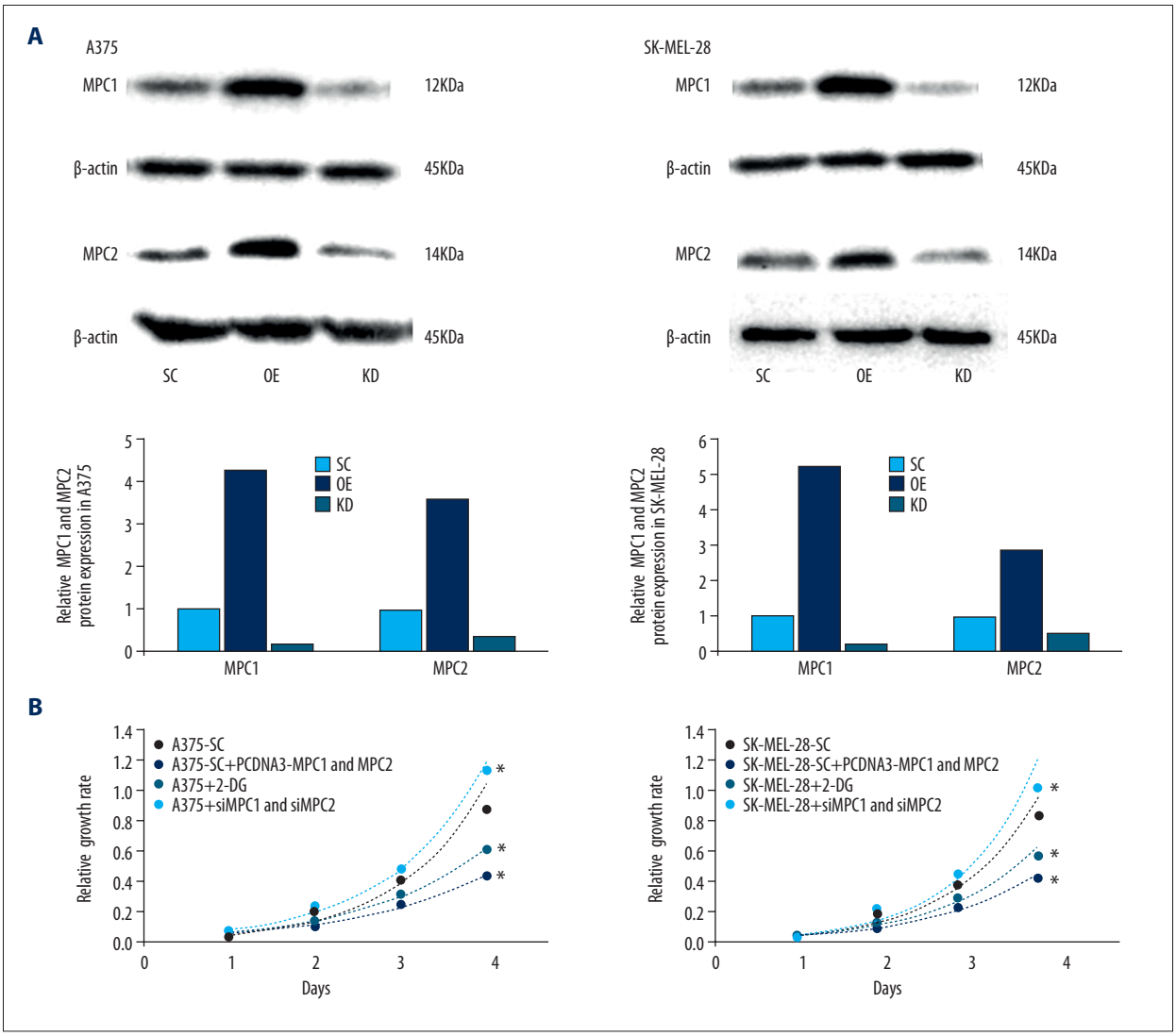
by the elevated NADH levels. Supporting this link, the mRNA levels and protein levels of the MPC1 and the MPC2 were decreased by the hypoxia treatment (Figure 3D).

NADH blockage rescues MPC1 and MPC2 expression in melanoma cells

Since the repression of MPC1 or MPC2 by CtBP1 is subject to NADH regulation, we investigated whether NADH blockage can be used for rescuing the MPC1 or MPC2 expression in melanoma cells. 2-DG is a nonmetabolizable structural analogue of glucose, which can be phosphorylated by hexokinase, leading to accumulation of the nonmetabolizable product, 2-deoxyglucose-phosphate (2-DG-P) [21]. A previous report showed 2-DG was able to inhibit glycolysis and reduce NADH level [22]. Consist with a previous report, the NADH level was decreased in A375 cells when treated with 10 mM 2-DG for 24 h (Figure 4A). Further, 2-DG treatment decreased the CtBP1 recruitment to the regulatory regions of MPC1 and MPC2 genes (Figure 4B). Subsequently, 2-DG treatment increased the mRNA and protein levels of MPC1 or MPC2 (Figure 4C), similar to the effect of the CtBP1 knockdown in melanoma cells (Figure 3A), suggesting that ablation of CtBP1's activity by blocking NADH rescues the MPC1 and MPC2 expression.

MPC1 and MPC2 expression repressed malignant properties of melanoma cells

To understand the biological functions of MPC1 and MPC2 in melanoma cells, we assayed melanoma cells growth and migration upon expression or knockdown of MPC1 and MPC2. We found that ectopic co-expression of MPC1 and MPC2 in melanoma cell lines, A375 and SK-MEL-28, inhibited their growth, migration, and invasion. Inversely, knockdown of MPC1 and MPC2 enhanced proliferation, migration and invasion of melanoma cells. Furthermore, melanoma cells that received 2-DG



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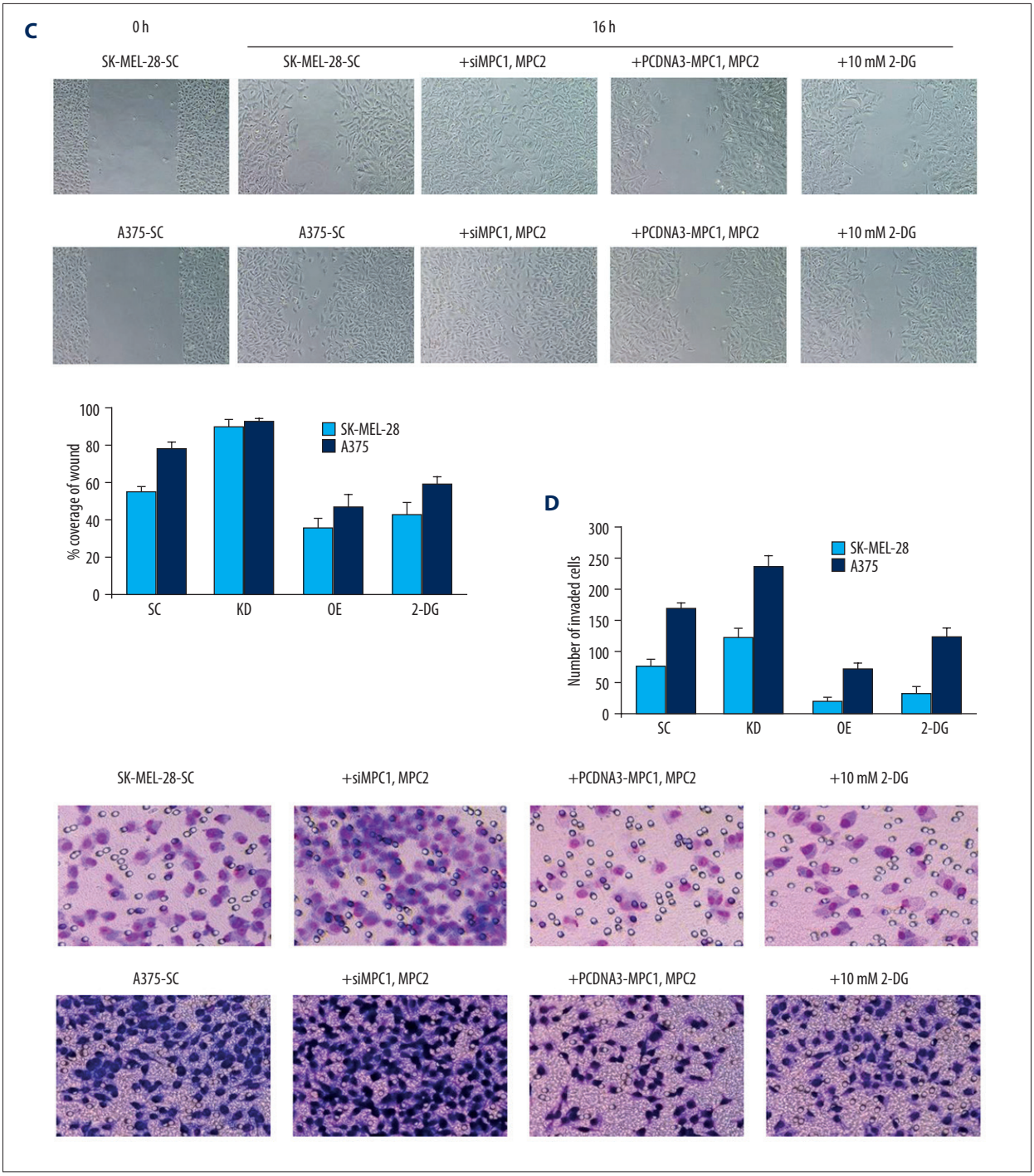

Figure 5. MPC1 and MPC2 repress melanoma cells proliferation and migration. (**A**) Western blot analysis showed overexpression or knockdown of MPC1 and MPC2 in A375 and SK-MEL-28 cells. The relative protein expression levels were quantified and are shown in the bar diagram. (**B**) Growth curves of A375 and SK-MEL-28 cells, which were transfected with MPC1 and MPC2, siRNAs against MPC1 and MPC2, scramble or treated with 10 mM 2-DG, respectively. * Indicates $P<0.05$ (**C**) Melanoma cell migration assay was performed after transfection of scramble, knockdown, or overexpression of MPC1 and MPC2 for 48 h, or 2-DG treatment for 16 h. Representative images show the wound at 0 h and 16 h with or without different treatments. The % of wound closure area shown in the bar diagram are mean ±SD from triplicate experiments ($P<0.05$). (**D**) Cell invasion assay showed that knockdown of MPC1 and MPC2 promoted melanoma cell invasive ability. Overexpression of MPC1 and MPC2 or 2-DG treatment inhibited melanoma cell invasion. The invaded cell numbers were calculated from 3 randomly chosen microscopic fields. Results are shown in the bar diagram as mean ±SD from triplicate experiments ($P<0.05$).

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treatment had inhibited malignant properties (Figure 5A–5D) that were similar to the effect of MPC1 and MPC2 knockdown. These data provide a therapeutic strategy to restore the mitochondrial pyruvate transportation and, in turn, restore NADH-mediated redox balance.

Discussion

A previous study revealed that oxygen (pO_2) within human cancer tissues is much lower than that of the adjacent normal tissue [23]. Rapidly growing cells experience hypoxia, which leads to glycolytic metabolism in response to reduced O_2 availability. Previous studies have mainly focussed on HIF1, c-Myc, and p53-mediated changes under hypoxia [24–26]. Our study revealed another switch in this adaptation, linked by NADH and CtBP1 regulation.

Compared to the sir2 family members, which are enzymes utilizing NAD [27], NADH increases CtBP1 binding to its partners and thus the suppression of target gene transcription. Loss of CtBP1-mediated transcription repression of the Bax gene has been reported to dampen mitochondrial functions, such as ATP production [28]. In this study, we also uncovered direct control of the lactate and pyruvate redox equilibration by CtBP1 via transcriptional control of the mitochondrial pyruvate transporter MPC1 and MPC2 genes. Loss of MPC1 and MPC2 forces the pyruvate from the glycolytic influx to the lactate conversion, leading to increased free NADH in the cytosol and nucleus.

Genetic deletion of MPC in yeast leads to growth defects [13]. Furthermore, disruption or deletion of MPC1 or MPC2 is lethal for mouse embryo development [29,30]. Expression of MPC1 and MPC2 inhibits cancer cell proliferation, migration, invasion, and stemness in many types of cancer cell [14,31,32]. To reveal the mechanism that regulates MPC1 and MPC2 expression, our study investigated the transcriptional control of these key players in metabolic regulation, and provides another regulatory pathway intimately linked to the metabolic alterations (feed-forward).

Low expression of MPC1 or MPC2 is associated with poor survival of patient with lung cancer, colon cancer, bladder cancer, and kidney cancer, indicating the critical roles of MPC1 and MPC2 expression level in cancers [14]. In this study, we identified CtBP1 as a regulator of MPC1 and MPC2, providing a new clue to understand how MPC1 and MPC2 expression is regulated in melanoma cells.

CtBP1 is actively engaged in transcriptional repression. Our previous study reported CtBP1 was overexpressed in melanoma tissues and transcriptionally repressed Brca1 and P16, leading to abnormalities in melanoma cell DNA damage repair and cell cycle. In this study, we demonstrated CtBP1 is a key metabolic regulator and effector linking melanoma cell metabolism to transcriptional regulation, which regulates cell proliferation, migration, and invasion. Overexpression or activation of CtBP1 is highly associated with melanoma cell malignant properties and represses a panel of tumor suppressors in melanoma cells, which may allow CtBP1-targeted therapy for melanoma treatment in the future.

CtBP1 interacts with partner proteins with the PxDLX binding domain to transcriptionally regulate target genes that contribute to cancer cell proliferation, migration, and EMT [20]. Since CtBP1 has been found to be overexpressed in multiple cancer types, it is emerging as a cancer therapeutic target [33]. Small molecules or short peptides have been designed for blocking CtBP1's PxDLX binding domain to interfere in the interaction between CtBP1 and its partner proteins, and showed promising anti-cancer effects [33–35]. Our study showed the results of elevated CtBP1 and the consequently elevated NADH level, via the CtBP1-mediated blockage of the MPC1 and MPC2 genes, and the CtBP1-NADH 'avalanche', and led to cell tumorigenesis by transcriptional and metabolic reprogramming. Blocking CtBP1 either by glucose metabolism inhibitors like 2-DG or by CtBP1 depletion will put a brake on this land slide. These findings provide another clue for CtBP1-targeted drug design and development.

Overall, our results demonstrate that CtBP1 functions as an NADH generator in addition to being an NADH sensor, thus showing that CtBP1 in centrally involved in tumor metabolism and transcription control. Our findings may aid in future research on the potential of CtBP1-NADH targeting for cancer therapy.

Conclusions

Our work reveals that MPC1 and MPC2 are transcriptionally repressed by CtBP1 and thus provide positive feedback to CtBP1's functions. Higher expression levels of MPC1 and MPC2 inhibit melanoma cell proliferation and migration, suggesting the potential tumor-suppressor roles of MPC1 and MPC2 in melanoma cells.

Conflict of interest

None.

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References:

1. Hanahan D, Weinberg RA: Hallmarks of cancer: The next generation. Cell, 2011; 144: 646–74
2. Yin C, Qie S, Sang N: Carbon source metabolism and its regulation in cancer cells. Crit Rev Eukaryot Gene Expr, 2012; 22: 17–35
3. Roudier E, Perrin A: Considering the role of pyruvate in tumor cells during hypoxia. Biochim Biophys Acta, 2009; 1796: 55–62
4. Chinnadurai G: The transcriptional corepressor CtBP: A foe of multiple tumor suppressors. Cancer Res, 2009; 69: 731–34
5. Nadauld LD, Phelps R, Moore BC et al: Adenomatous polyposis coli control of C-terminal binding protein-1 stability regulates expression of intestinal retinol dehydrogenases. J Biol Chem, 2006; 281: 37828–35
6. Deng H, Liu J, Deng Y et al: CtBP1 is expressed in melanoma and represses the transcription of p16INK4a and Brca1. J Invest Dermatol, 2013; 133(5): 1294–301
7. Deng Y, Deng H, Liu J et al: Transcriptional down-regulation of Brca1 and E-cadherin by CtBP1 in breast cancer. Mol Carcinog, 2012; 51(6): 500–7
8. Deng Y, Liu J, Han G et al: Redox-dependent Brca1 transcriptional regulation by an NADH-sensor CtBP1. Oncogene, 2010; 29(50): 6603–8
9. Birts CN, Harding R, Soosaipillai G et al: Expression of CtBP family protein isoforms in breast cancer and their role in chemoresistance. Biol Cell, 2011; 103: 1–19
10. Di LJ, Byun JS, Wong MM et al: Genome-wide profiles of CtBP link metabolism with genome stability and epithelial reprogramming in breast cancer. Nat Commun, 2013; 4: 1449
11. Li M, Riddle S, Zhang H et al: Metabolic reprogramming regulates the proliferative and inflammatory phenotype of adventitial fibroblasts in pulmonary hypertension through the transcriptional corepressor C-terminal binding protein-1. Circulation, 2016; 134: 1105–21
12. Zhang Q, Wang SY, Nottke AC et al: Redox sensor CtBP mediates hypoxia-induced tumor cell migration. Proc Natl Acad Sci USA, 2006; 103: 9029–33
13. Bricker DK, Taylor EB, Schell JC et al: A mitochondrial pyruvate carrier required for pyruvate uptake in yeast, Drosophila, and humans. Science, 2012; 337: 96–100
14. Schell JC, Olson KA, Jiang L et al: A role for the mitochondrial pyruvate carrier as a repressor of the Warburg effect and colon cancer cell growth. Mol Cell, 2014; 56: 400–13
15. Zhang Q, Yoshimatsu Y, Hildebrand J et al: Homeodomain interacting protein kinase 2 promotes apoptosis by downregulating the transcriptional corepressor CtBP. Cell, 2003; 115: 177–86
16. Zhang Q, Piston DW, Goodman RH: Regulation of corepressor function by nuclear NADH. Science, 2002; 295: 1895–97
17. Williamson DH, Lund P, Krebs HA: The redox state of free nicotinamide-adenine dinucleotide in the cytoplasm and mitochondria of rat liver. Biochem J, 1967; 103: 514–7
18. Dcona MM, Morris BL, Ellis KC, Grossman SR: CtBP– an emerging oncogene and novel small molecule drug target: Advances in the understanding of its oncogenic action and identification of therapeutic inhibitors. Cancer Biol Ther, 2017; 18: 379–91
19. Byun JS, Gardner K: C-terminal binding protein: A molecular link between metabolic imbalance and epigenetic regulation in breast cancer. Int J Cell Biol, 2013; 2013: 647975
20. Chinnadurai G: CtBP, an unconventional transcriptional corepressor in development and oncogenesis. Mol Cell, 2002; 9: 213–24
21. Aft RL, Zhang FW, Gius D: Evaluation of 2-deoxy-D-glucose as a chemotherapeutic agent: Mechanism of cell death. Br J Cancer, 2002; 87: 805–12
22. Garriga-Canut M, Schoenike B, Qazi R et al: 2-Deoxy-D-glucose reduces epilepsy progression by NRSF-CtBP-dependent metabolic regulation of chromatin structure. Nat Neurosci, 2006; 9: 1382–87
23. Vaupel P, Mayer A: Hypoxia in cancer: Significance and impact on clinical outcome. Cancer Metastasis Rev, 2007; 26: 225–39
24. van Uden P, Kenneth NS, Rocha S: Regulation of hypoxia-inducible factor-1alpha by NF-kappaB. Biochem J, 2008; 412: 477–84
25. Kilic-Eren M, Boylu T, Tabor V: Targeting PI3K/Akt represses Hypoxia inducible factor-1alpha activation and sensitizes Rhabdomyosarcoma and Ewing's sarcoma cells for apoptosis. Cancer Cell Int, 2013; 13: 36
26. Richard DE, Berra E, Gothie E et al: p42/p44 mitogen-activated protein kinases phosphorylate hypoxia-inducible factor 1alpha (HIF-1alpha) and enhance the transcriptional activity of HIF-1. J Biol Chem, 1999; 274: 32631–37
27. Imai S, Armstrong CM, Kaeberlein M, Guarente L: Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. Nature, 2000; 403: 795–800
28. Kim JH, Youn HD: C-terminal binding protein maintains mitochondrial activities. Cell Death Differ, 2009; 16: 584–92
29. Vanderperre B, Herzig S, Krznar P et al: Embryonic lethality of mitochondrial pyruvate carrier 1 deficient mouse can be rescued by a ketogenic diet. PLoS Genet, 2016; 12: e1006056
30. Vigueira PA, McCommis KS, Schweitzer GG et al: Mitochondrial pyruvate carrier 2 hypomorphism in mice leads to defects in glucose-stimulated insulin secretion. Cell Rep, 2014; 7: 2042–53
31. Zhou X, Xiong ZJ, Xiao SM et al: Overexpression of MPC1 inhibits the proliferation, migration, invasion, and stem cell-like properties of gastric cancer cells. Onco Targets Ther, 2017; 10: 5151–63
32. Wang L, Xu M, Qin J et al: MPC1, a key gene in cancer metabolism, is regulated by COUPTFII in human prostate cancer. Oncotarget, 2016; 7: 14673–83
33. Dcona MM, Morris BL, Ellis KC, Grossman SR: CtBP – an emerging oncogene and novel small molecule drug target: Advances in the understanding of its oncogenic action and identification of therapeutic inhibitors. Cancer Biol Ther, 2017; 18: 379–91
34. Blevins MA, Huang M, Zhao R: The role of CtBP1 in oncogenic processes and its potential as a therapeutic target. Mol Cancer Ther, 2017; 16: 981–90
35. Blevins MA, Zhang C, Zhang L et al: CPP-E1A fusion peptides inhibit CtBP-mediated transcriptional repression. Mol Oncol, 2018; 12: 1358–73

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