BBA - Reviews on Cancer 1878 (2023) 188886

Contents lists available at ScienceDirect

BBA - Reviews on Cancer

journal homepage: www.elsevier.com/locate/bbacan

Review

CtBP: A global regulator of balancing acts and homeostases

Alok Jaiswal, Raghvendra Singh *

Department of Chemical Engineering, Indian Institute of Technology Kanpur, Kanpur 208016, India

ARTICLE INFO

Keywords:
CtBP
Homeostasis
Repression motif
Cell surveillance
p53
NFkB
NADH/NAD+ ratio

ABSTRACT

The classical role of C-terminal binding protein (CtBP) is that of a global corepressor. However, its exact mechanism of repression is not known. In this review, we elucidate the repression motif used by CtBP. Further, we provide other unifying features of its mechanism of action. For example, in the presence of a high NADH/NAD+ ratio in the cell, causing a low glycolytic condition, the NADH-bound dimeric form of CtBP causes global repression, maintaining balances and homeostases of many cellular processes, under the cell surveillance of p53 and NFkB. In contrast, in the presence of a low NADH/NAD+ ratio, causing a high glycolytic condition, the NADH-free monomeric form of CtBP blocks p53 function and NFkB-mediated transcription. Further, a low NADH/NAD+ ratio upsets the homeostases and balances in the absence of the cell surveillances of p53 and NFkB, causing global instability, the dominant outcome of CtBP's action in carcinogenesis, in cells in a high glycolytic state.

1. Introduction

C-terminal binding proteins (CtBPs), which are highly conserved among vertebrates and invertebrates, share amino acid homology with NAD-dependent 2-hydroxy acid dehydrogenases (Fig. 1A) [1]. CtBP1 was originally identified based on its interaction with the C-terminal region of the human adenovirus E1A proteins [1]. Subsequently, a highly homologous human protein CtBP2 was identified [1]. Further, an N-terminally truncated version of CtBP1 (named CtBP3), exhibiting acyltransferase activity, has been found [1] [2]. Using acyl CoA in Golgi, CtBP3 selectively catalyzes the acylation of lysophosphatidic acid [1] [2].

CtBP links DNA/histone modifying proteins to sequence-specific DNA binding proteins and functions as a corepressor [3]. The corepressor activity of CtBP is NADH-dependent [4]. NADH induces dimerization of CtBP, thereby, regulating its function [5], which differs from that of the monomeric form of the protein. Further, CtBPs act as sensors of the oxygen level of the microenvironment [6]. The oxygen level of a cell's microenvironment is inversely correlated with the NADH concentration within the cell [7]. Thus, the hypoxic conditions increase NADH levels and dimerization of CtBP in the cell.

Several corepressors function by inducing the removal of the acetyl group from the N-terminal tails of histones through histone deacetylases (HDACs), causing chromatin condensation and restricting access of the transcription factors to the promoter [1]. CtBP can interact with histone deacetylase HDAC1 [8]. Further, CtBP has been shown to act as a corepressor along with the ternary complex factor, Net [9]. CtBP-Net complex represses target genes in a deacetylase activity-dependent manner [9]. On the other hand, it can also repress some promoters in deacetylase independent manner [10] [11]. Thus, there are different mechanisms of repression used by CtBP, and further clarity on its mechanism of action is required.

Repressors can be distinguished based on the range of their repressor activity [12]. Short-range repressors bind close to ($\leq$100 bp) the activators within enhancers, acting on the element only to which they are directly bound [12]. On the other hand, long-range repressors can repress over distances of $>$1 kbp and can repress many enhancer elements simultaneously [12] [13]. Short-range repressors usually recruit evolutionarily conserved corepressor CtBP [12]. Thus, CtBP may be called a global corepressor.

Here, we found that CtBP uses a common regulatory motif for repression in many different contexts (Fig. 1B). A common structure of its repression is that, at the promoter, both an activator complex and a repressor complex, having CtBP as a corepressor, form and both complexes share a protein. The likely role of this motif is to establish a balance between gene activation and repression due to the limiting availability of the shared protein, controlling the transcription rate. On the other hand, disturbing this balance may act as a switch. In agreement, we found that a common feature of CtBP-mediated repression is to maintain balances and regulate homeostasis of different kinds.

* Corresponding author.
E-mail address: raghvend@iitk.ac.in (R. Singh).

https://doi.org/10.1016/j.bbcan.2023.188886
Received 9 December 2022; Received in revised form 6 March 2023; Accepted 9 March 2023
Available online 29 March 2023

Due to its dependence on NADH, CtBP's function and activity are different in different glycolytic states, caused by the NADH/NAD+ ratio in the cells. Normal cells are usually in a low glycolytic state in which the maintenance of the balances of various biological processes, under proper cell surveillance, is important. On the other hand, cancer cells may be characterized by broken/overridden cell surveillance in a high glycolytic state. We elucidate the difference in the function of CtBP in high vs. low glycolytic states of the cells. These observations bring a unifying portrayal of the CtBP-mediated repressions, including that of the motif of the repression, the regulation of CtBP by NADH, the role of the oligomeric states of CtBP, and the biological function regulated by these states.

2. The motif of repression used by CtBP

Here, we have collated different phenomena of repression mediated by CtBP and found that it uses a common motif in many different kinds of repression (Fig. 1B). This motif is involved in balancing the transcription of the target genes and maintaining homeostasis.

2.1. The control of the Wnt signaling in epidermal cells

The homeodomain interacting protein kinase 2 (HIPK2) forms a complex with CtBP and represses β-catenin/LEF-1 mediated transcription of cyclin D1, which is involved in epidermal stem cell proliferation and increases susceptibility to develop squamous cell carcinoma [14]. HIPK2 forms a complex with β-catenin/LEF1 and CtBP and represses β-catenin/LEF1 mediated transcription [14] (Fig. 2A). Appropriate Wnt pathway activity is important in maintaining a balance between epidermal stem cell compartment and epidermal differentiation [14] [15]. This balance is maintained by the transcriptional activator β-catenin/LEF1 and the repressor complex LEF1/HIPK2/CtBP [14] [15] acting together on the Wnt target genes. The balance of the Wnt signaling is implicated in epidermal homeostasis [15], which is important in preventing squamous cell carcinoma development. Further, in this regulation, LEF1 is shared between the activator and the repressor complexes (Fig. 2A).

2.2. The control of the Wnt signaling in gastrointestinal cancer cells

Gastrins cause the proliferation of gastrointestinal cancer cells [16]. The gastrins, Gamide and Ggly, caused the dissociation of β-catenin from E-cadherin, translocation of β-catenin to the nucleus, and association of β-catenin to TCF4 in PAK1 dependent manner [16]. On the other hand, TCF4 also binds with CtBP and functions as a corepressor of Wnt target genes [17]. Thus, TCF4 is shared between the Wnt activator and the repressor complexes (Fig. 2B). Further, a kinase-inactive mutant of PAK1 blocked the effect of gastrins on β-catenin's association with TCF4 and expression of β-catenin target genes, c-Myc and cyclin D1 [16]. Furthermore, PAK1 binds and phosphorylates CtBP at the Ser158 position, causes its cellular redistribution, and blocks its corepressor function [3]. Thus, the activator complex β-catenin-TCF4/LEF and the repressor complex TCF4-CtBP maintain a balance of the Wnt signaling and PAK1 can tilt this balance in the favor of the transcription of Wnt target genes by blocking the corepressor function of CtBP (Fig. 2B). Therefore, PAK1 acts as a signaling switch in the TCF4 and CtBP implemented balance of the Wnt signaling in gastrointestinal cancer cells.

2.3. The control of cell cycle by BRCA1

RB Binding Protein 8 (RBBP8)/CtIP, a protein involved in DNA double-strand break repair, interacts with CtBP and BRCA1, increases cyclin D1 and CDK4 levels facilitating G1-S transition, and is upregulated in gastric cancer tissues [18]. Further, BRCA1 transactivates p21 [19]. On the other hand, CtIP-CtBP-BRCA1 causes p21 promoter deacetylation, inhibiting p21 transcription and promoting the G1-S transition of gastric cancer cells [18]. Thus, CtBP maintains a balance of cell cycle through the control of p21 transcription (Fig. 2C) and BRCA1 is shared between the activator and the repressor complexes.

2.4. The control of TGFβ signaling

TGFβ signaling is important in balancing the differentiation and proliferation of cells [20]. In this context, TGFβ/BMP signaling causes activation and nuclear translocation of Smad, which activates transcription by recruiting P/CAF and p300 [20] (Fig. 2D). Further, TGFβ/BMP signaling is affected in opposite ways by the EB family of zinc finger proteins, ZEB1 and ZEB2 [20]. ZEB1 helps the Smad-mediated transcriptional activation by binding with p300, while ZEB2 represses it by making a complex with CtBP [20]. Further, ZEB1 also forms a repressor complex with CtBP [21]. Thus, ZEB proteins form an activator complex, Smad-p300-P/CAF-ZEB1, and a repressor complex, ZEB1/ZEB2-CtBP, balancing TGFβ signaling (Fig. 2D). This model of regulation of the TGFβ signaling by ZEB proteins is found in Xenopus development [20].

2.5. The control of E-cadherin gene expression

Pinin/DRS (Pnn), a nuclear protein involved in mRNA processing and cell adhesion, associates with transcription machinery causing E-cadherin expression while CtBP1 represses the E-cadherin promoter [22] (Fig. 2E). On the other hand, Pnn binds CtBP1 and blocks CtBP mediated repression of E-cadherin [22], acting as a switch (Fig. 2E). Further, Pnn positively affects E-cadherin mRNA splicing whereas CtBP affects it negatively [23]. Thus, Pnn and CtBP maintain a balance of E-cadherin expression both at the transcription and post-transcription levels, and the Pnn-mediated block of the CtBP repression activity tilts this balance in the favor of E-cadherin transcription (Fig. 2E).

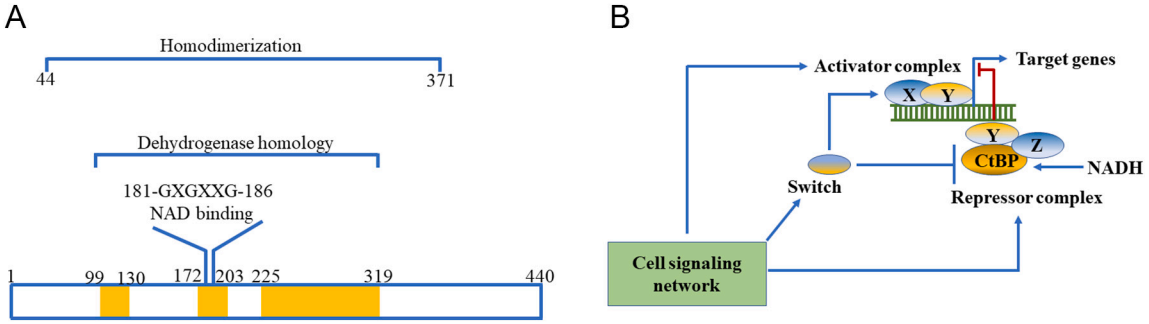

Fig. 1. Domain structure of CtBP and the general regulatory motif used by CtBP in repression. (A) Domain structure of human CtBP1 [1] (More detailed structure in [1]). The regions of highest homology with 2-hydroxy acid dehydrogenases have been shown in yellow [1] (B) The regulatory motif used in CtBP-mediated repression. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

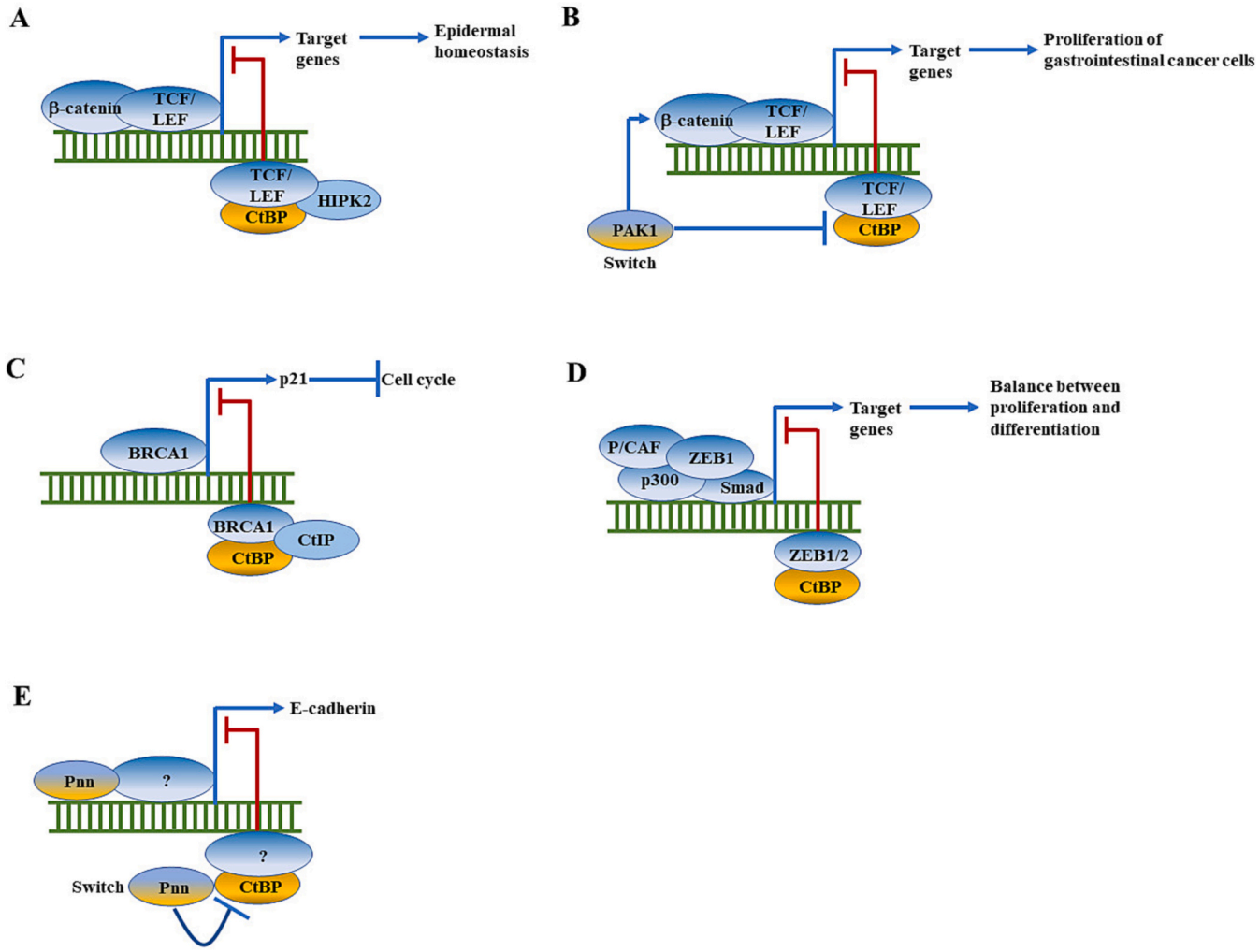

Fig. 2. The motif used in CtBP-mediated repressions. (A) The motif used in epidermal homeostasis. (B) The motif used in the control of the Wnt signaling in gastrointestinal cancer cells. (C) The motif used in the cell cycle control by BRCA1 (D) The motif used in the TGFβ signaling (E) The motif used in the control of E-cadherin gene expression.

In the section above, we have shown that CtBP-mediated repression uses a common motif, sharing a protein between the activator and the repressor complexes regulating a gene (Fig. 1B). This factor may connect the repression motif to additional regulatory networks (Fig. 1B). Further, in this motif, CtBP may be negatively regulated, blocking its repressor activity and creating a switch (Fig. 1B). Notably, CtBP represses Wnt target genes, which are pro-proliferation. In contrast, it also represses p21, which blocks cell cycle progression. Thus, CtBP's role in a cell may be diverse and complex while it maintains a balance between activation and repression of a gene, controlling its transcription rate.

2.6. *The role of the dimeric form of CtBP*

NAD+ and NADH regulate the binding of CtBP to partner proteins, which makes the repression complex with CtBP [24]. In this process, NADH is 2-3 orders of magnitude more effective in promoting CtBP-partner (e.g., ZEB) interaction than NAD+ [24]. Further, CtBP has at least a 100-fold higher affinity for NADH than for NAD+, supporting the role of NADH in affecting CtBP-mediated repression [25]. Further, since NADH/NAD+ ratio affects the repression mediated by CtBP, CtBP acts as a redox sensor for the transcription [24]. Consistent with the role of NADH in promoting CtBP's repressor function, the hypoxic condition induced NADH levels in cancer cells and promoted CtBP's binding to the E-cadherin promoter causing the repression of the E-cadherin gene, reducing cell-cell adhesion, promoting cell migration and metastasis [7]. These effects were inhibited by pyruvate, which prevents NADH increase [7]. Notably, NADH causes CtBP dimerization [5] [26]. Thus, CtBP is in the monomeric form under a low NADH/NAD+ ratio and a dimeric form under a high NADH/NAD+ ratio in the cell. Further, the usual corepression activity of CtBP is due to its dimeric form [26].

2.7. *A reduction in NADH/NAD+ ratio has been implicated in cancer*

High rates of glycolysis correspond to a low NADH/NAD+ ratio because a high NADH/NAD+ ratio inhibits GAPDH [27], which is required for glycolysis. Thus, there is an inverse relationship between the glycolysis rate and NADH/NAD+ ratio. Further, cancer cells are characterized by high rates of glycolysis [27], corresponding to a low NADH/NAD+ ratio and the presence of the monomeric form of CtBP. In this context, cytosolic malate dehydrogenase 1 (MDH1) regenerates NAD+ from NADH [28], decreasing the NADH/NAD+ ratio. Further, MDH1 is overexpressed in human tumors and correlates with poor prognosis [28], suggesting a role of the decreased NADH/NAD+ ratio in tumor progression. Similarly, when mitochondrial oxidation of NADH by the respiratory chain is impaired, cytosolic carboxylation of glutamine, as a part of the MDH1 catalyzed regeneration of NAD+ in the cytosol, takes place so that NADH/NAD+ ratio decreases and the rate of glycolysis increases in the cells [29]. Further, the hydride transfer

complex (HTC), a multi-enzymatic complex of malate dehydrogenase 1, malic enzyme 1, and cytosolic pyruvate carboxylase, transfers reducing equivalents from NADH to NADP+, thereby regenerating NAD+ [30]. Furthermore, HTC blocks senescence and confers fitness to the cells under hypoxia or mitochondrial dysfunction [30]. Moreover, the HTC-mediated rewiring of metabolism may reduce the NADH/NAD+ ratio in the cell and have a role in carcinogenesis [30]. Thus, a reduction in NADH/NAD+ ratio has been implicated in cancer.

2.8. The mechanisms of CtBP-mediated repression

2.8.1. The histone deacetylase-dependent repression

An inappropriate expression of Evi1, a zinc finger nuclear protein, has been implicated in leukemic transformation [31]. Further, Evi1 represses the TGFβ signaling through CtBP1 in a histone deacetylase (HDAC)-dependent manner [31]. Similarly, the activity of the transcription factor, myocyte enhancer factor-2 (MEF2), is repressed by MEF2-interacting transcription repressor (MITR) through CtBP in a class II histone deacetylase (HDAC) dependent manner [32]. Further, CtBP has been shown to recruit cofactor ZEB and HDAC [33]. This complex represses in a CtBP-dimer-dependent manner while the monomer is defective in its corepression activity [33]. Interestingly, mutant CtBP that could not form dimer was unable to repress Dpp signaling in *Drosophila* [34]. Although there is considerable support for the idea of the involvement of the dimeric form of CtBP in HDAC-dependent corepressor activity, the question that remains to be addressed is whether, in HDAC-dependent corepressor activity of CtBP, the mechanism requiring a dimerized form of CtBP is dispensable (Fig. 3A). Since CtBP dimerization is caused by NADH, linking the cell metabolism with transcriptional regulation, the resolution of the question of the oligomeric state of CtBP will help clarify the functional relevance of CtBP-mediated repression and its link with metabolism.

2.8.2. Involvement of HIC1 and SIRT1

Hypermethylated in cancer 1 (HIC1) is epigenetically inactivated in cancer and acts as a corepressor [35]. HIC1 causes the nuclear translocation of CtBP1 [35]. Further, HIC1 binds with the histone deacetylase sirtuin-1 (SIRT1) and the HIC1-SIRT1 complex represses the SIRT1 promoter [36] (Fig. 3B), creating a feedback loop. Similarly, a high NADH/NAD+ ratio decreases the expression and activity of the SIRT1 [37]. Thus, NADH and HIC1 independently decrease SIRT1 expression and CtBP involves with both NADH and HIC1, suggesting an NADH-dependent involvement of CtBP in the HIC1-mediated repression. In agreement, the HIC1-CtBP complex binds with the SIRT1 promoter [38]. Although, further investigation is required to clarify the role of NADH and the dimeric CtBP in HIC1-mediated repression (Fig. 3B). In this context, it is suggested that in the HIC1-mediated repression, the involvement of the CtBP makes the repressor complex more effective [35] although CtBP may be dispensable in HIC1-mediated repression.

2.9. The histone deacetylase independent repression

2.9.1. The involvement of CtIP

E2F-Rb or E2F-p130 complexes repress in both HDAC-dependent and -independent manners [39]. In HDAC-independent repression activity, they recruit CtBP and CtIP [39] (Fig. 3C). CtIP contains LXCXE sequence, which is required for its binding to Rb, and PLDLS sequence, which is required for its binding to CtBP [39]. These interactions may overlap [39]. Thus, the CtBP dimer may be required for the CtIP-dependent repression activity of CtBP [39]. Similarly, Ikaros can repress both in HDAC-dependent and -independent manners [11]. The recruitment of CtBP by Ikaros is an HDAC-independent mechanism of Ikaros-mediated repression [11]. In this mechanism, Ikaros also recruits CtIP along with CtBP [40]. Thus, the involvement of CtIP is an HDAC-independent mechanism of CtBP-mediated repression and the role of the oligomeric state of CtBP in this mechanism requires further investigation (Fig. 3C).

2.10. Other mechanisms in which CtBP does not participate as a corepressor but affects the transcription

2.10.1. Monomeric form of CtBP blocks NFkB-mediated transcription

Monomeric CtBP directly binds with acetyltransferase p300 and inhibits the p300-mediated histone acetylation and transcriptional activation [41]. In agreement, the monomeric form of CtBP, which is found when the NADH/NAD+ ratio is low, inhibits NF-kB transcriptional activity and activation of pro-inflammatory gene expression in macrophages and microglia in a p300-dependent manner [42], blocking the innate immune response. Consistently, inhibition of the CtBP dimerization replicated the effect of the reduced NADH/NAD+ ratio [42], suggesting an involvement of the monomeric form of CtBP in blocking

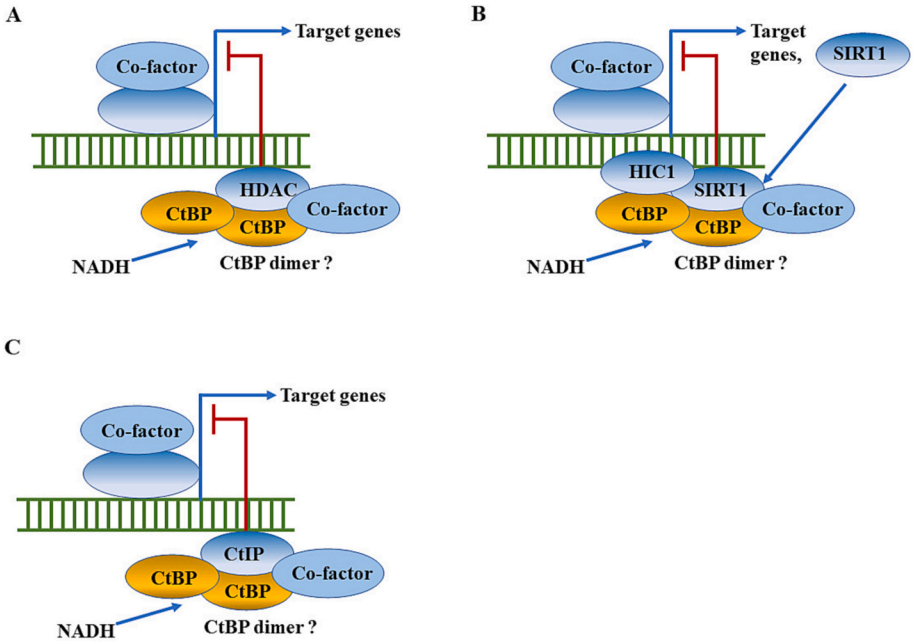

Fig. 3. The mechanisms of CtBP-mediated repression. (A) HDAC-dependent mechanism. (B) SIRT1-dependent mechanism. (C) HDAC-independent mechanism.

NFkB transcriptional activity. Further, the monomeric form of CtBP blocks NFkB transcriptional activity by directly affecting p300 and not by functioning as a corepressor (Fig. 4A).

In contrast, when NADH/NAD+ ratio is high due to a low level of occludin, which is an NADH oxidase, the expression, and activity of the histone deacetylase sirtuin-1 (SIRT1), which deacetylates NFkB, is low while the level of the acetylated, transcriptionally active NFkB is high [37]. Thus, a high NADH/NAD+ ratio increases the NFkB transcriptional activity by reducing the histone deacetylase SIRT1 activity. Further, a high NADH/NAD+ ratio increases the NFkB transcriptional activity by relieving the inhibition of the acetyltransferase p300 by the monomeric CtBP (Fig. 4A), since the monomeric CtBP is not found in the high NADH/NAD+ ratio condition. In contrast, a low NADH/NAD+ ratio blocks the NFkB activity.

2.10.2. Monomeric form of CtBP blocks p53 activity

The dimeric form of CtBP, found in a high NADH/NAD+ condition, causes p53 accumulation, implementing a glycolytic stress response [27]. In contrast, the monomeric form, found in the cells with a low NADH/NAD+ ratio, blocks the p53 function through HDM2 (Fig. 4B), which causes the degradation and nuclear expulsion of p53. Similarly, the inactivation of HIC1 [36] or a low NADH/NAD+ [37] causes SIRT1 upregulation (Fig. 3B), resulting in deacetylation and inactivation of p53, allowing cells to bypass apoptosis during DNA damage. Thus, in the cells that are in a high glycolytic state with a low NADH/NAD+ ratio e.g., during carcinogenesis, the p53-mediated surveillance mechanism is absent due to either the monomeric form of CtBP affecting p53 through HDM2 or a low NADH/NAD+ ratio causing the p53 deacetylation through SIRT1.

In agreement, metformin, which inhibits the NADH dehydrogenase, the mitochondrial complex I, reduced proliferation and induced apoptosis in cancer cells [43]. Since metformin inhibits the mitochondrial complex I, its effect on cell proliferation and apoptosis may be due to the increased NADH/NAD+ in the cell under metformin treatment. Further, silencing of lactate dehydrogenase-A (LDH-A), which oxidizes NADH to NAD+, increased the NADH/NAD+ ratio and caused p53-dependent apoptosis in cancer cells [44]. Further, LDH-A silencing decreased SIRT1 activity and increased the accumulation of acetylated/active form of p53 [44].

In summary, the dimeric form of CtBP regulates the global repression activity of CtBP implementing balances and homeostases while the monomeric form or the absence of the dimeric form upsets the cell's balances. Further, the monomeric form of CtBP does not participate as a corepressor but affects transcription negatively by other miscellaneous mechanisms e.g., by interfering with the action of the histone acetyltransferase p300. Interestingly, the NADH-free form of the monomeric CtBP blocks both the p53 function, which implements a stress response, and the NFkB transcriptional activity, which implements the innate immune response. Thus, two critical cell surveillance mechanisms are compromised by the monomeric CtBP in cells with a low NADH/NAD+ ratio or high glycolysis rate.

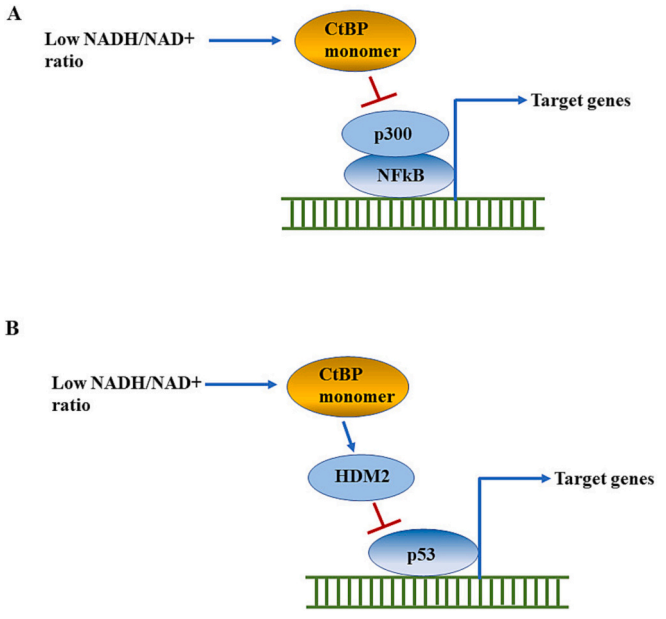

Fig. 4. The role of the monomeric CtBP in transcription. (A) The effect on NF-kB mediated transcription (B) The effect on p53 activity.

3. The balancing acts of CtBP in stem cells

3.1. The maintenance of lateral inhibition between intestinal stem cells and enteroblasts in Drosophila

In the *Drosophila* midgut, when intestinal stem cells (ISCs) divide into two daughter cells, one cell retains the expression of delta, a notch ligand, and remains ISC while the other daughter cell loses delta expression through lateral inhibition and becomes enteroblast (EB) [45]. In ISCs, CtBP represses the targets of the *suppressor of hairless*, Su (H), the *Drosophila* homolog of the notch pathway activator RBP-Jk, maintaining the ISC fate [45] (Fig. 5A). On the other hand, the EB fate is maintained due to an inhibition of the delta expression. In EBs, delta expression is inhibited by Groucho (Gro), another evolutionarily conserved corepressor, through its cooperation with the *Enhancer of split complex*, E(spl)-C, inhibiting their cell-cycle entry and causing their differentiation. Thus, while CtBP-mediated repression of the notch pathway maintains the ISCs fate, Groucho-mediated inhibition (the lateral inhibition) of the delta expression in EBs, maintains the EB cells fate [45], and the two repressors maintain ISCs-EBs homeostasis (Fig. 5A). The Groucho-mediated inhibition of the delta expression in EBs is called a lateral inhibition because this inhibition is caused by the activation of the notch pathway in EBs due to the expression of delta in the neighboring ISCs (Fig. 5A). Further, at the promoter of the notch target genes, both an activator complex, involving Su (H)/RBP-Jk, and a repressor complex, involving Su (H)/RBP-Jk-CtBP, form (Fig. 1 in [15]) and the repressor complex is required for maintaining the ISC fate.

3.2. The maintenance of antineuronal specification of the roof plate of the neural tube

A high level of HES1, a notch target gene, is required to block neurogenesis in the roof plate region of the neural tube [46]. The oxygen level in the roof plate of the neural tube is higher than that in the neurogenic regions [6]. The high oxygen level decreases the NADH level and the repression of HES1, caused by CtBP [6] involved in the notch repressor complex, maintaining antineuronal specification of the roof plate of the neural tube (Fig. 5B). Since a high level of oxygen blocks the notch repressor complex, which represses HES1 through CtBP, the oxygen level acts as a switch in this regulation of the neural tube, causing HES1 transcription, blocking neurogenesis in the roof plate.

3.3. The maintenance of cyst and germline stem cells and their niche in Drosophila testis

Hub cells, the primary component of the stem cell niche in *Drosophila* testis, are required to regulate the cyst stem cells (CySCs) and the germline stem cells (GSCs) [47]. In this context, the evolutionarily conserved transcription factor Escargot (Esg) maintains the CySC state and causes the proliferation of CySCs [48] (Fig. 5C). On the other hand, Esg through its interaction with CtBP is required for the maintenance of the hub cell fate [47], the niche of CySCs and GSCs (Fig. 5C). The niche, in turn, maintains the two types of the stem cells. Consistently, the depletion of Esg causes the hub cells to convert to CySCs and

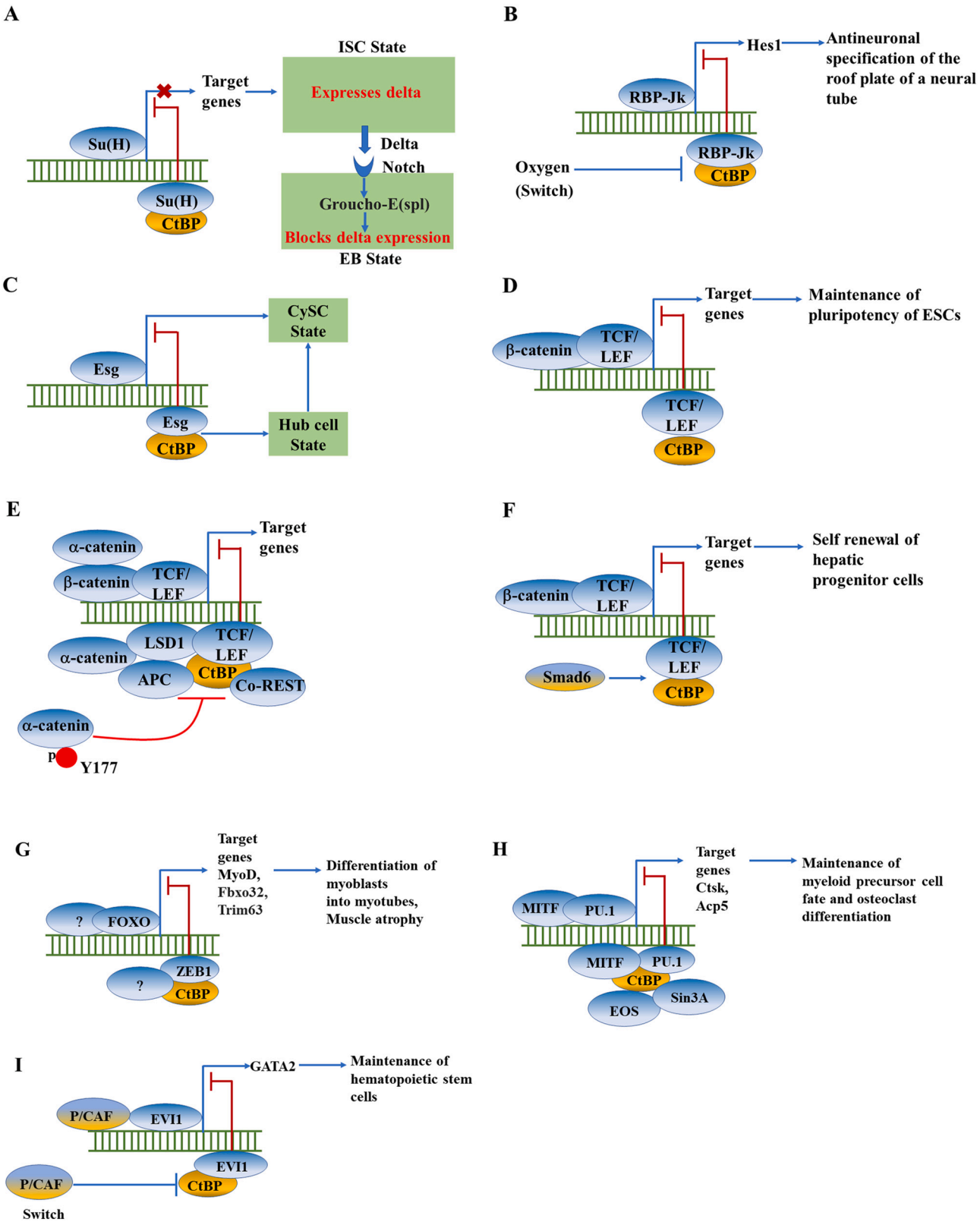

Fig. 5. The role of CtBP in stem cells. (A) The motif used in the maintenance of lateral inhibition between the intestinal stem cells and the enteroblasts in *Drosophila* (B) The motif used in the maintenance of antineuronal specification of roof plate of the neural tube (C) The motif used in the maintenance of cyst and germline stem cells and their niche in *Drosophila* testis (D) The motif used in the maintenance of pluripotency of embryonic stem cells (E) The motif used in the α-catenin-mediated control of the Wnt target genes through CtBP in human embryonic stem cells (F) The motif used in the maintenance of self-renewal of the hepatic progenitor cells (G) The motif used in the control of the differentiation of myoblasts, the maintenance of skeletal muscle development, and the control of muscle atrophy genes (H) The motif used in the control of osteoclast differentiation (I) The motif used in the maintenance of the hematopoietic stem cells.

differentiate as cyst cells, causing a complete loss of the hub cells as well as those of the two types of stem cells, CySCs, and GSCs [47]. Thus, Esg and Esg-CtBP together maintain the cyst and the germline stem cells, and their niche, the hub cells (Fig. 5C) in *Drosophila* testis.

3.4. The maintenance of pluripotency of embryonic stem cells

In mouse embryonic stem cells, the Wnt pathway regulates their pluripotency positively. On the other hand, the Wnt pathway effector TCF3 represses pluripotency factor OCT4 in corepressors Groucho and CtBP dependent manner, suggesting that CtBP affects the maintenance of pluripotency of these cells negatively [49] (Fig. 5D). Consistently, in these cells, CtBP2 causes β-catenin destruction and CtBP depletion prevents an exit from pluripotency of ESCs [50]. Upon CtBP knockdown, β-catenin was enriched at the *Nanog*, *Oct4*, *Rex1*, *Esrrb*, *Klf2, Nr5a2*, and *Dax1* loci, also the sites of CtBP2 occupation, preventing an exit of the ESCs from pluripotency [50]. Therefore, CtBPs are involved in the exit from pluripotency of ESCs cells, causing their fate specification, and maintaining ESC-progeny homeostasis. In mouse embryonic stem cells (mESCs), CtBP2 is highly expressed and its expression decreases during differentiation while CtBP1 is expressed at a low but constant level [51]. Both isoforms cause an exit from pluripotency in mESCs [51].

3.5. The α-catenin-mediated control of Wnt target genes through CtBP in human embryonic stem cells

Catenin inhibitory domain (CID) in adenomatous polyposis coli (APC) is essential for β-catenin degradation [52]. α-catenin interacts with APC CID and stabilizes APC-β-catenin interaction promoting β-catenin ubiquitination and degradation [52]. Further, α-catenin recruits both β-catenin-LEF1/TCF and APC in complex with CtBP-CoREST-LSD1 [52]. Thus, α-catenin helps form both an activator complex involving β-catenin and a repressor complex involving APC and CtBP, regulating transcription of Wnt target genes (Fig. 5E). Further, APC negatively regulates the Wnt signaling both by the direct degradation of β-catenin and by repression of the target genes through CtBP. On the other hand, the phosphorylation of α-catenin at the Y177 position prevents its association with APC in the repressor complex but not with β-catenin in the activator complex, and prevents repression of the Wnt target genes [52]. Thus, phosphorylation of α-catenin at the Y177 position tilts this balance toward higher Wnt activity and may act as a switch regulating the Wnt pathway.

Further, the depletion of α-catenin in hESCs prevents repression of Wnt target genes and promotes endodermal differentiation [52], which requires the Wnt activation [53], suggesting that α-catenin is dispensable for the activator complex but not for the repressor complex. Thus, the activator and the repressor complex mediated control of the Wnt target genes plays an important role in the hESC fate maintenance and differentiation, and α-catenin is shared between the two complexes.

3.6. The maintenance of self-renewal of hepatic progenitor cells

β-catenin signaling is required for the expansion and self-renewal of hepatic progenitor cells (HPCs) [54]. On the other hand, Smad6 inhibited the proliferation and self-renewal of these cells by promoting interaction between TCF and CtBP, repressing β-catenin target genes [54]. Thus, like the pluripotency in ESCs, in HPCs, CtBP inhibits the self-renewal of the cells by repressing the Wnt pathway (Fig. 5F). Further, in HPCs, Smad6 connects the Wnt pathway regulation to the TGFβ signaling network through CtBP.

3.7. The control of the differentiation of myoblasts, the maintenance of skeletal muscle development, and the control of muscle atrophy genes

Myogenic factor MyoD regulates skeletal muscle development [55]. Further, MyoD promotes myoblasts' differentiation into myotubes [56]. MyoD is a target gene of the transcription factor FOXO [57]. In contrast, ZEB1, a transcriptional repressor, represses the muscle differentiation genes through CtBP as a corepressor in myoblasts [55] (Fig. 5G). Indeed, the depletion of ZEB1 causes an accelerated formation of myotubes and precocious expression of myogenic differentiation genes [55].

Like the differentiation of myoblasts into myotubes, the muscle atrophy genes are activated by FOXO transcription factors while ZEB1 through CtBP represses these genes [58]. For example, ZEB1 represses the muscle atrophy genes Fbxo32 and Trim63 [58]. Especially, ZEB1 represses Fbxo32 in undifferentiated myoblasts and atrophic myotubes [58]. Thus, ZEB1-CtBP-mediated repression regulates myoblast fate and muscle atrophy (Fig. 5G) in myoblasts and atrophic myotubes.

3.8. The control of osteoclast differentiation

During osteoclast differentiation, the transcription factors MITF and PU.1 increase the expression of cathepsin K (Ctsk) and acid phosphatase 5 (Acp5) [59]. However, in myeloid precursor cells, which form either macrophages or osteoclasts, MITF and PU.1 form a repressor complex with EOS, CtBP, and Sin3A [59] (Fig. 5H). This complex represses the expression of Ctsk and Acp5 [59], maintaining myeloid precursor cell fate (Fig. 5H). Thus, in committed myeloid progenitors before the initiation of osteoclast differentiation, the repressor complex, involving CtBP, represses the differentiation genes [59]. On the other hand, during osteoclast differentiation, the association of the repressor complex with Ctsk and Acp5 promoter decreases [59], suggesting the involvement of a switch mechanism in this regulation.

3.9. The maintenance of hematopoietic stem cells

GATA2 is important for the maintenance of hematopoietic stem cells [60]. Further, ecotropic viral integration site 1 (EVI1) activates GATA2 [60]. Furthermore, the p300/CBP association factor (P/CAF) causes the acetylation of EVI1 and the acetylation is important for its activator function [60]. On the other hand, EVI1 also forms a repressor complex with the corepressor CtBP [60] (Fig. 5I). Further, P/CAF relieves the repressive effect of EVI1-CtBP complex on GATA2, maintaining the hematopoietic stem cells [60]. Thus, P/CAF acts as a switch that tilts the balance toward GATA2 activation and maintenance of hematopoietic stem cells (Fig. 5I).

In summary, since CtBP is involved in maintaining homeostasis of processes in stem cells, an aberration, leading to a lower NADH/NAD+ ratio, may disturb the homeostasis maintained by CtBP and cause carcinogenesis.

4. CtBP and Cancer

4.1. The malignant transformation of the hematopoietic stem cells

AML1 is important for hematopoietic cell development in the fetal liver and the differentiation of hematopoietic cells in adults [61]. Further, EVI1 is expressed at a very low level in normal hematopoietic cells but is highly expressed in chronic myelocytic leukemia [61]. AML1-EVI1 chimeric gene plays an important role in hematopoietic stem cell malignancies e.g., chronic myelocytic leukemia [61]. The repressive effect of AML1-EVI1 in association with CtBP on AML1-induced transcription is a possible cause of the malignant transformation of hematopoietic stem cells [61] (Fig. 6A).

Further, the AML1-EVI1 chimeric protein causes a differentiation block of malignant myeloid progenitors inducing leukemic transformation in hematopoietic stem cell tumors [62]. In this context, AML1-EVI1 associates with C/EBPalpha, a transcription factor that causes granulocytic differentiation, and has a dominant negative effect on C/EBPalpha transcriptional activity via CtBP [62]. CtBP may induce its repressive effect via the recruitment of histone deacetylase, affecting the DNA binding activity of C/EBPalpha [62]. This repression of the C/

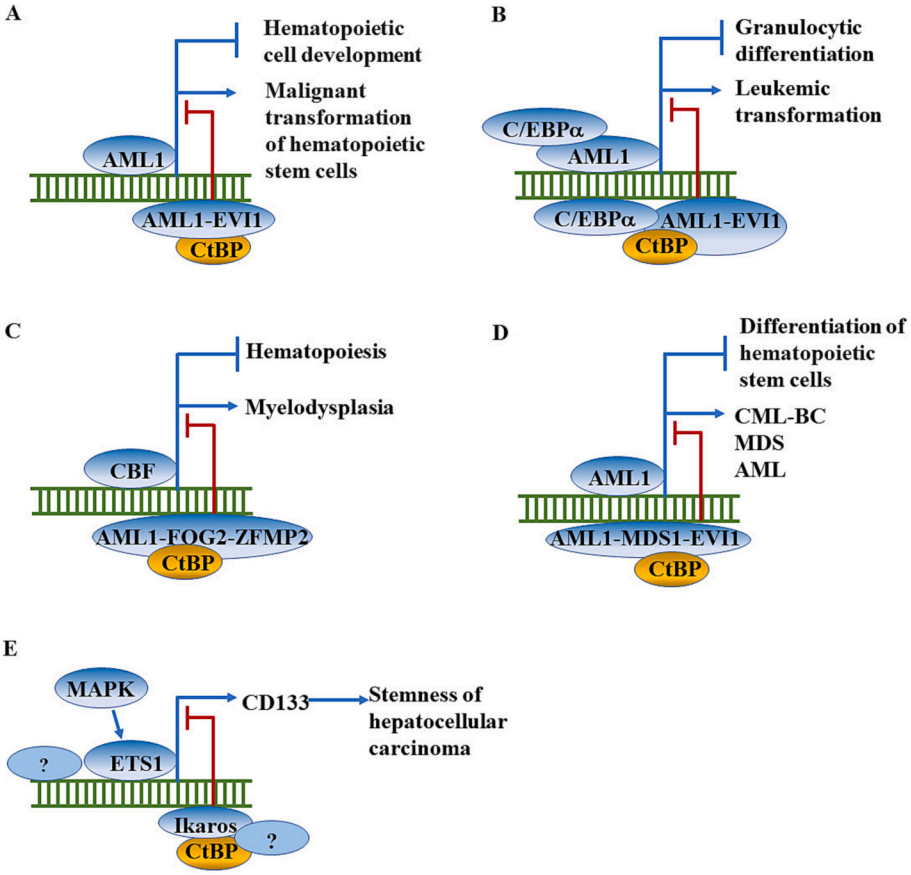

Fig. 6. The CtBP-mediated repression in cancer. (A, B, C, D) The motif used in the malignant transformation of the hematopoietic stem cells (E) The motif used in the control of CD133 expression in hepatocellular carcinoma.

EBPalpha function may be the cause of the leukemogenic potential of AML1-EVI1 [62] (Fig. 6B).

Furthermore, the core binding factor (CBF) is involved in specifying the hematopoietic stem cell and regulates hematopoiesis [63]. Translocation or point mutation in AML1/RUNX1, the DNA binding subunit of CBF, plays a critical role in the development of acute myeloid leukemia and myelodysplasia [63]. In a myelodysplasia patient, AML1 was found to be fused with FOG2/ZFPM2, and the fusion protein recruits CtBP [63]. This complex represses the transcriptional activity of CBF and GATA1 [63] (Fig. 6C).

In addition, the chimeric transcription factor AML1-MDS1-EVI1 (AME) encodes a protein found in patients with chronic myelogenous leukemia during the blast crisis (CML-BC), with myelodysplastic syndrome (MDS), and with acute myeloid leukemia (AML) [64]. AME physically interacts with corepressors CtBP1 and HDAC1 [64]. The interaction between AME and CtBP1 causes growth upregulation and abnormal differentiation of murine bone marrow progenitors [64] (Fig. 6D).

4.2. The control of CD133 expression in hepatocellular carcinoma

Transcription factor ETS1 causes the transcription of CD133 [65], a cell surface protein and cancer stem cell marker in hepatocellular carcinoma (HCC) [66]. On the other hand, Ikaros forms a repressor complex with CtBP and represses CD133 in these cells [66]. Further, Ikaros was upregulated by ETS1, which is regulated by the MAPK pathway [66]. Thus, the MAPK pathway through ETS1 affects CD133 expression positively while ETS1 negatively regulates the CD133 expression through the repression complex involving Ikaros and CtBP, establishing a balance of CD133 expression in HCC (Fig. 6E). Consistently, the reduction in Ikaros expression correlated with poor survival in HCC patients [66]. Thus, in HCC, CtBP has an anti-cancer effect.

In summary, CtBP is a corepressor that may have both pro- and anti-cancer effects.

5. CtBP and homeostasis

5.1. The maintenance of a balanced inflammatory response of microglia and astrocytes in CNS

Microglia and astrocytes are important in maintaining homeostasis of inflammatory responses within the central nervous system (CNS) [67]. 5-androsten-3β,17β-diol (ADIOL) through the estrogen receptor (ER)β recruits CtBP to AP-1-dependent promoters, repressing genes that amplify inflammatory responses of microglia and astrocytes and activate Th17 T cells [67]. In agreement, the addition of ADIOL or synthetic ERβ-specific ligands prevented experimental autoimmune encephalomyelitis in an Erβ and CtBP-dependent manner [67]. Thus, ADIOL/ERβ/CtBP maintains a balanced inflammatory response of microglia and astrocytes in CNS [67].

5.2. The maintenance of pH homeostasis in breast cancer cells

Cancer cells maintain high levels of anabolism through glycolysis [68]. However, they also accumulate acidic metabolites such as pyruvate and lactate due to incomplete glycolysis [68]. On the other hand, glutamine consumption by cancer cells is important in releasing the acidification pressure through the production of ammonia during glutaminolysis, resisting acidification due to incomplete glycolysis [68]. CtBP plays an important role in metabolism and pH homeostasis by

repressing SIRT4, a repressor of glutaminolysis [68]. Consistently, tumor samples of breast cancer patients show a high level of CtBP expression while SIRT4 expression in these tissues was abolished [68]. Thus, cancer cells reduce acidification pressure through the promotion of glutaminolysis, regulated positively by CtBP through its repression of SIRT4, and maintain growth by maintaining metabolic homeostasis [68].

5.3. The maintenance of cholesterol homeostasis in breast cancer cells

Reduction of the cholesterol amount leads to epithelial-to-mesenchymal transition (EMT) and increased migration of breast cancer cells [69]. CtBP maintains cholesterol homeostasis in these cells by forming a complex with ZEB1 and repressing the sterol regulatory element-binding transcription factor 2 (SREBF2) [69], which activates the biosynthesis of cholesterol. In this context, TGFβ decreases the intracellular cholesterol level through ZEB1 and CtBP complex repressing the SREBF2 promoter [69], causing migration of the cells. Further, CtBP increases the activity of TGFβ signaling by reducing the membrane cholesterol level in these cells [69]. On the other hand, cholesterol reduces the stability of TGBβ receptors, affecting the EMT and migration of breast cancer cells negatively. These interactions between TGFβ signaling and cholesterol homeostasis in which CtBP is a major player orchestrate metastasis of breast cancer cells [69].

5.4. The maintenance of skeletal muscle homeostasis

Skeletal muscle maintains homeostasis by maintaining a balance between protein synthesis and proteolysis by balancing hypertrophic and atrophic signals [21]. Skeletal muscle atrophy is caused by the atrogenes including those of the ubiquitin-proteasome and autophagy-lysosomal systems induced by FOXO transcription factors [21]. ZEB1 forms a complex with CtBP that represses the FOXO3 transcriptional activity [21]. Consistently, ZEB1 deficiency in mice induced a number of atrogenes, including Atrogin-1/Fbxo32, Psma1, MuRF1/Trim63, Gabarapl1, Ctsl, 4ebp1, and Nrf2 and caused higher muscle atrophy [21]. Thus, FOXO transcription factors activate the atrogenes while ZEB1/CtBP complex represses their promoter, maintaining skeletal muscle homeostasis [21].

6. Conclusions

The paper shows that a specific type of motif is used repeatedly in CtBP-mediated repressions. In this motif, both an activator and a repressor complex form at the promoter of the target genes, and a cofactor is shared between the two complexes. Thus, the amount of this limiting cofactor may tilt the balance toward gene activation or repression, controlling the transcription rate. Similarly, other mechanisms of switches may also be present in this motif. Moreover, the cofactor and the switch may be further regulated through their link to the broader cell signaling network (Fig. 1B).

CtBP is prominently involved in homeostatic processes, especially in stem cells, including the ESCs. In these cells, it maintains their stemness and differentiation. CtBP is also involved in hematopoietic malignancies. In this context, the transcription factor AML1 regulates hematopoietic cell development. On the other hand, some chimeric/translocation proteins involving AML1 make repressor complexes with the corepressor CtBP. These repressor complexes disrupt the normal hematopoietic cell development by AML1 and cause hematopoietic malignancies.

There are two kinds of repression mechanisms of CtBP: (1) a specific global mechanism implemented by the dimeric CtBP. CtBP dimerization is caused by a high NADH/NAD+ ratio during the low glycolytic condition usually found in normal cells (2) miscellaneous mechanisms of the monomeric CtBP found in the cells having a low NADH/NAD+ ratio during the high glycolytic condition.

In the former mechanism, CtBP is involved in balancing acts and maintaining homeostasis while the cell surveillance mechanisms, implemented by the stress response of p53 and innate immune response of NFkB, are present. In contrast, in the latter mechanism, in high glycolytic conditions, especially present in cancer cells, the monomeric CtBP blocks the responses implemented by p53 and NFkB. Moreover, the high glycolytic conditions with a low NADH/NAD+ ratio upsets the homeostasis and balancing acts of the dimeric CtBP, by decreasing its affinity for the binding partner in the repressor complex. Thus, the high glycolytic condition causes global instability in the cell by disturbing the homeostatic processes and blocking cell surveillance, which may be linked to carcinogenesis.

On the other hand, hypoxia, which causes epithelial-to-mesenchymal transition and metastasis of cancer cells, increases the NADH level [7], causing repression of E-cadherin by the dimeric CtBP and migration of cells [7]. Due to an increase in NADH level, the creation of the hypoxic condition corresponds to a switch of cancer cells from a high glycolytic state to a low glycolytic state, restoring the homeostases implemented by the dimeric CtBP. This switch also restores the cell surveillances, blocked by the monomeric CtBP, while causing cell migration due to the repression of E-cadherin caused by the dimeric CtBP. It may be interesting to understand why restoration of the homeostases and cell surveillance mechanisms are important for cancer cells during EMT and metastasis. Intuitively, the underlying cellular stability may be useful during such profound changes in cell behavior.

Further, since CtBP dimer-monomer induced stability-instability has a link with metabolism and cellular oxidative state, further explorations of this angle will be the key to understanding the CtBP-produced underlying instabilities that cause carcinogenesis. Furthermore, in the processes in which CtBP is involved, the delineation of the role of its oligomeric state, the cellular NADH level, and the metabolic and oxidative states of the cell will be crucial in substantiating the role of CtBP in carcinogenesis.

Since cancer cells lack or have poor cell surveillance, they are more sensitive to agents that negatively affect their stability. In agreement, stem cell-like breast cancer cells that are resistant to metformin are sensitive to the inhibition of NADH-dependent CtBP dimerization [5], which maintains cellular stability by maintaining the underlying cellular balances and homeostases.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

References

[1] G. Chinnadurai, CtBP, an unconventional transcriptional corepressor in development and oncogenesis, Mol. Cell 9 (2002) 213–224, https://doi.org/10.1016/S1097-2765(02)00443-4.
[2] R. Welgert, M.G. Silletta, S. Spanò, G. Turacchie, C. Cericola, A. Colanzi, S. Senatore, R. Mancini, E.V. Pollshchuk, M. Salmona, F. Facchiano, K.N.J. Burger, A. Mironov, A. Luini, D. Corda, CtBP/BARS induces fission of Golgi membranes by acylating lysophosphatidic acid, Nature 402 (1999) 6760, 429–433, https://doi.org/10.1038/46587, 429–433.
[3] C.J. Barnes, R.K. Vadlamudi, S.K. Mishra, R.H. Jacobson, F. Li, R. Kumar, Functional inactivation of a transcriptional corepressor by a signaling kinase, Nat. Struct. Mol. Biol. 10 (2003) 8, 622–628, https://doi.org/10.1038/nsb957.
[4] S. Chen, J.R. Whetstine, S. Ghosh, J.A. Hanover, R.R. Gali, P. Grosu, Y. Shi, The conserved NAD(H)-dependent corepressor CTBP-1 regulates Caenorhabditis elegans life span, Proc. Natl. Acad. Sci. U. S. A. 106 (2009) 1496–1501, https://doi.org/10.1073/PNAS.0802674106/SUPPL_FILE/0802674106SI.PDF.
[5] A. Banerjee, C.N. Birts, M. Darley, R. Parker, A.H. Mirnezami, J. West, R.I. Cutress, S.A. Beers, M.J.J. Rose-Zerilli, J.P. Blaydes, Stem cell-like breast cancer cells with acquired resistance to metformin are sensitive to inhibitors of NADH-dependent

CtBP dimerization, Carcinogenesis. 40 (2019) 871–882, https://doi.org/10.1093/CARCIN/BGY174.
[6] J.M. Dias, S. Ilkhanizadeh, E. Karaca, J.K. Duckworth, V. Lundin, M.G. Rosenfeld, J. Ericson, O. Hermanson, A.I. Teixeira, CtBPs sense microenvironmental oxygen levels to regulate neural stem cell state, Cell Rep. 8 (2014) 665–670, https://doi.org/10.1016/J.CELREP.2014.06.057.
[7] Q. Zhang, S.Y. Wang, A.C. Nottke, J.V. Rocheleau, D.W. Piston, R.H. Goodman, Redox sensor CtBP mediates hypoxia-induced tumor cell migration, Proc. Natl. Acad. Sci. 103 (2006) 9029–9033, https://doi.org/10.1073/PNAS.0603269103.
[8] A. Sundqvist, K. Sollerbrant, C. Svensson, The carboxy-terminal region of adenovirus E1A activates transcription through targeting of a C-terminal binding protein-histone deacetylase complex, FEBS Lett. 429 (1998) 183–188, https://doi.org/10.1016/S0014-5793(98)00588-2.
[9] P. Criqui-Filipe, C. Ducret, S.M. Maira, B. Wasylyk, Net, a negative Ras-switchable TCF, contains a second inhibition domain, the CID, that mediates repression through interactions with CtBP and de-acetylation, EMBO J. 18 (1999) 3392–3403, https://doi.org/10.1093/EMBOJ/18.12.3392.
[10] A.R. Meloni, E.J. Smith, J.R. Nevins, A mechanism for Rb/p130-mediated transcription repression involving recruitment of the CtBP corepressor, Proc. Natl. Acad. Sci. U. S. A. 96 (1999) 9574–9579, https://doi.org/10.1073/PNAS.96.17.9574.
[11] J. Koipally, K. Georgopoulos, Ikaros interactions with CtBP reveal a repression mechanism that is independent of histone deacetylase activity, J. Biol. Chem. 275 (2000) 19594–19602, https://doi.org/10.1074/jbc.M000254200.
[12] M. Sutrias-Grau, D.N. Arnosti, CtBP contributes quantitatively to Knirps repression activity in an NAD binding-dependent manner, Mol. Cell. Biol. 24 (2004) 5953–5966, https://doi.org/10.1128/MCB.24.13.5953-5966.2004/ASSET/077CD43F-7047-4D16-AAAA-86E7441EE6BD/ASSETS/GRAPHIC/ZMB0130441930007.JPEG.
[13] S. Barolo, M. Levine, Hairy mediates dominant repression in the Drosophila embryo, EMBO J. 16 (1997) 2883–2891, https://doi.org/10.1093/EMBOJ/16.10.2883.
[14] G. Wei, S. Ku, G.K. Ma, S. Saito, A.A. Tang, J. Zhang, J.H. Mao, E. Appella, A. Balmain, E.J. Huang, HIPK2 represses β-catenin-mediated transcription, epidermal stem cell expansion, and skin tumorigenesis, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 13040–13045, https://doi.org/10.1073/PNAS.0703213104/SUPPL_FILE/03213FIG 13.PDF.
[15] A. Jaiswal, R. Singh, Homeostases of epidermis and hair follicle, and development of basal cell carcinoma, Biochim. Biophys. Acta Rev. Cancer (2022), 188795, https://doi.org/10.1016/J.BBCAN.2022.188795.
[16] H. He, A. Shulkes, G.S. Baldwin, PAK1 interacts with β-catenin and is required for the regulation of the β-catenin signalling pathway by gastrins, Biochim. Biophys. Acta Mol. Cell Res. 1783 (2008) 1943–1954, https://doi.org/10.1016/J.BBAMCR.2008.04.016.
[17] T. Valenta, J. Lukas, V. Korinek, HMG box transcription factor TCF-4's interaction with CtBP1 controls the expression of the Wnt target Axin2/Conductin in human embryonic kidney cells, Nucleic Acids Res. 31 (2003) 2369–2380, https://doi.org/10.1093/NAR/GKG346.
[18] Y. Yu, L. Chen, G. Zhao, H. Li, Q. Guo, S. Zhu, P. Li, L. Min, S. Zhang, RBBP8/CtIP suppresses P21 expression by interacting with CtBP and BRCA1 in gastric cancer, Oncogene 39 (2019) 6, 1273–1289, https://doi.org/10.1038/s41388-019-1060-7.
[19] K. Somasundaram, H. Zhang, Y.X. Zeng, Y. Mouvras, Y. Peng, H. Zhang, G.S. Wu, J. D. Licht, B.L. Weber, W.S. El-Deiry, Arrest of the cell cycle by the tumour-suppressor BRCA1 requires the CDK-inhibitor p21WAF1/CiP1, Nature. 389 (1997) 187–190, https://doi.org/10.1038/38291.
[20] A.A. Postigo, J.L. Depp, J.J. Taylor, K.L. Kroll, Regulation of Smad signaling through a differential recruitment of coactivators and corepressors by ZEB proteins, EMBO J. 22 (2003) 2453–2462, https://doi.org/10.1093/EMBOJ/CDG226.
[21] C. Ninfali, L. Siles, D.S. Darling, A. Postigo, Regulation of muscle atrophy-related genes by the opposing transcriptional activities of ZEB1/CtBP and FOXO3, Nucleic Acids Res. 46 (2018) 10697–10708, https://doi.org/10.1093/NAR/GKY835.
[22] R. Alpatov, G.C. Munguba, P. Caton, J.H. Joo, Y. Shi, Y. Shi, M.E. Hunt, S. P. Sugrue, Nuclear speckle-associated protein Pnn/DRS binds to the transcriptional corepressor CtBP and relieves CtBP- mediated repression of the E-cadherin gene, Mol. Cell. Biol. 24 (2004) 10223–10235, https://doi.org/10.1128/MCB.24.23.10223-10235.2004/ASSET/2026092D-5C8C-47A5-B05D-C4E904B52FB4/ASSETS/GRAPHIC/ZMB0230445750008.JPEG.
[23] R. Alpatov, Y. Shi, G.C. Munguba, B. Moghimi, J.-H. Joo, J. Bungert, S.P. Sugrue, Corepressor CtBP and nuclear speckle protein Pnn/DRS differentially modulate transcription and splicing of the E-cadherin gene, Mol. Cell. Biol. 28 (2008) 1584–1595, https://doi.org/10.1128/MCB.00421-07/ASSET/2A0BD603-6A3E-40EE-AE79-3B26464611AC/ASSETS/GRAPHIC/ZMB0050873330007.JPEG.
[24] Q. Zhang, D.W. Piston, R.H. Goodman, Regulation of corepressor function by nuclear NADH, Science 295 (2002) (1979) 1895–1897, https://doi.org/10.1126/SCIENCE.1069300/SUPPL_FILE/1069300S2_THUMB.GIF.
[25] C.C. Fjeld, W.T. Birdsong, R.H. Goodman, Differential binding of NAD+ and NADH allows the transcriptional corepressor carboxyl-terminal binding protein to serve as a metabolic sensor, Proc. Natl. Acad. Sci. 100 (2003) 9202–9207, https://doi.org/10.1073/PNAS.1633591100.
[26] S.S.C. Thio, J.V. Bonventre, S.I.H. Hsu, The CtBP2 co-repressor is regulated by NADH-dependent dimerization and possesses a novel N-terminal repression domain, Nucleic Acids Res. 32 (2004) 1836–1847, https://doi.org/10.1093/NAR/GKH344.
[27] B. CN, B. A, D. M, D. CR, N. S, N. SK, P. R, W. J, T. A, R.-Z. MJJ, B, JP, p53 is regulated by aerobic glycolysis in cancer cells by the CtBP family of NADH-dependent transcriptional regulators, Sci. Signal. 13 (2020), https://doi.org/10.1126/SCISIGNAL.AAU9529.
[28] E.A. Hanse, C. Ruan, M. Kachman, D. Wang, X.H. Lowman, A. Kelekar, Cytosolic malate dehydrogenase activity helps support glycolysis in actively proliferating cells and cancer, Oncogene 36 (2017) 27, 3915–3924, https://doi.org/10.1038/onc.2017.36.
[29] E. Gaude, C. Schmidt, P.A. Gammage, A. Dugourd, T. Blacker, S.P. Chew, J. Saez-Rodriguez, J.S. O'Neill, G. Szabadkai, M. Minczuk, C. Frezza, NADH shuttling couples cytosolic reductive carboxylation of glutamine with glycolysis in cells with mitochondrial dysfunction, Mol. Cell 69 (2018) 581–593.e7, https://doi.org/10.1016/J.MOLCEL.2018.01.034.
[30] S. Igelmann, F. Lessard, O. Uchenunu, J. Bouchard, A. Fernandez-Ruiz, M. C. Rowell, S. Lopes-Paciencia, D. Papadopoli, A. Fouillen, K.J. Ponce, G. Huot, L. Mignacca, M. Benfdil, P. Kalegari, H.M. Wahba, J. Pencik, N. Vuong, J. Quenneville, J. Guillon, V. Bourdeau, L. Hulea, E. Gagnon, L. Kenner, R. Moriggl, A. Nanci, M.N. Pollak, J.G. Omichinski, I. Topisirovic, G. Ferbeyre, A hydride transfer complex reprograms NAD metabolism and bypasses senescence, Mol. Cell 81 (2021) 3848–3865.e19, https://doi.org/10.1016/J.MOLCEL.2021.08.028.
[31] K. Izutsu, M. Kurokawa, Y. Imai, K. Maki, K. Mitani, H. Hirai, The corepressor CtBP interacts with Evi-1 to repress transforming growth factor β signaling, Blood. 97 (2001) 2815–2822, https://doi.org/10.1182/BLOOD.V97.9.2815.
[32] C.L. Zhang, T.A. McKinsey, J.R. Lu, E.N. Olson, Association of COOH-terminal-binding protein (CtBP) and MEF2-interacting transcription repressor (MITR) contributes to transcriptional repression of the MEF2 transcription factor, J. Biol. Chem. 276 (2001) 35–39, https://doi.org/10.1074/jbc.M007364200.
[33] L.J. Zhao, M. Kuppuswamy, S. Vijayalingam, G. Chinnadurai, Interaction of ZEB and histone deacetylase with the PLDLS-binding cleft region of monomeric C-terminal binding protein 2, BMC Mol. Biol. 10 (2009) 89, https://doi.org/10.1186/1471-2199-10-89/FIGURES/7.
[34] C. Bi, F. Meng, L. Yang, L. Cheng, P. Wang, M. Chen, M. Fang, H. Xie, CtBP represses Dpp signaling as a dimer, Biochem. Biophys. Res. Commun. 495 (2018) 1980–1985, https://doi.org/10.1016/J.BBRC.2017.12.018.
[35] T. Valenta, J. Lukas, L. Doubravska, B. Fafilek, V. Korinek, HIC1 attenuates Wnt signaling by recruitment of TCF-4 and β-catenin to the nuclear bodies, EMBO J. 25 (2006) 2326–2337, https://doi.org/10.1038/SJ.EMBOJ.7601147.
[36] Y.C. Wen, D.H. Wang, C.Y. RayWhay, J. Luo, W. Gu, S.B. Baylin, Tumor suppressor HIC1 directly regulates SIRT1 to modulate p53-dependent DNA-damage responses, Cell. 123 (2005) 437–448, https://doi.org/10.1016/J.CELL.2005.08.011.
[37] V. Castro, L. Bertrand, M. Luethen, S. Dabrowski, J. Lombardi, L. Morgan, N. Sharova, M. Stevenson, I.E. Blasig, M. Toborek, V. Castro, L. Bertrand, M. Luethen, S. Dabrowski, J. Lombardi, L. Morgan, N. Sharova, M. Stevenson, Occludin controls HIV transcription in brain pericytes via regulation of SIRT-1 activation, FASEB J. 30 (2016) 1234–1246, https://doi.org/10.1096/FJ.15-277673.
[38] C. van Rechem, G. Boulay, S. Pinte, N. Stankovic-Valentin, C. Guérardel, D. Leprince, Differential regulation of HIC1 target genes by CtBP and NuRD, via an acetylation/SUMOylation switch, in quiescent versus proliferating cells, Mol. Cell. Biol. 30 (2010) 4045–4059, https://doi.org/10.1128/MCB.00582-09/ASSET/D922EA7D-F52E-4642-8CB3-221063A6F0DE/ASSETS/GRAPHIC/ZMB9991087250009.JPEG.
[39] A.R. Meloni, E.J. Smith, J.R. Nevins, A mechanism for Rb/p130-mediated transcription repression involving recruitment of the CtBP corepressor, Proc. Natl. Acad. Sci. 96 (1999) 9574–9579, https://doi.org/10.1073/PNAS.96.17.9574.
[40] J. Koipally, K. Georgopoulos, Ikaros-CtIP interactions do not require C-terminal binding protein and participate in a deacetylase-independent mode of repression, J. Biol. Chem. 277 (2002) 23143–23149, https://doi.org/10.1074/jbc.M202079200.
[41] J.H. Kim, E.J. Cho, S.T. Kim, H.D. Youn, CtBP represses p300-mediated transcriptional activation by direct association with its bromodomain, Nat. Struct. Mol. Biol. 12 (2005) 5, 423–428, https://doi.org/10.1038/nsmb924.
[42] Y. Shen, D. Kapfhamer, A.M. Minnella, J.E. Kim, S.J. Won, Y. Chen, Y. Huang, L. H. Low, S.M. Massa, R.A. Swanson, Bioenergetic state regulates innate inflammatory responses through the transcriptional co-repressor CtBP, Nat. Commun. 8 (2017) 1, 1–13, https://doi.org/10.1038/s41467-017-00707-0.
[43] W.W. Wheaton, S.E. Weinberg, R.B. Hamanaka, S. Soberanes, L.B. Sullivan, E. Anso, A. Glasauer, E. Dufour, G.M. Mutlu, G.R. Scott Budigner, N.S. Chandel, Metformin inhibits mitochondrial complex I of cancer cells to reduce tumorigenesis, Elife. (2014), https://doi.org/10.7554/ELIFE.02242.
[44] S.J. Allison, J.R.P. Knight, C. Granchi, R. Rani, F. Minutolo, J. Milner, R.M. Phillips, Identification of LDH-A as a therapeutic target for cancer cell killing via (i) p53/NAD(H)-dependent and (ii) p53-independent pathways, Oncogenesis 3 (2014) 5, e102–e102, https://doi.org/10.1038/oncsis.2014.16.
[45] X. Guo, H. Huang, Z. Yang, T. Cai, R. Xi, Division of labor: roles of Groucho and CtBP in notch-mediated lateral inhibition that controls intestinal stem cell differentiation in Drosophila, Stem Cell Rep. 12 (2019) 1007–1023, https://doi.org/10.1016/J.STEMCR.2019.03.005.
[46] J.H. Baek, J. Hatakeyama, S. Sakamoto, T. Ohtsuka, R. Kageyama, Persistent and high levels of Hes1 expression regulate boundary formation in the developing central nervous system, Development. 133 (2006) 2467–2476, https://doi.org/10.1242/DEV.02403.
[47] J. Voog, S.L. Sandall, G.R. Hime, L.P.F. Resende, M. Loza-Coll, A. Aslanian, J. R. Yates, T. Hunter, M.T. Fuller, D.L. Jones, Escargot restricts niche cell to stem cell conversion in the Drosophila testis, Cell Rep. 7 (2014) 722–734, https://doi.org/10.1016/J.CELREP.2014.04.025.
[48] R. Sênos Demarco, B.J. Stack, A.M. Tang, J. Voog, S.L. Sandall, T.D. Southall, A. H. Brand, D.L. Jones, Escargot controls somatic stem cell maintenance through the

attenuation of the insulin receptor pathway in Drosophila, Cell Rep. 39 (2022), 110679, https://doi.org/10.1016/J.CELREP.2022.110679.
[49] W.-L. Tam, C.Y. Lim, J. Han, J. Zhang, Y.-S. Ang, H.-H. Ng, H. Yang, B. Lim, T-cell factor 3 regulates embryonic stem cell pluripotency and self-renewal by the transcriptional control of multiple lineage pathways, Stem Cells 26 (2008) 2019–2031, https://doi.org/10.1634/STEMCELLS.2007-1115.
[50] T.W. Kim, S. Kwak, J. Shin, B.H. Kang, S.E. Lee, M.Y. Suh, J.H. Kim, I.Y. Hwang, J. H. Lee, J. Choi, E.J. Cho, H.D. Youn, Ctbp2-mediated β-catenin regulation is required for exit from pluripotency, Exp. Mol. Med. 49 (2017), https://doi.org/10.1038/EMM.2017.147.
[51] M.Y. Suh, T.W. Kim, H.T. Lee, J. Shin, J.H. Kim, H. Jang, H.J. Kim, S.T. Kim, E. J. Cho, H.D. Youn, Abundance of C-terminal binding protein isoform is a prerequisite for exit from pluripotency in mouse embryonic stem cells, FASEB J. 32 (2018) 6423–6435, https://doi.org/10.1096/FJ.201700837RRRR.
[52] S.H. Choi, C. Estarás, J.J. Moresco, J.R. Yates, K.A. Jones, α-Catenin interacts with APC to regulate β-catenin proteolysis and transcriptional repression of Wnt target genes, Genes Dev. 27 (2013) 2473–2488, https://doi.org/10.1101/GAD.229062.113.
[53] O. Naujok, U. Diekmann, S. Lenzen, The generation of definitive endoderm from human embryonic stem cells is initially independent from Activin A but requires canonical Wnt-signaling, Stem Cell Rev. Rep. 10 (2014) 480–493, https://doi.org/10.1007/S12015-014-9509-0/FIGURES/12.
[54] D. ZY, L. HF, J. GN, C. WX, W. W, D. PK, Z. MZ, Z. B, C. XP, Smad6 suppresses the growth and self-renewal of hepatic progenitor cells, J. Cell. Physiol. 229 (2014), https://doi.org/10.1002/JCP.24488.
[55] L. Siles, E. Sánchez-Tilló, J.-W. Lim, D.S. Darling, K.L. Kroll, A. Postigo, ZEB1 imposes a temporary stage-dependent inhibition of muscle gene expression and differentiation via CtBP-mediated transcriptional repression, Mol. Cell. Biol. 33 (2013) 1368–1382, https://doi.org/10.1128/MCB.01259-12.
[56] B. Langley, M. Thomas, A. Bishop, M. Sharma, S. Gilmour, R. Kambadur, Myostatin inhibits myoblast differentiation by down-regulating MyoD expression, J. Biol. Chem. 277 (2002) 49831–49840, https://doi.org/10.1074/jbc.M204291200.
[57] P. Hu, K.G. Geles, J.H. Paik, R.A. DePinho, R. Tjian, Codependent activators direct myoblast-specific MyoD transcription, Dev. Cell 15 (2008) 534–546, https://doi.org/10.1016/J.DEVCEL.2008.08.018.
[58] C. Ninfali, L. Siles, D.S. Darling, A. Postigo, Regulation of muscle atrophy-related genes by the opposing transcriptional activities of ZEB1/CtBP and FOXO3, Nucleic Acids Res. 46 (2018) 10697–10708, https://doi.org/10.1093/NAR/GKY835.
[59] H. R, S. SM, B. A, S. R, S. U, O. MC, Eos, MITF, and PU.1 recruit corepressors to osteoclast-specific genes in committed myeloid progenitors, Mol. Cell. Biol. 27 (2007), https://doi.org/10.1128/MCB.01839-06.
[60] A. Shimahara, N. Yamakawa, I. Nishikata, K. Morishita, Acetylation of lysine 564 adjacent to the C-terminal binding protein-binding motif in EVI1 is crucial for transcriptional activation of GATA2, J. Biol. Chem. 285 (2010) 16967–16977, https://doi.org/10.1074/JBC.M110.102046.
[61] K. Mitani, Molecular mechanisms of leukemogenesis by AML1/EVI-1, Oncogene. 23 (2004) 4263–4269, https://doi.org/10.1038/SJ.ONC.1207777.
[62] K. Tokita, K. Maki, K. Mitani, RUNX1/EVI1, which blocks myeloid differentiation, inhibits CCAAT-enhancer binding protein alpha function, Cancer Sci. 98 (2007) 1752–1757, https://doi.org/10.1111/J.1349-7006.2007.00597.X.
[63] E.M. Chan, E.M. Comer, F.C. Brown, K.E. Richkind, M.L. Holmes, B.H. Chong, R. Shiffman, D.E. Zhang, M.L. Slovak, C.L. Willman, C.T. Noguchi, Y. Li, D. J. Heiber, L. Kwan, R.J. Chan, G.H. Vance, H.C. Ramsey, R.A. Hromas, AML1-FOG2 fusion protein in myelodysplasia, Blood. 105 (2005) 4523–4526, https://doi.org/10.1182/BLOOD-2004-07-2762.
[64] V. Senyuk, S. Chakraborty, F.M. Mikhail, R. Zhao, Y. Chi, G. Nucifora, The leukemia-associated transcription repressor AML1/MDS1/EVI1 requires CtBP to induce abnormal growth and differentiation of murine hematopoietic cells, Oncogene. 21 (2002) 3232–3240, https://doi.org/10.1038/SJ.ONC.1205436.
[65] S. Ohnishi, O. Maehara, K. Nakagawa, A. Kameya, K. Otaki, Hypoxia-inducible factors activate CD133 promoter through ETS family transcription factors, PLoS One 8 (2013) 66255, https://doi.org/10.1371/journal.pone.0066255.
[66] L. Zhang, H. Li, C. Ge, M. Li, F. Yu Zhao, H. Lei Hou, M. Xin Zhu, H. Tian, L. Xing Zhang, T. Yang Chen, G. Ping Jiang, H. Yang Xie, Y. Cui, M. Yao, J. Jun Li, Inhibitory effects of transcription factor Ikaros on the expression of liver cancer stem cell marker CD133 in hepatocellular carcinoma, Oncotarget. 5 (2014) 10621–10635, https://doi.org/10.18632/ONCOTARGET.2524.
[67] K. Saijo, J.G. Collier, A.C. Li, J.A. Katzenellenbogen, C.K. Glass, An ADIOL-ERβ-CtBP transrepression pathway negatively regulates microglia-mediated inflammation, Cell. 145 (2011) 584–595, https://doi.org/10.1016/J.CELL.2011.03.050.
[68] L. Wang, H. Zhou, Y. Wang, G. Cui, L.J. Di, CtBP maintains cancer cell growth and metabolic homeostasis via regulating SIRT4, Cell Death Dis. 6 (2015), https://doi.org/10.1038/CDDIS.2014.587.
[69] Z. Zhao, D. Hao, L. Wang, J. Li, Y. Meng, P. Li, Y. Wang, C. Zhang, H. Zhou, K. Gardner, L. Jun Di, CtBP promotes metastasis of breast cancer through repressing cholesterol and activating TGF-β signaling, Oncogene. 38 (2019) 2076–2091, https://doi.org/10.1038/S41388-018-0570-Z.