MOLECULAR BIOLOGY

Transcription factor E4F1 as a regulator of cell life and disease progression

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E4F transcription factor 1 (E4F1), a member of the GLI-Kruppel family of zinc finger proteins, is now widely recognized as a transcription factor. It plays a critical role in regulating various cell processes, including cell growth, proliferation, differentiation, apoptosis and necrosis, DNA damage response, and cell metabolism. These processes involve intricate molecular regulatory networks, making E4F1 an important mediator in cell biology. Moreover, E4F1 has also been implicated in the pathogenesis of a range of human diseases. In this review, we provide an overview of the major advances in E4F1 research, from its first report to the present, including studies on its protein domains, molecular mechanisms of transcriptional regulation and biological functions, and implications for human diseases. We also address unresolved questions and potential research directions in this field. This review provides insights into the essential roles of E4F1 in human health and disease and may pave the way for facilitating E4F1 from basic research to clinical applications.

INTRODUCTION

The E4F transcription factor 1 (*E4F1*) gene, located in the 16p13.3 region of the human genome, encodes a GLI-Kruppel family zinc finger (ZF) protein that is expressed ubiquitously in both humans and mice (*1*). E4F1 was initially recognized as a cellular target of the adenovirus protein E1A and is responsible for regulating the transcription of the adenovirus *E4* gene (*2*–*5*). The full-length E4F1 protein, $p120^{E4F}$, can generate a 50-kDa N-terminal fragment, $p50^{E4F}$, in the presence of E1A. Both forms of the E4F1 protein have transcriptional regulation functions, but $p120^{E4F}$ is the most abundantly expressed form in humans (*3*, *5*, *6*). This review provides an overview of both forms of the E4F1 protein. Notably, $p50^{E4F}$ is explicitly mentioned in this review, and all other E4F1 proteins are referred to as $p120^{E4F}$.

E4F1 is a gene essential for embryonic development, as demonstrated by the death of embryos in the peri-implantation period of *E4f1* knockout mice and its crucial role in mitosis during early embryos (*7*). To bypass this embryonic lethality, a study using a mouse model with a conditional knockout of the *E4f1* gene demonstrated that *E4f1* deletion impaired Sertoli cell proliferation, resulting in increased apoptosis of germ cells, which, in turn, reduced testis size and fertility (*8*). A zebrafish model also revealed that E4f1 acts as a positive regulator in tail development and coordinates with Wnt signaling to regulate the expression of *cdx4*, a key gene in caudal tissue specification and erythropoiesis (*9*). In addition, E4F1 plays a role in maintaining the characteristics of human embryonic stem cells, and its transcripts are properly spliced by the spliceosome-associated factor SON (*10*). Overall, E4F1 is a key regulator of cell life and embryonic development. This review summarizes the protein domains, interacting partners, evolution, and conservation of E4F1 and discusses the molecular mechanisms of E4F1 transcriptional regulation, its various biological functions, and its important role in various human diseases.

E4F1 PROTEIN DOMAINS

The *E4F1* gene encodes a full-length protein consisting of 784 amino acids, which contains nine ZF motifs (C_2H_2 or C_2HC). The N-terminal region of E4F1, known as $p50^{E4F}$ (amino acids 1 to 357), contains a ubiquitin E3 ligase active (E3) region (amino acids 41 to 85) that has been found to play a crucial role in promoting the formation of polyubiquitin chains and E4F1-mediated ubiquitination of p53 tumor protein (p53) both in vitro and in vivo. Although E4F1 has been shown to act as a ubiquitin ligase to regulate p53 effector functions, it does not affect its degradation (*11*). The $p50^{E4F}$ fragment also contains a DNA binding domain (amino acids 194 to 253) composed of two C_2H_2 and one C_2HC ZF motifs that are essential for E4F1-DNA binding (*12*). The remaining six ZF motifs of E4F1 are located close to the C-terminal region of the full-length protein (amino acids 437 to 600) (*13*) (Fig. 1A).

Numerous proteins have been reported to interact with E4F1, and using E4F1 truncations, specific binding regions on E4F1 have been validated (Fig. 1B) (*13*–*25*). Figure 1B illustrates that the binding of E4F1 to these proteins is dependent on multiple regions within the full-length E4F1, including the C-terminal fragment that excludes $p50^{E4F}$, indicating a different role of $p120^{E4F}$ in controlling diverse biological functions. The proteins whose binding regions with E4F1 are located at the N-terminal containing DNA binding domain can affect (inhibit or enhance) the transcriptional activity of E4F1 (*14*, *18*, *20*–*22*). Furthermore, proteins with overlapping binding regions on E4F1 may influence their ability to bind to E4F1, but limited research has been conducted on this aspect. Further exploration is needed to clarify the effects of proteins with overlapping regions on their ability to bind E4F1 to each other (Fig. 1B). Subsequent paragraphs will discuss the specific effects and functions of these proteins after binding to E4F1.

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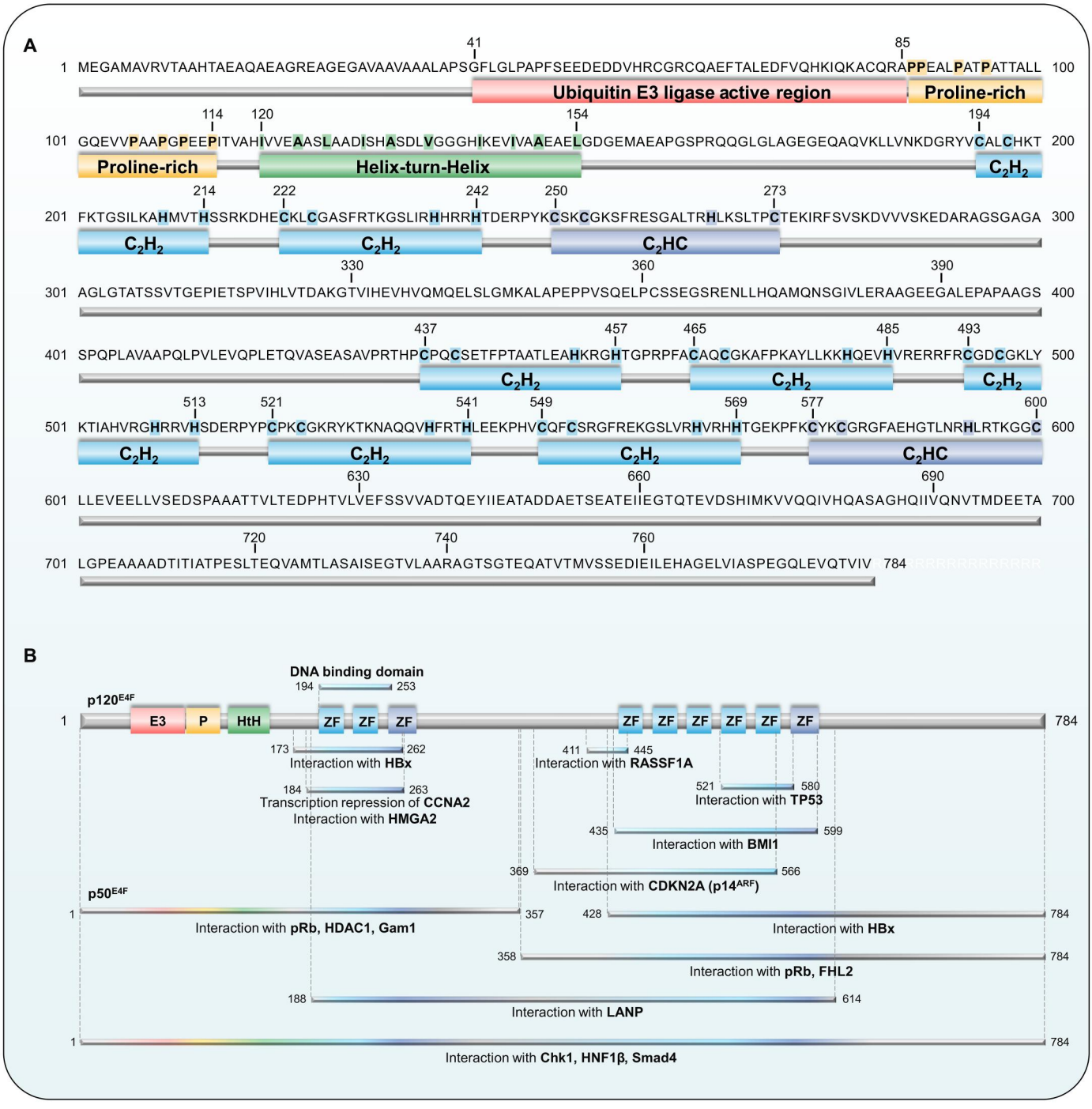


Fig. 1. Schematic structure of E4F1 protein. (**A**) Structural motif characteristics of p120^{E4F} full-length amino acids (1 to 784). The ubiquitin E3 region and the proline-rich (P) region are located at residues 41 to 85 and 86 to 114, respectively, with the proline residues highlighted in yellow. A potential Helix-turn-Helix (HtH) motif lies at residues 120 to 154, where hydrophobic residues in the form of "4-3" repeat are highlighted in green. The ZF motifs span residues 194 to 273 and 437 to 600, consisting of seven C_2H_2 and two C_2HC zinc finger motifs. The conserved cysteines (C) and histidines (H) required for zinc coordination are highlighted in blue or purple. (**B**) Proteins that interact with E4F1 and their specific binding regions. The regions in (B) correspond to the regions of the same color as in (A). Proteins with unknown E4F1 binding regions are drawn as the same length as p120^{E4F}. TP53, tumor protein p53; CDKN2A, cyclin-dependent kinase inhibitor 2A.

In addition, the E4F1 protein sequences from six classes of chordates (Mammalia, Reptilia, Aves, Amphibia, Osteichthyes, and Chondrichthyes) were aligned and analyzed, and a circle tree was constructed to illustrate the potential evolutionary history and routes of E4F1 among the 107 species of chordates (Fig. 2A). Subsequently, the amino acid sequences of the E3 region and ZF motifs on E4F1 protein were compared on the basis of 19 randomly selected species (mostly Mammalia) from the six classes of chordates. The comparison indicated that the E3 region exhibits high conservation in Mammalia, but not as much across chordate classes, particularly for residues whose mutation may be essential for the ubiquitin E3 ligase activity of E4F1 on p53 (Fig. 2B). This suggests that E4F1-mediated ubiquitination of p53 may be limited to a few species of chordates, mainly Mammalia. Importantly, the ZF motifs of E4F1 were found to be highly conserved among chordates, especially in the region containing the DNA binding domain (amino acids 194 to 253), suggesting that the transcriptional regulatory role of E4F1 may be ubiquitous in chordates (Fig. 2B).

E4F1 IS A TRANSCRIPTIONAL REGULATOR

An initial study on the E4F1 protein as an intracellular transcriptional regulator reported that both the full-length $p120^{E4F}$ and the hydrolysis product $p50^{E4F}$ are regulated by the adenovirus protein E1A. E1A induces their phosphorylation, which increases the DNA binding activity of $p50^{E4F}$ but reduces that of $p120^{E4F}$ (*3*). In vitro, both forms of E4F1 recognize the same DNA motif [RTGACGT(C/A)AY]. However, in vivo, $p120^{E4F}$ has additional C-terminal residues compared with $p50^{E4F}$ and exhibits different functions and regulatory characteristics. Specifically, $p50^{E4F}$ transactivates the adenovirus *E4* gene in the presence of E1A, whereas $p120^{E4F}$ represses the *E4* promoter transcription in the absence of E1A (Fig. 3A) (*5*, *26*).

On the basis of validation of the transcriptional activation or repression properties of E4F1, further studies have revealed regulators of its transcriptional activity. $p120^{E4F}$ interacts with the retinoblastoma-associated protein (pRb), which negatively controls the cell cycle and is a crucial modulator connecting growth processes to the transcriptional regulation of several genes (*27*–*32*). This interaction enhances the DNA binding activity of E4F1, resulting in transcriptional repression of its target genes (*18*). $p120^{E4F}$ also forms a complex with the leucine-rich acidic nuclear protein (LANP), which inhibits histone acetylation and transcription and interacts with ataxin-1 (*33*–*35*). This complex results in increased binding of $p120^{E4F}$ to the promoters of target genes. Notably, ataxin-1 protein can compete with $p120^{E4F}$ for LANP, reducing the LANP-$p120^{E4F}$ complex formation and consequently weakening the transcriptional repression mediated by $p120^{E4F}$ (*21*). In addition, histone deacetylase 1 (HDAC1) forms a complex with $p120^{E4F}$, enhancing its transcriptional repressive activity. However, the avian adenoviral chicken embryo lethal orphan (CELO) protein, Gam1, responsible for viral replication and transcription activation (*36*–*38*), can inactivate HDAC1 by forming Gam1-HDAC1-$p120^{E4F}$ complexes, leading to impaired transcriptional repression by $p120^{E4F}$ (*20*).

To date, multiple studies have provided strong evidence that $p120^{E4F}$ functions as a crucial transcriptional regulator during the mammalian cell cycle, with its binding to the promoter of *CCNA2*, a gene responsible for cell cycle progression through the production of cyclin A2 protein (*39*, *40*), resulting in the repression of cyclin A2 expression (*14*, *41*, *42*). Interestingly, several proteins have been identified as capable of modulating $p120^{E4F}$-mediated transcriptional repression of *CCNA2*. For instance, RASSF1A (ras association domain family member 1A), a tumor suppressor and cell cycle regulator found in many human cancers (*43*–*46*), has been shown to promote the interaction between $p120^{E4F}$ and the *CCNA2* promoter (*42*). In contrast, the binding of $p120^{E4F}$ to the *CCNA2* promoter can be impaired by its interaction with HMGA2 (high mobility group AT-hook 2), an architectural transcription factor that promotes cell proliferation and tumorigenesis (*47*–*49*). This impairment of $p120^{E4F}$'s binding ability leads to a decrease in its transcriptional repressive effect on *CCNA2*, which, in turn, promotes the transcriptional activation of *CCNA2* mediated by the activating transcription factor/adenosine 3′,5′-monophosphate response element–binding protein family members (*14*, *50*, *51*). Figure 3B presents the transcriptional regulatory profile of the $p120^{E4F}$-dependent *CCNA2* gene, which has been fully validated on the basis of the aforementioned studies.

Chromatin immunoprecipitation sequencing (ChIP-seq) analyses have revealed multiple potential target genes controlled by E4F1, indicating its extensive involvement in various biological processes. E4F1 acts as a transcriptional activator of multiple related genes involved in genome integrity, mitochondrial homeostasis, and energy metabolism, which are discussed in detail in the following sections on "DNA damage response" (DDR) and "cell metabolism" (*23*, *52*, *53*).

Currently, many investigations have focused on the transcriptional repression function of E4F1 on *CCNA2*. However, advanced transcription–related technologies such as ChIP, CUT&RUN, and CUT&Tag should be used to explore other direct targets beyond *CCNA2*. Although mainly characterized as a transcriptional repressor, recent studies have demonstrated that E4F1 can also serve as a transcriptional activator. Furthermore, elucidating the mechanism by which E4F1 plays these divergent roles remains a critical outstanding question.

BIOLOGICAL FUNCTIONS OF E4F1

E4F1 has been reported to participate in multiple cellular biological functions, with the most extensively studied functions being cell growth and proliferation. Furthermore, E4F1 has been implicated in cell differentiation, apoptosis and necrosis, DDR, and cell metabolism.

E4F1 in cell growth and proliferation

Numerous studies have established that E4F1 is a critical regulator of cell growth and proliferation. Validation experiments have shown that the ectopic expression of $p120^{E4F}$, but not $p50^{E4F}$, inhibits cell growth by blocking the cell cycle in the G_1 phase (Fig. 4) (*13*, *15*, *16*, *18*, *41*, *54*). Mechanistically, $p120^{E4F}$ can inhibit the transcription of the cell cycle–regulating gene *CCNA2*, resulting in lower levels of cyclin A2, and subsequent cell cycle arrest in G_1 (*41*). In addition, binding of pRb, RASSF1A, or p53 to $p120^{E4F}$ inhibits the progression from G_1 to S phase, which eventually leads to cell growth arrest (*13*, *15*, *18*, *42*). Notably, a study unexpectedly revealed that p53 is indispensable for $p120^{E4F}$-mediated growth arrest (*13*). However, there are opposing results that suggest $p120^{E4F}$ negatively regulates cell proliferation through p53-independent pathways (*18*, *54*). It is

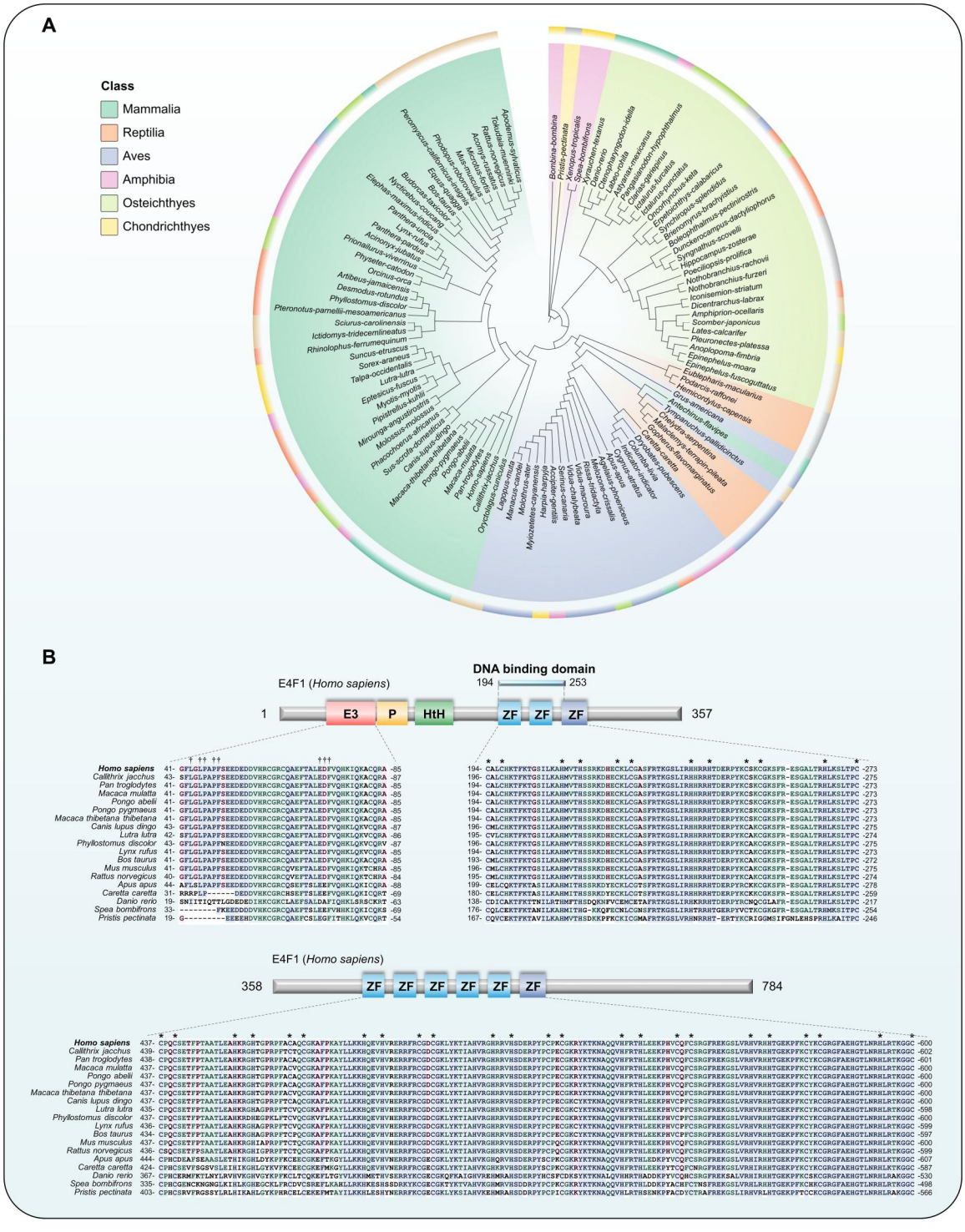


Fig. 2. Evolutionary analysis of E4F1 and protein sequence comparison among different species. (**A**) Phylogenetic tree of E4F1 for 107 species of chordates. The corresponding protein sequences searched from the National Center for Biotechnology Information database were aligned by the ClustalW method, and the circle tree was generated through neighbor-joining analysis with 1000 bootstrap replications. The 107 species of chordates belong to six classes (Mammalia, Reptilia, Aves, Amphibia, Osteichthyes, and Chondrichthyes), presented by different colors in the inner circle, and 37 orders are shown by colors in the outer circle. (**B**) Protein sequence comparison of the ubiquitin E3 region and the ZF motifs of E4F1 among different species. Residues that may play an essential role in E4F1 ubiquitin E3 ligase activity on p53 are denoted by † (*11*). The ZF motifs, including C_2H_2 and C_2HC, are denoted by *.

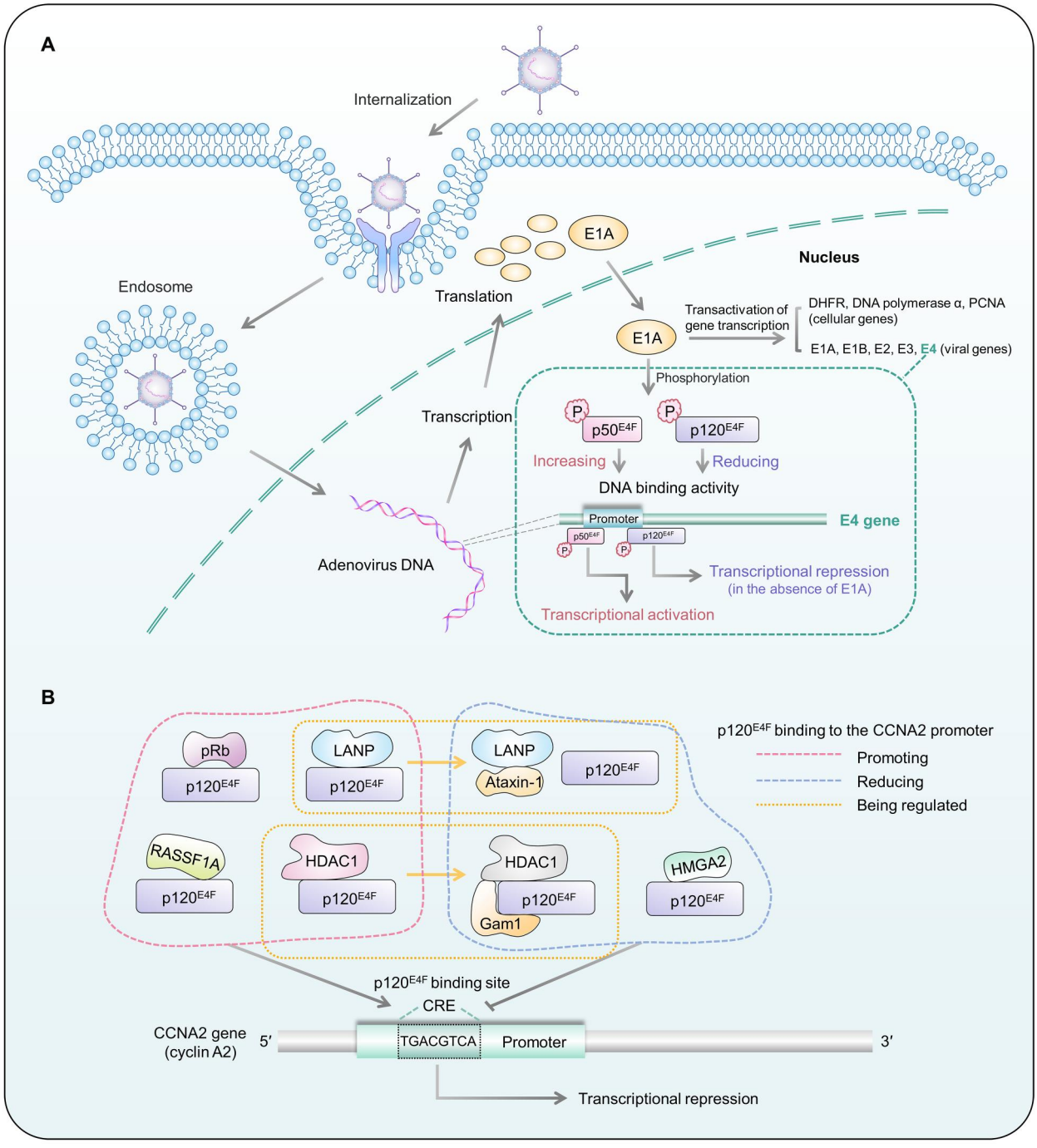


Fig. 3. Schematic diagram of E4F1 transcriptional regulation. (**A**) Upon adenovirus infection, the expression of host cell gene *E4F1* is markedly elevated alongside the expression of viral gene *E1A* via transcription and translation (*5*, *129*, *130*). E1A protein phosphorylates p50^{E4F}, promoting its binding to sequences in the *E4* promoter and activating adenovirus *E4* gene transcription (*4*, *5*, *131*). However, p120^{E4F}, which can repress adenovirus *E4* gene transcription in the absence of E1A, experiences a reduction in its DNA binding activity due to E1A-induced phosphorylation. (**B**) The binding of p120^{E4F} to the promoter region of *CCNA2*, which involves the CRE element (TGACGTCA) (*50*, *132*), is enhanced by hypophosphorylated pRb, LANP, RASSF1A, and HDAC1, thereby promoting p120^{E4F}-dependent transcriptional repression of the *CCNA2* gene. Conversely, ataxin-1 protein reduces the formation of LANP-E4F1 complexes by competing with p120^{E4F} for LANP. In addition, the Gam1 protein inactivates HDAC1 by forming Gam1-E4F1-HDAC1 complexes. Furthermore, HMGA2 interferes with p120^{E4F} binding to the CRE element. Therefore, ataxin-1, Gam1, and HMGA2 relieve the transcriptional repression of *CCNA2* by reducing p120^{E4F} binding to the *CCNA2* CRE site. DHFR, dihydrofolate reductase; PCNA, proliferating cell nuclear antigen; CRE, adenosine 3′,5′-monophosphate response element.

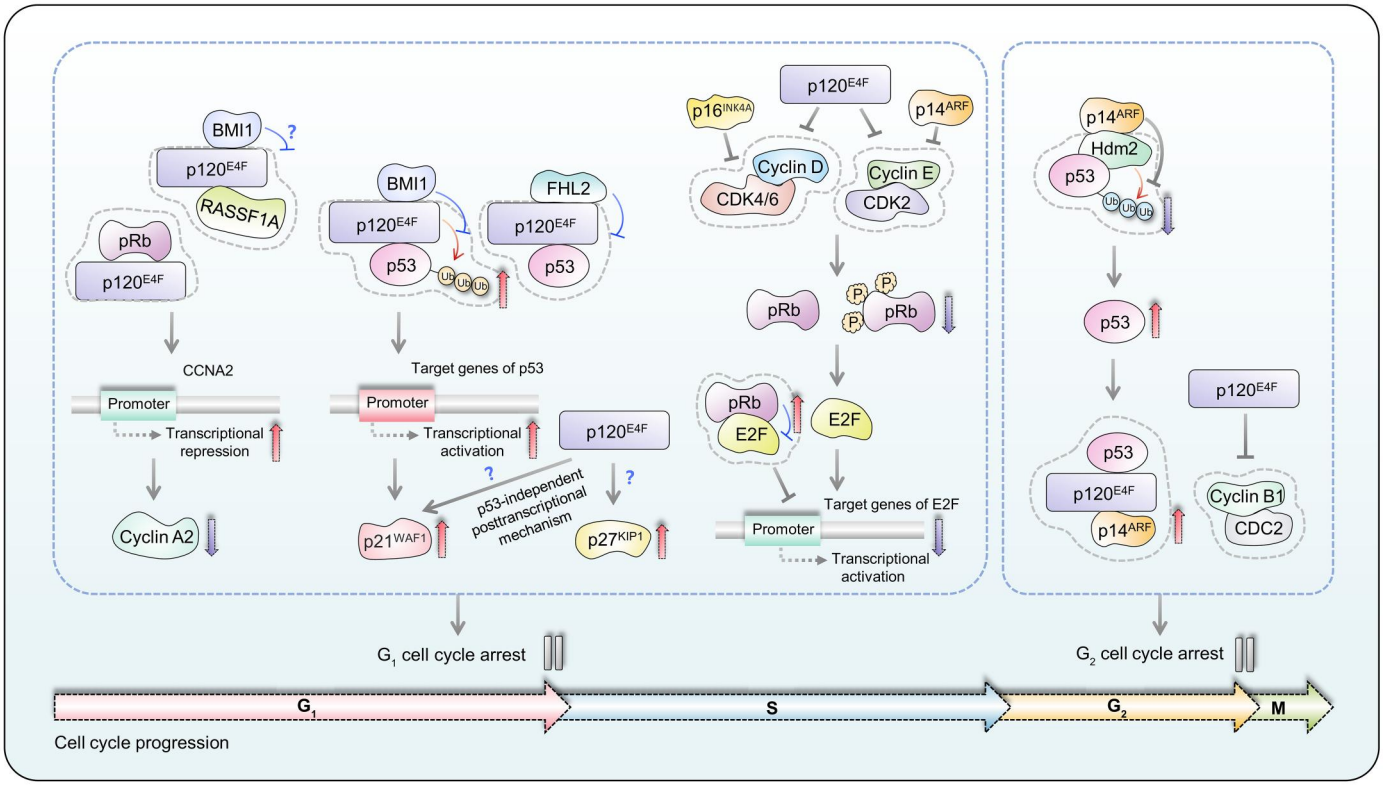


Fig. 4. Molecular mechanisms of p120^{E4F}-induced cell cycle arrest. The G_1 cell cycle arrest induced by p120^{E4F} is mainly mediated by multiple pathways. First, p120^{E4F}-pRb or p120^{E4F}-RASSF1A interaction can enhance p120^{E4F}-dependent transcriptional repression of the *CCNA2* gene, resulting in the downregulation of cyclin A2 protein. Second, p120^{E4F} can induce an atypical ubiquitination of p53 (independent of degradation), triggering a p53-dependent transcriptional program including cell cycle arrest–related genes such as *p21^{WAF1}*. In addition, the p120^{E4F}-FHL2 interaction can reduce nuclear p120^{E4F}-p53 complexes, relieving the effect of p120^{E4F}-induced G_1 cell cycle arrest. Moreover, overexpression of p120^{E4F} leads to the up-regulation of p21^{WAF1} and p27^{KIP1} protein levels, and the effect on p21^{WAF1} occurs through an unknown posttranscriptional regulation independent of p53. Last, p120^{E4F} can inhibit the activity of CDK4/6 and CDK2 kinases, leading to an increase in nonphosphorylated pRb that enhances the binding of pRb and E2F. The regulation suppresses the transcriptional activation of E2F-targeted genes, which promotes the transition from G_1 to S phase of the cell cycle. Interestingly, the pathways of G_2 cell cycle arrest mediated by p120^{E4F} have also been reported. The formation of a ternary complex (p53-p120^{E4F}-p14^{ARF}) enhances the inhibition of G_2-M transition induced by p14^{ARF}, while p14^{ARF} stabilizes p53 protein by inactivating Hdm2 (ubiquitin E3 ligase of p53) and promoting the nucleolar import of Hdm2. Furthermore, p120^{E4F} can induce G_2 cell cycle arrest by reducing CDC2 kinase activity. p21^{WAF1}, CDK inhibitor 1A; p27^{KIP1}, CDK inhibitor 1B; p16^{INK4A}, CDK inhibitor 2A; p14^{ARF}, tumor suppressor ARF; CDC2, cell division control protein 2 homolog.

also interesting to note that p120^{E4F} can induce atypical ubiquitylation of p53, which leads to increased recruitment of p53 to the promoter of *p21^{WAF1}*, a gene associated with cell cycle inhibition and directly controlled by p53 (*55*), eventually resulting in G_1 to S cell cycle arrest (*11*). Furthermore, the LIM-only protein FHL2 (four and a half LIM domains 2), which is a coactivator or corepressor of several transcription factors (*56*–*63*), can interact directly with the full-length protein p120^{E4F} rather than p50^{E4F} in the nucleus. This binding reduces the nuclear p120^{E4F}-p53 complexes and inhibits the ability of p120^{E4F} to block cell cycle in the G_1 phase. (*19*). p120^{E4F} was also found to interact with the polycomb complex protein BMI-1 (BMI1), which is a major component of the polycomb group complex 1 that is involved in the proliferation and self-renewal of hematopoietic stem cells (*64*–*67*). *p120^{E4F}* knockdown can rescue the growth ability of BMI1-deficient hematopoietic cells. However, the mechanism by which BMI1 regulates p120^{E4F} in cell proliferation remains incompletely understood. The authors postulate that BMI1 might inhibit the atypical ubiquitin E3 ligase activity or impair the interaction of p120^{E4F} with microtubule-associated proteins such as RASSF1A to ensure normal chromosome separation and cell cycle progression (*17*).

Interestingly, it has been reported that the overexpression of p120^{E4F} leads to the up-regulation of p21^{WAF1} levels through posttranscriptional regulation, independent of p53. This overexpression also results in the up-regulation of the cyclin-dependent kinase inhibitor p27^{KIP1} and reduced activity of cyclin-dependent kinase 2 (CDK2) and CDK4/6 kinases. These kinases play a crucial role in regulating the G_1-S transition (*68*–*70*). Consequently, overexpression of p120^{E4F} leads to the suppression of genes promoting G_1-S transition that are regulated by E2F, a family of multiple members that directly control the transcriptional regulation of several genes involved in cell cycle progression (*54*, *71*–*74*).

A role for p120^{E4F} in G_2-M arrest has been revealed, similar to its regulatory function in the G_1-S transition (Fig. 4). The tumor suppressor p14^{ARF} can interact with Hdm2, a ubiquitin ligase that negatively regulates p53 (*75*, *76*). Through inactivating Hdm2 and inducing nucleolar import of the p14^{ARF}-Hdm2 complexes, p14^{ARF} facilitates the stabilization of p53 levels (*77*–*80*). p120^{E4F} interacts with p14^{ARF} and p53, forming ternary complexes with these

partners, resulting in the enhancement of $p14^{ARF}$-induced G_2 block in a p53-dependent manner (*16*). In addition, CDC2 is a critical kinase that promotes the G_2-M transition of the cell cycle by forming a complex with cyclin B1. $p120^{E4F}$ can induce G_2-M cell cycle arrest by inhibiting the activity of CDC2 kinase (*71*, *81*, *82*).

In conclusion, E4F1 appears to have a role in cell cycle regulation and cell growth. This section clearly demonstrated its ability to arrest the cell cycle, particularly in the G_1-S transition and also in the G_2 phase. However, the E4F1-related pathways depicted in Fig. 4 require further research to better understand how its scattered interacting partners form a precise regulatory network.

E4F1 in cell differentiation

In addition to the key role of E4F1 in cell growth and proliferation, an interesting report has demonstrated the involvement of $p120^{E4F}$ in cell differentiation, specifically in inducing osteoblastic differentiation through the formation of $p120^{E4F}$-Smad4 complexes. Smad4 is a crucial downstream protein for bone morphogenetic protein signaling (*83*, *84*). These findings highlight the need for additional studies on E4F1, with a focus on its potential significance in muscle development and regeneration in vivo (Fig. 5A) (*25*).

E4F1 in cell death

Beyond its role in cell growth and differentiation, E4F1 can act as a cell death regulator in both $p50^{E4F}$ and $p120^{E4F}$. Cells overexpressing $p50^{E4F}$ and exposed to E1A become sensitive to cell death signals, but the mechanisms of $p50^{E4F}$-induced apoptosis and necrosis require further investigation (*85*). Interestingly, $p120^{E4F}$ expression is inhibited by miR-33-3p up-regulation induced by selenium deficiency in vein endothelial cells, resulting in apoptosis. This study provides valuable insight into our understanding of vascular diseases (Fig. 5B) (*86*).

E4F1 in DDR

The DDR recruits key factors to DNA breaks and involves the intricate interactions of numerous DNA repair proteins. DNA repair relies on poly[adenosine 5′-diphosphate(ADP)–ribose] polymerase 1 (PARP-1), a member of the PARP protein family that is responsible for sensing DNA lesions and facilitating subsequent DNA repair (*87*–*91*). In addition, checkpoint kinase 1 (Chk1) is a DNA damage checkpoint protein that plays a crucial role in maintaining genomic stability and has been widely studied (*92*–*96*).

Several studies have shed light on the mechanisms involved in how E4F1 can orchestrate the DDR process with several proteins. Through poly(ADP-ribose) (PAR), $p120^{E4F}$ interacts with PARP-1 and is subsequently recruited to DNA breaks, facilitating ATR (ATR serine/threonine kinase)–CHK1 signaling and DNA repair, including DNA-end resection and homologous recombination (*97*). Similar to this, $p120^{E4F}$ was found to interact with SMARCA4, the catalytic subunit of the chromatin remodeling switch/sucrose non-fermentable (SWI/SNF) complex, promoting its recruitment, along with PARP-1, to DNA lesions. This emphasizes the important role of E4F1 in repairing DNA double-strand breaks, maintaining genomic integrity, and supporting cell survival (*97*). Moreover, $p120^{E4F}$ can also act as a transcriptional activator of *CHK1* and stabilize Chk1 protein levels by reducing its degradation in response to DDR. This leads to increased genomic stability by up-regulating Chk1 expression (Fig. 5C) (*23*, *98*).

E4F1 in cell metabolism

The roles of E4F1 in cell metabolism have also been observed. Interestingly, $p120^{E4F}$ was first described as a transcriptional activator and regulates the expression of genes responsible for encoding mitochondrial proteins such as *Taz* (tafazzin), *Dnajc19* [DnaJ heat shock protein family (Hsp40) member C19], *Hax1* (HCLS1-associated protein X-1), *Mrpl15* (mitochondrial ribosomal protein L15), *Ndufs5* [NADH dehydrogenase (ubiquinone) iron-sulfur protein 5], *Dlat* (dihydrolipoamide *S*-acetyltransferase), *Brp44l* (brain protein 44-like), *Kifbp* (kinesin family binding protein), and *Pdpr* [pyruvate dehydrogenase (PDH) phosphatase regulatory subunit] in mouse embryonic fibroblasts. This transcriptional regulation directly controls mitochondrial homeostasis and metabolic functions (Fig. 5D). Specifically, deficiency of $p120^{E4F}$ can result in notable mitochondrial dysfunction characterized by elevated levels of reactive oxygen species (ROS) and energy stress (*23*).

Recent progress has shown that E4F1 plays a crucial role in pyruvate metabolism. E4F1 exerts its metabolic functions through transcriptional activation of genes essential for mitochondrial PDH activity, which is a mediator of pyruvate oxidation that fuels the tricarboxylic acid cycle (Fig. 5D). *E4F1* knockout leads to a notable decrease in PDH activity and severely disrupts pyruvate metabolism (*53*). Another study has emphasized the critical role of E4F1 in maintaining skin homeostasis, which depends on its transcriptional regulation of genes controlling PDH activity (*99*).

E4F1 IS IMPLICATED IN VARIOUS DISEASES

Abnormal E4F1 expression contributes to various diseases by disrupting cellular functions. Given that E4F1-related cancer is more extensively reported compared to other specific disease categories, we divide E4F1-related disorders into cancer and other diseases for discussion in this section. Table 1 summarizes E4F1-related regulations in these diseases according to major human body systems, presenting the corresponding organs, diseases, regulating axes, and effects or functions in an orderly manner.

Cancer

E4F1 has been investigated in the context of hepatitis B virus (HBV) infection and HBV-related liver cancer. Specifically, through its binding to the HBV enhancer II region, $p120^{E4F}$ exerts transcriptional repression on HBV (*22*). However, the binding of $p120^{E4F}$ and HBV X protein (HBx), a multifunctional regulator that modulates viral replication and hepatocyte functions (*100*–*105*), can reduce $p120^{E4F}$'s transcriptional repressive activity, leading to activated HBV transcription and thereby ensuring virus DNA replication and survival (*22*). Interestingly, another study suggested that $p120^{E4F}$ may have oncogenic properties in HBV-mediated carcinogenesis by inhibiting HBx-induced autophagy and maintaining mitochondrial function and proliferation in HBV-positive hepatocellular carcinoma (HCC) cells (*106*). Briefly, these studies suggest that the binding of HBx and $p120^{E4F}$ may impair their respective functions and play a role in HBV infection and HCC progression. Notably, recent research has demonstrated that $p120^{E4F}$ promotes invasion, migration, and epithelial-mesenchymal transition of HCC cells by transcriptionally activating high mobility group box 1 (HMGB1), an oncoprotein that facilitates HCC progression (*107*–*110*), in response to angiotensin II stimulation (*111*).

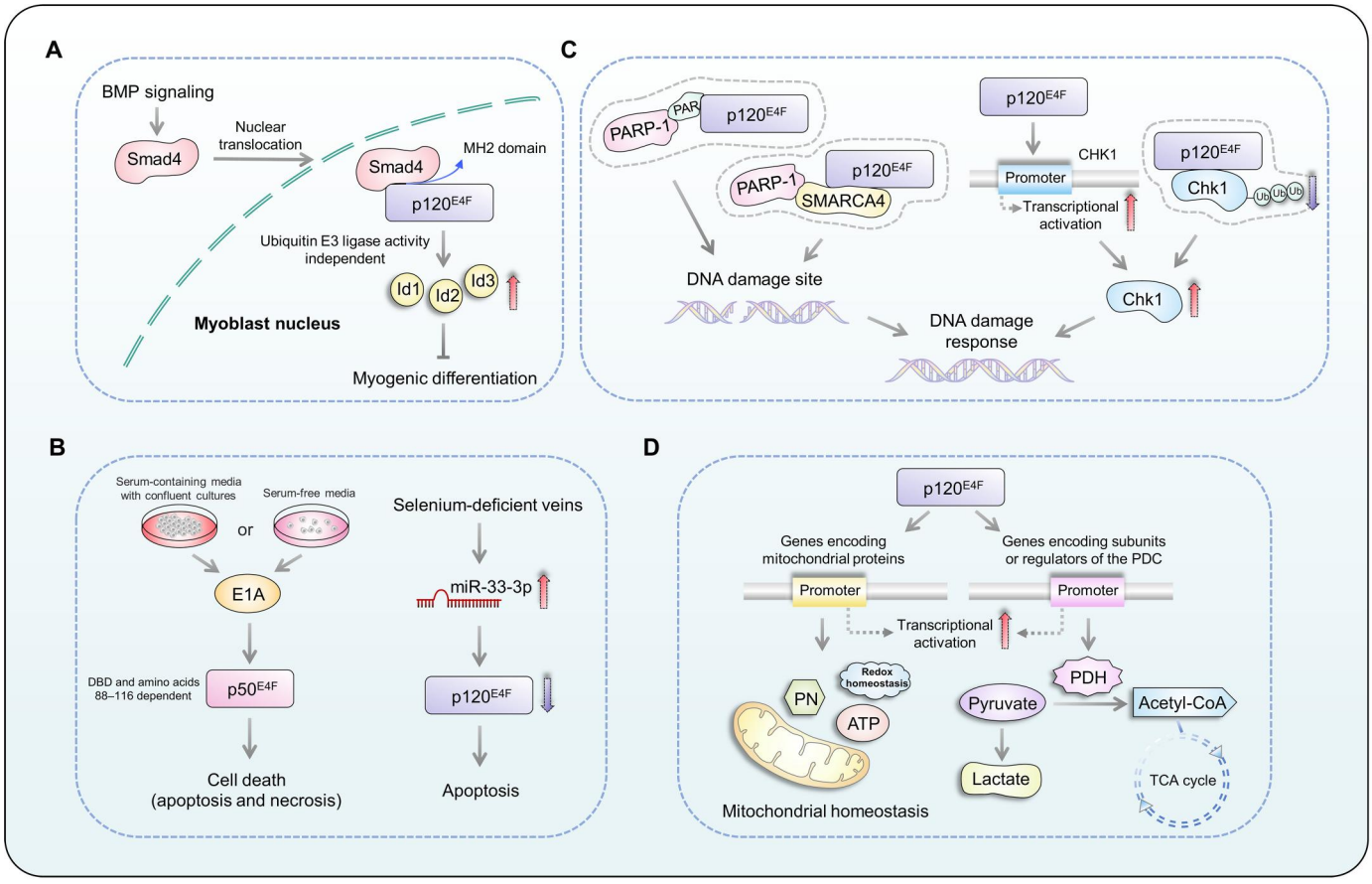


Fig. 5. Molecular mechanisms of E4F1 in various cell biological processes. (**A**) p120^{E4F} collaborates with Smad4 to inhibit myogenic differentiation in myoblasts. Upon bone morphogenetic protein (BMP) signaling, Smad4 translocates into the nucleus where it may bind to p120^{E4F} via its Mad homology 2 (MH2) domain. Subsequently, p120^{E4F} promotes the expression of inhibitor of differentiation or inhibitor of DNA binding (Ids), leading to the inhibition of myogenic differentiation. (**B**) E4F1 regulates cell death. p50^{E4F} sensitizes E1A-expressing cells to death signals such as confluent cultures and serum starvation, inducing cell death. Moreover, miR-33-3p up-regulation by selenium deficiency leads to the inhibition of p120^{E4F} expression, resulting in apoptosis of vein endothelial cells. (**C**) p120^{E4F} plays a key role in response to DNA damage. p120^{E4F} indirectly binds to PARP-1 through PAR and is recruited by PARP-1 to sites of DNA damage. Furthermore, SMARCA4 is rapidly recruited to DNA lesions in a p120^{E4F} and PARP-1–dependent manner to promote DNA damage repair. p120^{E4F} activates *CHK1* transcription and reduces the polyubiquitination of Chk1 to protect it from degradation, thereby up-regulating Chk1 levels in response to DNA breaks. (**D**) p120^{E4F} controls transcriptional programs essential for cell metabolism. p120^{E4F} activates the transcription of genes encoding mitochondria-associated proteins, regulating mitochondrial homeostasis. In addition, p120^{E4F} acts as a transcriptional activator for genes encoding subunits or regulators of the pyruvate dehydrogenase complex (PDC), which maintains PDH activity, the key enzyme in the oxidative decarboxylation of pyruvate to acetyl–coenzyme A (CoA), thus fueling the tricarboxylic acid (TCA) cycle. DBD, DNA binding domain; PN, pyrimidine nucleotide.

A recent study found that *TOB1-AS1* (TOB1 antisense RNA 1), a long noncoding RNA gene that exerts tumor suppressor activity (*112*–*115*), modulates p120^{E4F} in non–small cell lung cancer (NSCLC). Patients with NSCLC with high expression of p120^{E4F} were found to have a longer overall survival time, suggesting that p120^{E4F}, which is up-regulated by *TOB1-AS1*, is an essential regulator in preventing the progression of NSCLC (*116*). However, high expression of p120^{E4F} was observed in most acute myeloid leukemic cells. Its inactivation induced mitochondrial dysfunction and increased ROS in this cancer, leading to autophagic cell death, indicating that p120^{E4F} plays a positive role in regulating the progression of acute myeloid leukemia (*117*, *118*).

A study on triple-negative breast cancer (TNBC) used ChIP-seq and RNA sequencing analyses to reveal three targets, namely, *CHK1*, protein phosphatase 5 catalytic subunit (*PPP5C*), and TELO2-interacting protein 2 (*TTI2*), that are directly controlled by p120^{E4F} and are involved in ATM (ATM serine/threonine kinase)/ATR–Chk1 signaling, which drives the response to DDR (*119*). Previous studies also supported the role of p120^{E4F} in DDR through the regulation of *CHK1* (*97*, *98*). Depletion of p120^{E4F} leads to reduced DDR and chemotherapy resistance, providing insight into the mechanisms of TNBC development, driven in part by p120^{E4F}-mediated activation of the ATM/ATR-Chk1 pathway (*119*).

As outlined above, studies on E4F1 in human cancers are still limited. To expand our understanding of this topic, we used large tumor-related databases to analyze *E4F1* gene alterations, including mutation, amplification, deep deletion, and structural variant. The analysis of *E4F1* gene alterations in all cancer types was carried out using cBioPortal on the The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC) databases. According to the findings, invasive breast carcinoma has the highest

Table 1. Regulatory pathways and roles of E4F1 in human body systems. ALKBH5, AlkB homolog 5 (RNA demethylase); Ang-II, angiotensin II; EMT, epithelial-mesenchymal transition; ESC, epidermal stem cell; INK4a/ARF, cyclin-dependent kinase inhibitor 2A; KDM4A, lysine demethylase 4A; OS, overall survival; Ref., reference; SCD1, steaoryl–coenzyme A desaturase 1; ↑increase or promote; ↓decrease.

System	Organ/tissue	Disease/ development	$p120^{E4F}$			
			Upstream	Direct target	Regulatory axis	Effect/function
Digestive system	Liver	Hepatitis B (*22*, *133*)	N/A	Binding to the HBV enhancer II region	$p120^{E4F}$ → HBV enhancer II region → genes encoding the HBx protein and surface proteins I/II	HBV transcription↓
			Interaction with HBx protein	N/A	HBx → $p120^{E4F}$	The HBx inhibitory effect on the transcriptional function of $p120^{E4F}$
		HCC (*106*) (HBV positive)	N/A	Binding to HBx protein	$p120^{E4F}$ → HBx → p53	Metabolic and growth arrest↓
		HCC (*111*)	Stimulated by Ang-II	Transcriptional activation of *HMGB1* gene	Ang-II → $p120^{E4F}$→ HMGB1	HCC cell invasion, migration, and EMT↑
Respiratory system	Lung	NSCLC (*116*)	*TOB1-AS1*	N/A	*TOB1-AS1* → $p120^{E4F}$	The expression of $p120^{E4F}$ is positively correlated with *TOB1-AS1* and associated with a higher OS rate
Urinary system	Kidney	Nephrogenesis (*24*)	N/A	Binding to HNF1β protein	N/A	Pronephros abnormality↑(overexpression of $p120^{E4F}$)
Circulatory system	Blood vessel	Atherosclerosis (*122*)	Estrogen	N/A	Estrogen → estrogen receptor → $p120^{E4F}$	Estrogen-related atheroprotection↑
	Bone marrow	Myeloid leukemia (*117*, *118*)	N/A	N/A	$p120^{E4F}$ → mitochondria→ROS	Cell death (autophagy)↓tumor development↑
Reproductive system	Breast	TNBC (*119*)	N/A	Transcriptional activation of *CHK1*, *PPP5C*, and *TTI2* genes, etc.	$p120^{E4F}$ → ATM/ ATR→ Chk1	DNA damage-stress response↑chemotherapy sensitivity↓
Endocrine system	Adipose tissue	Obesity andinsulin resistance (*124*)	N/A	Binding to p53 protein	$p120^{E4F}$ → p53 → SCD1	Maintenance of lipid metabolisminsulin sensitivity↑
Locomotor system	Fibrocartilage	Intervertebral disc degeneration (*126*)	DNMT3B	N/A	KDM4A → ALKBH5→ DNMT3B → $p120^{E4F}$	Senescence of nucleus pulposus cells↓
	Bone	Bony defects (*127*)	N/A	N/A	N/A	Involvement in skeletal regulation
Others	Skin	Epidermal defects (*128*)	N/A	N/A	$p120^{E4F}$ → BMI1→INK4a/ ARF → p53	ESC-dependent maintenance of skin homeostasis
	–	Mitochondrial disorders (*23*, *125*)	N/A	Transcriptional activation of *DNAJC19*, *DLAT*, *PDPR*, and *CHK1*	$p120^{E4F}$ → genes encoding mitochondria-related proteins	Maintenance of mitochondrial metabolism

alteration frequency of the *E4F1* gene (6.62%) among all cancers, with amplification being the main type of alteration (5.88%). In addition, mutation and amplification were found to be the main types of *E4F1* gene alterations in all cancers (Fig. 6A). The amino acid mutations of the full-length E4F1 protein were also analyzed using cBioPortal based on the same studies. The mutation sites are widely distributed throughout the full-length E4F1 protein, with missense being the main mutation type and R167 being the most commonly mutated site (Fig. 6B). Moreover, the somatic mutation frequency in E4F1 is 0.2%. However, studies on E4F1 mutations at these sites have yet to be reported.

In addition to analyzing the genomic alteration of *E4F1*, we investigated its potential impact on cancer prognosis (overall survival time) across 33 cancer types using the TCGA database. Using the

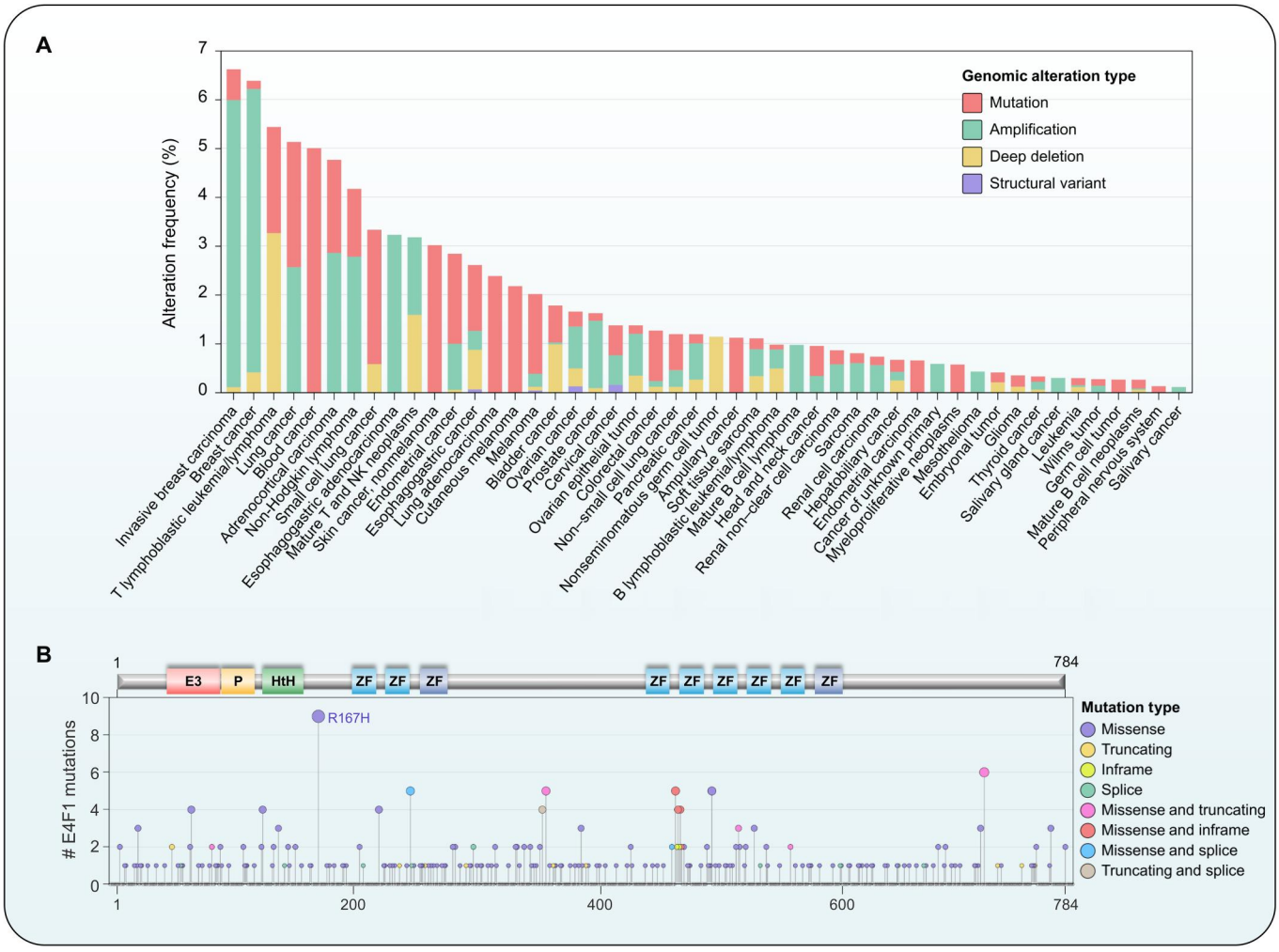


Fig. 6. Genomic alteration of E4F1. (**A**) A stacked bar plot of the genomic alteration (mutation, amplification, deep deletion, and structural variant) of *E4F1* across various cancer types. The analysis of data was carried out using cBioPortal and included 172,970 patients (182 cancer types) in 351 studies from the TCGA and the ICGC databases. The plot includes cancer types with total cases of >20 and alteration frequencies of >0.01%. (**B**) A lollipop plot of the amino acid mutations of the full-length E4F1 protein. The mutations were analyzed using the same studies as in (A) by cBioPortal. The somatic mutation frequency in E4F1, measured as the percentage of samples with somatic mutations, is 0.2%. Mutation diagram circles are colored with respect to the corresponding mutation types. NK, natural killer.

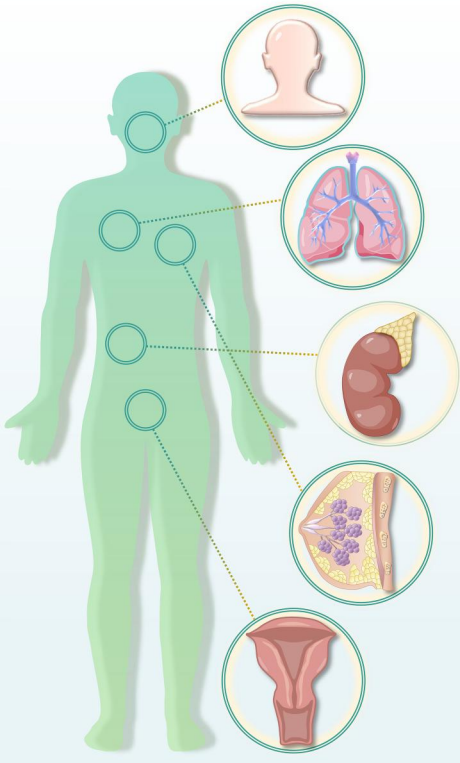
online tool TIMER2.0, we observed that E4F1 may affect the prognosis of various cancer types, including head and neck squamous cell carcinoma, lung squamous cell carcinoma, mesothelioma, adrenocortical carcinoma, kidney renal clear cell carcinoma, breast invasive carcinoma, cervical squamous cell carcinoma, and endocervical adenocarcinoma. Notably, there are no published or investigated studies on E4F1 in these human cancers (Fig. 7). These findings highlight the potential for E4F1 to act as either an inhibitor or a promoter in cancer development. Given the obvious differences in the impact of E4F1 on prognosis across certain cancers (Fig. 7), further research is warranted to elucidate the diverse functions and complex mechanisms of E4F1 in cancer, particularly within the cancer types shown in Fig. 7, to obtain a more comprehensive understanding of its opposing roles in different cancer types.

Other diseases

A study investigating proteins that interact with hepatocyte nuclear factor 1β (HNF1β), a protein responsible for congenital anomalies of the kidney and urinary tract (CAKUT) (*120*, *121*), has identified $p120^{E4F}$ as one of its interaction partners critical for nephrogenesis. Overexpressing $p120^{E4F}$ in embryos affects the size and morphology of the pronephros, resulting in kidney malformations. These findings suggest that *E4F1* could be considered a CAKUT gene (*24*).

E4F1 has also been reported to be implicated in vascular disease. Microarray analysis revealed that $p120^{E4F}$, which is involved in inhibiting the proliferation of vascular smooth muscle cells mediated by estrogen receptor α, may serve as an estrogen-responsive protector against human atherosclerosis (*122*).

In addition to the metabolic functions of E4F1 mentioned above, several studies have investigated its implications in various metabolic diseases. A meta-analysis demonstrated that $p120^{E4F}$ may play a crucial role in the progression of type 2 diabetes mellitus, indicating



Cancer	Number	E4F1 HR (*P* value)		
		5 years	10 years	15 years
HNSC	522	0.76 (***)	0.75 (****)	0.77 (***)
HNSC-HPV⁻	422	0.80 (**)	0.78 (**)	0.81 (**)
HNSC-HPV⁺	98	NS	0.66 (*)	N/A
LUSC	501	0.83 (**)	0.86 (*)	NS
MESO	87	1.32 (*)	1.54 (***)	N/A
ACC	79	2.22 (**)	1.78 (**)	1.79 (**)
KIRC	533	NS	1.17 (*)	1.17 (*)
BRCA	1100	0.83 (*)	NS	NS
BRCA-LumA	568	NS	NS	NS
BRCA-LumB	219	0.64 (*)	0.66 (*)	0.70 (*)
BRCA-Basal	191	NS	NS	NS
BRCA-Her2	82	NS	NS	NS
CESC	306	NS	NS	0.782 (*)

Fig. 7. The impact of E4F1 on cancer prognosis. These data were obtained from the TCGA database, wherein the relationships between the expression of *E4F1* gene and overall survival time (5, 10, and 15 years) of patients with cancer were analyzed using the online tool TIMER2.0. Among the 33 cancer types examined, the expression of *E4F1* gene affected disease prognosis in seven cancer types [head and neck squamous cell carcinoma (HNSC), lung squamous cell carcinoma (LUSC), mesothelioma (MESO), adrenocortical carcinoma (ACC), kidney renal clear cell carcinoma (KIRC), breast invasive carcinoma (BRCA), and cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC)]. The table highlights the cancer names marked in blue and red, which suggest negative [hazard ratio (HR) < 1, $P < 0.05$] and positive (HR > 1, $P < 0.05$) correlations, respectively, between *E4F1* gene expression and poor prognosis (shorter survival time) in patients with cancer. HPV, human papilloma virus; LumA/B, luminal A/B; Her2, human epidermal growth factor receptor 2; CESC, cervical squamous cell carcinoma and endocervical adenocarcinoma; NS, no statistical significance ($P > 0.05$); *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

p120^{E4F} as a potential therapeutic target for this chronic disease (*123*). Another study associated with obesity and insulin resistance revealed that p120^{E4F} regulates p53-dependent lipid metabolism in adipocytes, likely indicating an important role of p120^{E4F} in the development of diabetes (*124*). Moreover, homozygous mutations in *E4F1* have been found in several patients with mitochondrial disease, whereas proteins encoded by *E4F1* in mitochondria have not been reported (*125*). On the basis of previous findings (*23*) and quantitative polymerase chain reaction analysis, E4F1 was reconfirmed to positively regulate the transcriptional expression of several mitochondrial bioenergetics–related genes such as *DNAJC19*, *DLAT*, *PDPR*, and *CHK1*, which may offer insight into the mechanisms underlying E4F1 dysfunction–induced mitochondrial diseases (*125*). However, future studies will be needed to identify additional targets of E4F1 involved in mitochondrial and other metabolic disorders.

Notably, p120^{E4F} was found to be implicated in the regulation of fibrocartilage and bone. Reduced expression of E4F1, through *E4F1* promoter hypermethylation mediated by the DNA methyltransferase DNMT3B (DNA methyltransferase 3β), leads to nucleus pulposus cell senescence and subsequent degeneration of the intervertebral disc (*126*). A case report was presented regarding a child with bilateral radial ulnar synostosis and vertebral deformities. The child had a deletion at the gene region *16p13.3*, which includes *E4F1*. This suggests that E4F1 may have an important role in skeletal development; however, the exact mechanism is still unknown (*127*).

In addition, the function of E4F1 has been observed in the skin, where inactivation of p120^{E4F} leads to epidermal stem cell–dependent defects in skin homeostasis, including severe skin ulcerations, through its effect on the BMI1-p14^{ARF}-p53 axis. These findings suggest a potentially crucial role of p120^{E4F} in the development of skin carcinoma and malignant melanoma associated with these stem cells (*128*).

CONCLUDING REMARKS

In this review, we summarized the major advances in the regulation of cell life and disease progression by transcription factor E4F1 from its first research to the present. First, the E4F1 protein domain, its interacting partners, and the evolution and conservation of E4F1 are described. While E4F1 primarily acts as a transcriptional repressor, as unequivocally demonstrated in the *CCNA2* gene, it can also

function as a transcriptional activator regulating genes involved in DDR and cellular energy metabolism (*23*, *53*, *98*). For its biological functions, a summary of the literature on the molecular mechanisms by which E4F1 regulates cell growth and proliferation has led to the formation of an intricate E4F1-associated molecular regulatory network of the cell cycle, mainly involved in the G_1-S phase transition, along with the block in the G_2 phase. Interestingly, accumulating evidence suggests that E4F1 is a key regulator of certain cell differentiation, cell death, DDR, and cell metabolism. These functions provide valuable insights into its critical roles in cell life, indicating E4F1 as a powerful potential target for regulating human health and disease.

To further investigate the regulatory network of E4F1′s molecular functions and enhance our understanding of its complex orchestration in cell survival and death, we have compiled the following crucial questions. (i) In addition to p53, what other substrates does the atypical ubiquitin E3 ligase E4F1 target, and what are the specific rules and preferences governing this ubiquitination? (ii) Do multiple reported interacting proteins of E4F1 with overlapping binding regions on E4F1 have competitive, synergistic, or inhibitory relationships in the same space time? (iii) What are the specific trigger conditions and molecular pathways for the hydrolysis of $p120^{E4F}$ to $p50^{E4F}$? Because of their distinct functions in transcriptional regulation, the clarification of detailed mechanisms will give us a deep understanding of its complex orchestration in the balance of cell survival and death. (iv) What are the specific regulatory mechanisms of E4F1 as a transcriptional repressor or activator, and does this correlate with certain decisive cofactors, DNA motif characteristics of its substrates, or E4F1 conformational alterations mediated by certain regulators that allow E4F1 to function in transcriptional repression or activation? (v) As a DNA binding factor, how is E4F1 recruited to chromatin? Additional investigations are required to elucidate the signaling conditions and factors that induce E4F1 recruitment. (vi) Is E4F1-mediated cell cycle arrest dependent on p53 or other key proteins in various cells or tissues, and what are the central regulatory mechanisms? (vii) Further attention is required to investigate whether alterations in the *E4F1* gene, such as mutations and amplifications, affect the function of E4F1, especially mutations at the R167 site.

Given the importance of E4F1 in various cell biological processes, its implications for diseases have also received attention. As mentioned above, E4F1 is involved in the regulation of the onset and progression of disorders, including cancers, kidney malformations, vascular diseases, metabolic diseases, fibrocartilage or bone-related diseases, and possible skin diseases. Notably, E4F1′s "double-edged" role as a pro- or anticancerous regulator in cancers needs specific attention. For instance, E4F1 overexpression can promote cancer progression in HCC and acute myeloid leukemia but may also play an anticancerous role in NSCLC, as shown in the "Cancer" section. The heterogeneity of E4F1 on cancer prognosis was also observed between different cancer types based on the TCGA database and subsequent analyses by TIMER2.0. This suggests that different mechanisms of regulation in the balance between cell survival and death in different cancer types contribute to the heterogeneity of the biological outcomes of high or low E4F1 expression on cancer progression. Thus, when trying to develop drugs that modify E4F1 expression or activity for treating diseases, particularly cancer, we must focus our attention on the tissue types and developmental stages of the target diseases. E4F1 is an indispensable key regulator in cell survival. However, complete loss of E4F1-related biological effects (*E4F1* knockout) can result in cell death, and lower E4F1 expression (*E4F1* knockdown) might only contribute to the alteration in cell status, such as favoring or impairing cell survival, since the remaining E4F1 maintains basic biological functions. This difference suggests that the choice of knockdown or knockout techniques responsible for the downregulation of E4F1 expression in E4F1-related studies needs to be prudently determined, since it might cause completely distinct biological outcomes in the same cells or tissues. In addition, E4F1 has been reported to affect chemotherapy sensitivity, indicating its potential as a target for chemoresistance (*119*).

Overall, the appropriate expression and activity of E4F1 in different cells and tissues are required for health maintenance, and E4F1 may serve as a potential therapeutic target and prognostic biomarker, particularly for cancer. A deep investigation of the roles and molecular regulatory pathways of E4F1 in various diseases, as well as the development of drugs that can activate or inhibit E4F1, is likely to accelerate the progress of E4F1 from basic research into clinical applications.

REFERENCES AND NOTES

1. S. Saccone, P. Sandy, G. Meroni, M. Gostissa, G. Della Valle, G. Del Sal, Assignment of the E1A-regulated transcription factor E4F gene (E4F1) to human chromosome band 16p13.3 by in situ hybridization and somatic cell hybrids. *Cytogenet. Cell Genet.* **82**, 99–100 (1998).
2. C. Fognani, G. Della Valle, L. E. Babiss, Repression of adenovirus E1A enhancer activity by a novel zinc finger-containing DNA-binding protein related to the GLI-Kruppel protein. *EMBO J.* **12**, 4985–4992 (1993).
3. E. R. Fernandes, R. J. Rooney, The adenovirus E1A-regulated transcription factor E4F is generated from the human homolog of nuclear factor phiAP3. *Mol. Cell. Biol.* **17**, 1890–1903 (1997).
4. K. A. Lee, M. R. Green, A cellular transcription factor E4F1 interacts with an E1a-inducible enhancer and mediates constitutive enhancer function in vitro. *EMBO J.* **6**, 1345–1353 (1987).
5. P. Raychaudhuri, R. Rooney, J. R. Nevins, Identification of an E1A-inducible cellular factor that interacts with regulatory sequences within the adenovirus E4 promoter. *EMBO J.* **6**, 4073–4081 (1987).
6. J. R. Nevins, P. Raychaudhuri, A. S. Yee, R. J. Rooney, I. Kovesdi, R. Reichel, Transactivation by the adenovirus E1A gene. *Biochem. Cell Biol.* **66**, 578–583 (1988).
7. L. Le Cam, M. Lacroix, M. A. Ciemerych, C. Sardet, P. Sicinski, The E4F protein is required for mitotic progression during embryonic cell cycles. *Mol. Cell. Biol.* **24**, 6467–6475 (2004).
8. R.-G. Yan, Q.-L. Yang, Q.-E. Yang, E4 transcription factor 1 (E4F1) regulates sertoli cell proliferation and fertility in mice. *Animals (Basel)* **10**, 1691 (2020).
9. H. Ro, I. B. Dawid, Modulation of Tcf3 repressor complex composition regulates cdx4 expression in zebrafish. *EMBO J.* **30**, 2894–2907 (2011).
10. X. Lu, J. Goke, F. Sachs, P. E. Jacques, H. Liang, B. Feng, G. Bourque, P. A. Bubulya, H. H. Ng, SON connects the splicing-regulatory network with pluripotency in human embryonic stem cells. *Nat. Cell Biol.* **15**, 1141–1152 (2013).
11. L. Le Cam, L. K. Linares, C. Paul, E. Julien, M. Lacroix, E. Hatchi, R. Triboulet, G. Bossis, A. Shmueli, M. S. Rodriguez, O. Coux, C. Sardet, E4F1 is an atypical ubiquitin ligase that modulates p53 effector functions independently of degradation. *Cell* **127**, 775–788 (2006).
12. R. J. Rooney, K. Rothammer, E. R. Fernandes, Mutational analysis of p50E4F suggests that DNA binding activity is mediated through an alternative structure in a zinc finger domain that is regulated by phosphorylation. *Nucleic Acids Res.* **26**, 1681–1688 (1998).
13. P. Sandy, M. Gostissa, V. Fogal, L. D. Cecco, K. Szalay, R. J. Rooney, C. Schneider, G. Del Sal, p53 is involved in the p120E4F-mediated growth arrest. *Oncogene* **19**, 188–199 (2000).
14. M. A. Tessari, M. Gostissa, S. Altamura, R. Sgarra, A. Rustighi, C. Salvagno, G. Caretti, C. Imbriano, R. Mantovani, G. Del Sal, V. Giancotti, G. Manfioletti, Transcriptional activation of the cyclin A gene by the architectural transcription factor HMGA2. *Mol. Cell. Biol.* **23**, 9104–9116 (2003).
15. S. L. Fenton, A. Dallol, A. Agathanggelou, L. Hesson, J. Ahmed-Choudhury, S. Baksh, C. Sardet, R. Dammann, J. D. Minna, J. Downward, E. R. Maher, F. Latif, Identification of the

E1A-regulated transcription factor p120 E4F as an interacting partner of the RASSF1A candidate tumor suppressor gene. *Cancer Res.* **64**, 102–107 (2004).
16. H. Rizos, E. Diefenbach, P. Badhwar, S. Woodruff, T. M. Becker, R. J. Rooney, R. F. Kefford, Association of p14ARF with the p120E4F transcriptional repressor enhances cell cycle inhibition. *J. Biol. Chem.* **278**, 4981–4989 (2003).
17. J. Chagraoui, S. L. Niessen, J. Lessard, S. Girard, P. Coulombe, M. Sauvageau, S. Meloche, G. Sauvageau, E4F1: A novel candidate factor for mediating BMI1 function in primitive hematopoietic cells. *Genes Dev.* **20**, 2110–2120 (2006).
18. L. Fajas, C. Paul, O. Zugasti, L. Le Cam, J. Polanowska, E. Fabbrizio, R. Medema, M. L. Vignais, C. Sardet, pRB binds to and modulates the transrepressing activity of the E1A-regulated transcription factor p120E4F. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 7738–7743 (2000).
19. C. Paul, M. Lacroix, I. Iankova, E. Julien, B. W. Schafer, C. Labalette, Y. Wei, A. Le Cam, L. Le Cam, C. Sardet, The LIM-only protein FHL2 is a negative regulator of E4F1. *Oncogene* **25**, 5475–5484 (2006).
20. R. Colombo, G. F. Draetta, S. Chiocca, Modulation of p120E4F transcriptional activity by the Gam1 adenoviral early protein. *Oncogene* **22**, 2541–2547 (2003).
21. M. Cvetanovic, R. J. Rooney, J. J. Garcia, N. Toporovskaya, H. Y. Zoghbi, P. Opal, The role of LANP and ataxin 1 in E4F-mediated transcriptional repression. *EMBO Rep.* **8**, 671–677 (2007).
22. E. Rui, P. R. Moura, K. A. Goncalves, R. J. Rooney, J. Kobarg, Interaction of the hepatitis B virus protein HBx with the human transcription regulatory protein p120E4F in vitro. *Virus Res.* **115**, 31–42 (2006).
23. G. Rodier, O. Kirsh, M. Baraibar, T. Houles, M. Lacroix, H. Delpech, E. Hatchi, S. Arnould, D. Severac, E. Dubois, J. Caramel, E. Julien, B. Friguet, L. Le Cam, C. Sardet, The transcription factor E4F1 coordinates CHK1-dependent checkpoint and mitochondrial functions. *Cell Rep.* **11**, 220–233 (2015).
24. K. Dudziak, N. Mottalebi, S. Senkel, E. L. Edghill, S. Rosengarten, M. Roose, C. Bingham, S. Ellard, G. U. Ryffel, Transcription factor HNF1β and novel partners affect nephrogenesis. *Kidney Int.* **74**, 210–217 (2008).
25. J. Nojima, K. Kanomata, Y. Takada, T. Fukuda, S. Kokabu, S. Ohte, T. Takada, T. Tsukui, T. S. Yamamoto, H. Sasanuma, K. Yoneyama, N. Ueno, Y. Okazaki, R. Kamijo, T. Yoda, T. Katagiri, Dual roles of smad proteins in the conversion from myoblasts to osteoblastic cells by bone morphogenetic proteins. *J. Biol. Chem.* **285**, 15577–15586 (2010).
26. P. Raychaudhuri, S. Bagchi, J. R. Nevins, DNA-binding activity of the adenovirus-induced E4F transcription factor is regulated by phosphorylation. *Genes Dev.* **3**, 620–627 (1989).
27. N. Dyson, The regulation of E2F by pRB-family proteins. *Genes Dev.* **12**, 2245–2262 (1998).
28. X. Grana, J. Garriga, X. Mayol, Role of the retinoblastoma protein family, pRB, p107 and p130 in the negative control of cell growth. *Oncogene* **17**, 3365–3383 (1998).
29. C. Giacinti, A. Giordano, RB and cell cycle progression. *Oncogene* **25**, 5220–5227 (2006).
30. M. M. Kasten, A. Giordano, pRb and the cdks in apoptosis and the cell cycle. *Cell Death Differ.* **5**, 132–140 (1998).
31. N. J. Dyson, RB1: A prototype tumor suppressor and an enigma. *Genes Dev.* **30**, 1492–1502 (2016).
32. F. A. Dick, S. M. Rubin, Molecular mechanisms underlying RB protein function. *Nat. Rev. Mol. Cell Biol.* **14**, 297–306 (2013).
33. S. B. Seo, P. McNamara, S. Heo, A. Turner, W. S. Lane, D. Chakravarti, Regulation of histone acetylation and transcription by INHAT, a human cellular complex containing the set oncoprotein. *Cell* **104**, 119–130 (2001).
34. S. N. Kutney, R. Hong, T. Macfarlan, D. Chakravarti, A signaling role of histone-binding proteins and INHAT subunits pp32 and Set/TAF-Iβ in integrating chromatin hypoacetylation and transcriptional repression. *J. Biol. Chem.* **279**, 30850–30855 (2004).
35. S. B. Seo, T. Macfarlan, P. McNamara, R. Hong, Y. Mukai, S. Heo, D. Chakravarti, Regulation of histone acetylation and transcription by nuclear protein pp32, a subunit of the INHAT complex. *J. Biol. Chem.* **277**, 14005–14010 (2002).
36. S. Chiocca, A. Baker, M. Cotten, Identification of a novel antiapoptotic protein, GAM-1, encoded by the CELO adenovirus. *J. Virol.* **71**, 3168–3177 (1997).
37. J. B. Glotzer, M. Saltik, S. Chiocca, A. I. Michou, P. Moseley, M. Cotten, Activation of heat-shock response by an adenovirus is essential for virus replication. *Nature* **407**, 207–211 (2000).
38. S. Chiocca, V. Kurtev, R. Colombo, R. Boggio, M. T. Sciurpi, G. Brosch, C. Seiser, G. F. Draetta, M. Cotten, Histone deacetylase 1 inactivation by an adenovirus early gene product. *Curr. Biol.* **12**, 594–598 (2002).
39. M. Murphy, M. G. Stinnakre, C. Senamaud-Beaufort, N. J. Winston, C. Sweeney, M. Kubelka, M. Carrington, C. Brechot, J. Sobczak-Thepot, Delayed early embryonic lethality following disruption of the murine cyclin A2 gene. *Nat. Genet.* **15**, 83–86 (1997).
40. J. M. Blanchard, Cyclin A2 transcriptional regulation: Modulation of cell cycle control at the G1/S transition by peripheral cues. *Biochem. Pharmacol.* **60**, 1179–1184 (2000).
41. L. Fajas, C. Paul, A. Vie, S. Estrach, R. Medema, J. M. Blanchard, C. Sardet, M. L. Vignais, Cyclin A is a mediator of p120E4F-dependent cell cycle arrest in G1. *Mol. Cell. Biol.* **21**, 2956–2966 (2001).
42. J. Ahmed-Choudhury, A. Agathanggelou, S. L. Fenton, C. Ricketts, G. J. Clark, E. R. Maher, F. Latif, Transcriptional regulation of cyclin A2 by RASSF1A through the enhanced binding of p120E4F to the cyclin A2 promoter. *Cancer Res.* **65**, 2690–2697 (2005).
43. L. Shivakumar, J. Minna, T. Sakamaki, R. Pestell, M. A. White, The RASSF1A tumor suppressor blocks cell cycle progression and inhibits cyclin D1 accumulation. *Mol. Cell. Biol.* **22**, 4309–4318 (2002).
44. A. M. Richter, G. P. Pfeifer, R. H. Dammann, The RASSF proteins in cancer; from epigenetic silencing to functional characterization. *Biochim. Biophys. Acta* **1796**, 114–128 (2009).
45. H. Donninger, M. D. Vos, G. J. Clark, The RASSF1A tumor suppressor. *J. Cell Sci.* **120**, 3163–3172 (2007).
46. H. Donninger, J. A. Clark, M. K. Monaghan, M. L. Schmidt, M. Vos, G. J. Clark, Cell cycle restriction is more important than apoptosis induction for RASSF1A protein tumor suppression. *J. Biol. Chem.* **289**, 31287–31295 (2014).
47. B. Mansoori, A. Mohammadi, H. J. Ditzel, P. H. G. Duijf, V. Khaze, M. F. Gjerstorff, B. Baradaran, HMGA2 as a critical regulator in cancer development. *Genes (Basel)* **12**, 269 (2021).
48. S. Zhang, Q. Mo, X. Wang, Oncological role of HMGA2 (review). *Int. J. Oncol.* **55**, 775–788 (2019).
49. I. Cleynen, W. J. Van de Ven, The HMGA proteins: A myriad of functions (review). *Int. J. Oncol.* **32**, 289–305 (2008).
50. C. Desdouets, G. Matesic, C. A. Molina, N. S. Foulkes, P. Sassone-Corsi, C. Brechot, J. Sobczak-Thepot, Cell cycle regulation of cyclin A gene expression by the cyclic AMP-responsive transcription factors CREB and CREM. *Mol. Cell. Biol.* **15**, 3301–3309 (1995).
51. C. Desdouets, C. Ory, G. Matesic, T. Soussi, C. Brechot, J. Sobczak-Thepot, ATF/CREB site mediated transcriptional activation and p53 dependent repression of the cyclin A promoter. *FEBS Lett.* **385**, 34–38 (1996).
52. T. Houles, G. Rodier, L. Le Cam, C. Sardet, O. Kirsh, Description of an optimized ChIP-seq analysis pipeline dedicated to genome wide identification of E4F1 binding sites in primary and transformed MEFs. *Genom. Data* **5**, 368–370 (2015).
53. M. Lacroix, G. Rodier, O. Kirsh, T. Houles, H. Delpech, B. Seyran, L. Gayte, F. Casas, L. Pessemesse, M. Heuillet, F. Bellvert, J. C. Portais, C. Berthet, F. Bernex, M. Brivet, A. Boutron, L. Le Cam, C. Sardet, E4F1 controls a transcriptional program essential for pyruvate dehydrogenase activity. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 10998–11003 (2016).
54. E. R. Fernandes, J. Y. Zhang, R. J. Rooney, Adenovirus E1A-regulated transcription factor p120E4F inhibits cell growth and induces the stabilization of the cdk inhibitor p21WAF1. *Mol. Cell. Biol.* **18**, 459–467 (1998).
55. W. S. El-Deiry, p21(WAF1) mediates cell-cycle inhibition, relevant to cancer suppression and therapy. *Cancer Res.* **76**, 5189–5191 (2016).
56. R. Stilo, A. Leonardi, L. Formisano, B. Di Jeso, P. Vito, D. Liguoro, TUCAN/CARDINAL and DRAL participate in a common pathway for modulation of NF-κB activation. *FEBS Lett.* **521**, 165–169 (2002).
57. J. M. Muller, U. Isele, E. Metzger, A. Rempel, M. Moser, A. Pscherer, T. Breyer, C. Holubarsch, R. Buettner, R. Schule, FHL2, a novel tissue-specific coactivator of the androgen receptor. *EMBO J.* **19**, 359–369 (2000).
58. X. Du, P. Hublitz, T. Gunther, D. Wilhelm, C. Englert, R. Schule, The LIM-only coactivator FHL2 modulates WT1 transcriptional activity during gonadal differentiation. *Biochim. Biophys. Acta* **1577**, 93–101 (2002).
59. A. Morlon, P. Sassone-Corsi, The LIM-only protein FHL2 is a serum-inducible transcriptional coactivator of AP-1. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 3977–3982 (2003).
60. G. M. Fimia, D. De Cesare, P. Sassone-Corsi, A family of LIM-only transcriptional coactivators: Tissue-specific expression and selective activation of CREB and CREM. *Mol. Cell. Biol.* **20**, 8613–8622 (2000).
61. K. Kurakula, D. Sommer, M. Sokolovic, P. D. Moerland, S. Scheij, P. B. van Loenen, D. S. Koenis, N. Zelcer, C. M. van Tiel, C. J. de Vries, LIM-only protein FHL2 is a positive regulator of liver X receptors in smooth muscle cells involved in lipid homeostasis. *Mol. Cell. Biol.* **35**, 52–62 (2015).
62. Y. Wei, C. A. Renard, C. Labalette, Y. Wu, L. Levy, C. Neuveut, X. Prieur, M. Flajolet, S. Prigent, M. A. Buendia, Identification of the LIM protein FHL2 as a coactivator of β-catenin. *J. Biol. Chem.* **278**, 5188–5194 (2003).
63. P. McLoughlin, E. Ehler, G. Carlile, J. D. Licht, B. W. Schafer, The LIM-only protein DRAL/FHL2 interacts with and is a corepressor for the promyelocytic leukemia zinc finger protein. *J. Biol. Chem.* **277**, 37045–37053 (2002).
64. N. M. van der Lugt, J. Domen, K. Linders, M. van Roon, E. Robanus-Maandag, H. te Riele, M. van der Valk, J. Deschamps, M. Sofroniew, M. van Lohuizen, A. Berns, Posterior transformation, neurological abnormalities, and severe hematopoietic defects in mice with a targeted deletion of the bmi-1 proto-oncogene. *Genes Dev.* **8**, 757–769 (1994).

65. J. Lessard, A. Schumacher, U. Thorsteinsdottir, M. van Lohuizen, T. Magnuson, G. Sauvageau, Functional antagonism of the Polycomb-group genes eed and Bmi1 in hemopoietic cell proliferation. *Genes Dev.* **13**, 2691–2703 (1999).
66. I. K. Park, D. Qian, M. Kiel, M. W. Becker, M. Pihalja, I. L. Weissman, S. J. Morrison, M. F. Clarke, Bmi-1 is required for maintenance of adult self-renewing haematopoietic stem cells. *Nature* **423**, 302–305 (2003).
67. T. Konuma, H. Oguro, A. Iwama, Role of the polycomb group proteins in hematopoietic stem cells. *Dev. Growth Differ.* **52**, 505–516 (2010).
68. E. A. Nigg, Cyclin-dependent protein kinases: Key regulators of the eukaryotic cell cycle. *Bioessays* **17**, 471–480 (1995).
69. J. Boonstra, Progression through the G1-phase of the on-going cell cycle. *J. Cell. Biochem.* **90**, 244–252 (2003).
70. S. I. Reed, Control of the G1/S transition. *Cancer Surv.* **29**, 7–23 (1997).
71. R. J. Rooney, Cell cycle attenuation by p120E4F is accompanied by increased mitotic dysfunction. *Cell Growth Differ.* **12**, 505–516 (2001).
72. D. G. Johnson, R. Schneider-Broussard, Role of E2F in cell cycle control and cancer. *Front. Biosci.* **3**, d447–d458 (1998).
73. C. Stevens, N. B. La Thangue, E2F and cell cycle control: A double-edged sword. *Arch. Biochem. Biophys.* **412**, 157–169 (2003).
74. P. Lavia, P. Jansen-Durr, E2F target genes and cell-cycle checkpoint control. *Bioessays* **21**, 221–230 (1999).
75. R. Honda, H. Tanaka, H. Yasuda, Oncoprotein MDM2 is a ubiquitin ligase E3 for tumor suppressor p53. *FEBS Lett.* **420**, 25–27 (1997).
76. J. Momand, H. H. Wu, G. Dasgupta, MDM2 — Master regulator of the p53 tumor suppressor protein. *Gene* **242**, 15–29 (2000).
77. J. D. Weber, L. J. Taylor, M. F. Roussel, C. J. Sherr, D. Bar-Sagi, Nucleolar Arf sequesters Mdm2 and activates p53. *Nat. Cell Biol.* **1**, 20–26 (1999).
78. R. Honda, H. Yasuda, Association of p19(ARF) with Mdm2 inhibits ubiquitin ligase activity of Mdm2 for tumor suppressor p53. *EMBO J.* **18**, 22–27 (1999).
79. J. D. Weber, M. L. Kuo, B. Bothner, E. L. DiGiammarino, R. W. Kriwacki, M. F. Roussel, C. J. Sherr, Cooperative signals governing ARF-mdm2 interaction and nucleolar localization of the complex. *Mol. Cell. Biol.* **20**, 2517–2528 (2000).
80. C. J. Sherr, Tumor surveillance via the ARF-p53 pathway. *Genes Dev.* **12**, 2984–2991 (1998).
81. G. R. Stark, W. R. Taylor, Control of the G2/M transition. *Mol. Biotechnol.* **32**, 227–248 (2006).
82. V. A. Smits, R. H. Medema, Checking out the G2/M transition. *Biochim. Biophys. Acta* **1519**, 1–12 (2001).
83. K. Miyazono, Signal transduction by bone morphogenetic protein receptors: Functional roles of Smad proteins. *Bone* **25**, 91–93 (1999).
84. D. Chen, M. Zhao, G. R. Mundy, Bone morphogenetic proteins. *Growth Factors* **22**, 233–241 (2004).
85. E. R. Fernandes, R. J. Rooney, Suppression of E1A-mediated transformation by the p50E4F transcription factor. *Mol. Cell. Biol.* **19**, 4739–4749 (1999).
86. Y. Zhang, N. Wan, T. Pan, X. Hu, Q. Liu, S. Li, MicroRNA-33-3p regulates vein endothelial cell apoptosis in selenium-deficient broilers by targeting E4F1. *Oxid. Med. Cell. Longev.* **2019**, 6274010 (2019).
87. D. D'Amours, S. Desnoyers, I. D'Silva, G. G. Poirier, Poly(ADP-ribosyl)ation reactions in the regulation of nuclear functions. *Biochem. J.* **342**, 249–268 (1999).
88. F. R. Althaus, H. E. Kleczkowska, M. Malanga, C. R. Muntener, J. M. Pleschke, M. Ebner, B. Auer, Poly ADP-ribosylation: A DNA break signal mechanism. *Mol. Cell. Biochem.* **193**, 5–11 (1999).
89. J. C. Ame, C. Spenlehauer, G. de Murcia, The PARP superfamily. *Bioessays* **26**, 882–893 (2004).
90. A. A. E. Ali, G. Timinszky, R. Arribas-Bosacoma, M. Kozlowski, P. O. Hassa, M. Hassler, A. G. Ladurner, L. H. Pearl, A. W. Oliver, The zinc-finger domains of PARP1 cooperate to recognize DNA strand breaks. *Nat. Struct. Mol. Biol.* **19**, 685–692 (2012).
91. A. Ray Chaudhuri, A. Nussenzweig, The multifaceted roles of PARP1 in DNA repair and chromatin remodelling. *Nat. Rev. Mol. Cell Biol.* **18**, 610–621 (2017).
92. F. al-Khodairy, E. Fotou, K. S. Sheldrick, D. J. Griffiths, A. R. Lehmann, A. M. Carr, Identification and characterization of new elements involved in checkpoint and feedback controls in fission yeast. *Mol. Biol. Cell* **5**, 147–160 (1994).
93. N. C. Walworth, DNA damage: Chk1 and Cdc25, more than meets the eye. *Curr. Opin. Genet. Dev.* **11**, 78–82 (2001).
94. H. Zhao, H. Piwnica-Worms, ATR-mediated checkpoint pathways regulate phosphorylation and activation of human Chk1. *Mol. Cell. Biol.* **21**, 4129–4139 (2001).
95. J. W. Harper, S. J. Elledge, The DNA damage response: Ten years after. *Mol. Cell* **28**, 739–745 (2007).
96. J. Melo, D. Toczyski, A unified view of the DNA-damage checkpoint. *Curr. Opin. Cell Biol.* **14**, 237–245 (2002).
97. C. Moison, J. Chagraoui, M. C. Caron, J. P. Gagne, Y. Coulombe, G. G. Poirier, J. Y. Masson, G. Sauvageau, Zinc finger protein E4F1 cooperates with PARP-1 and BRG1 to promote DNA double-strand break repair. *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2019408118 (2021).
98. D. Grote, C. Moison, S. Duhamel, J. Chagraoui, S. Girard, J. Yang, N. Mayotte, Y. Coulombe, J. Y. Masson, G. W. Brown, S. Meloche, G. Sauvageau, E4F1 is a master regulator of CHK1-mediated functions. *Cell Rep.* **11**, 210–219 (2015).
99. P. Goguet-Rubio, B. Seyran, L. Gayte, F. Bernex, A. Sutter, H. Delpech, L. K. Linares, R. Riscal, C. Repond, G. Rodier, O. Kirsh, J. Touhami, J. Noel, C. Vincent, N. Pirot, G. Pavlovic, Y. Herault, M. Sitbon, L. Pellerin, C. Sardet, M. Lacroix, L. Le Cam, E4F1-mediated control of pyruvate dehydrogenase activity is essential for skin homeostasis. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 11004–11009 (2016).
100. H. Yuan, L. Zhao, Y. Yuan, H. Yun, W. Zheng, Y. Geng, G. Yang, Y. Wang, M. Zhao, X. Zhang, HBx represses WDR77 to enhance HBV replication by DDB1-mediated WDR77 degradation in the liver. *Theranostics* **11**, 8362–8378 (2021).
101. M. J. Bouchard, L. Wang, R. J. Schneider, Activation of focal adhesion kinase by hepatitis B virus HBx protein: Multiple functions in viral replication. *J. Virol.* **80**, 4406–4414 (2006).
102. X. Wang, Y. Li, A. Mao, C. Li, Y. Li, P. Tien, Hepatitis B virus X protein suppresses virus-triggered IRF3 activation and IFN-β induction by disrupting the VISA-associated complex. *Cell. Mol. Immunol.* **7**, 341–348 (2010).
103. C. Chan, T. Thurnherr, J. Wang, X. Gallart-Palau, S. K. Sze, S. Rozen, C. G. Lee, Global re-wiring of p53 transcription regulation by the hepatitis B virus X protein. *Mol. Oncol.* **10**, 1183–1195 (2016).
104. J. Wang, N. Li, Z. B. Huang, S. Fu, S. M. Yu, Y. M. Fu, P. C. Zhou, R. C. Chen, R. R. Zhou, Y. Huang, X. W. Hu, X. G. Fan, HBx regulates transcription factor PAX8 stabilization to promote the progression of hepatocellular carcinoma. *Oncogene* **38**, 6696–6710 (2019).
105. S. L. Chen, L. L. Liu, S. X. Lu, R. Z. Luo, C. H. Wang, H. Wang, S. H. Cai, X. Yang, D. Xie, C. Z. Zhang, J. P. Yun, HBx-mediated decrease of AIM2 contributes to hepatocellular carcinoma metastasis. *Mol. Oncol.* **11**, 1225–1240 (2017).
106. Y. Dai, M. P. Cros, C. Pontoizeau, B. Elena-Hermann, G. K. Bonn, P. Hainaut, Downregulation of transcription factor E4F1 in hepatocarcinoma cells: HBV-dependent effects on autophagy, proliferation and metabolism. *Carcinogenesis* **35**, 635–650 (2014).
107. M. Chen, Y. Liu, P. Varley, Y. Chang, X. X. He, H. Huang, D. Tang, M. T. Lotze, J. Lin, A. Tsung, High-mobility group box 1 promotes hepatocellular carcinoma progression through miR-21-mediated matrix metalloproteinase activity. *Cancer Res.* **75**, 1645–1656 (2015).
108. C. Hernandez, P. Huebener, J. P. Pradere, D. J. Antoine, R. A. Friedman, R. F. Schwabe, HMGB1 links chronic liver injury to progenitor responses and hepatocarcinogenesis. *J. Clin. Invest.* **128**, 2436–2451 (2018).
109. Y. Wei, X. Tang, Y. Ren, Y. Yang, F. Song, J. Fu, S. Liu, M. Yu, J. Chen, S. Wang, K. Zhang, Y. Tan, Z. Han, L. Wei, B. Zhang, Z. Cheng, L. Li, H. Wang, An RNA-RNA crosstalk network involving HMGB1 and RICTOR facilitates hepatocellular carcinoma tumorigenesis by promoting glutamine metabolism and impedes immunotherapy by PD-L1+ exosomes activity. *Signal Transduct. Target. Ther.* **6**, 421 (2021).
110. S. Tohme, H. O. Yazdani, Y. Liu, P. Loughran, D. J. van der Windt, H. Huang, R. L. Simmons, S. Shiva, S. Tai, A. Tsung, Hypoxia mediates mitochondrial biogenesis in hepatocellular carcinoma to promote tumor growth through HMGB1 and TLR9 interaction. *Hepatology* **66**, 182–197 (2017).
111. Y. Chen, X. He, F. Cheng, M. Li, X. Wu, C. Zhang, J. Li, B. Huang, M. Qi, Angiotensin II promotes EMT of hepatocellular carcinoma cells through high mobility group protein B1 mediated by E4F1. *Biochem. Biophys. Res. Commun.* **547**, 198–203 (2021).
112. Y. Jiao, K. K. Sun, L. Zhao, J. Y. Xu, L. L. Wang, S. J. Fan, Suppression of human lung cancer cell proliferation and metastasis in vitro by the transducer of ErbB-2.1 (TOB1). *Acta Pharmacol. Sin.* **33**, 250–260 (2012).
113. H. S. Lee, J. Kundu, R. N. Kim, Y. K. Shin, Transducer of ERBB2.1 (TOB1) as a tumor suppressor: A mechanistic perspective. *Int. J. Mol. Sci.* **16**, 29815–29828 (2015).
114. K. J. Ho, N. L. Do, H. H. Otu, M. J. Dib, X. Ren, K. Enjyoji, S. C. Robson, E. F. Terwilliger, S. J. Karp, Tob1 is a constitutively expressed repressor of liver regeneration. *J. Exp. Med.* **207**, 1197–1208 (2010).
115. Y. W. Zhang, R. E. Nasto, R. Varghese, S. A. Jablonski, I. G. Serebriiskii, R. Surana, V. S. Calvert, I. Bebu, J. Murray, L. Jin, M. Johnson, R. Riggins, H. Ressom, E. Petricoin, R. Clarke, E. A. Golemis, L. M. Weiner, Acquisition of estrogen independence induces TOB1-related mechanisms supporting breast cancer cell proliferation. *Oncogene* **35**, 1643–1656 (2016).
116. W. J. Shangguan, H. T. Liu, Z. J. Que, F. F. Qian, L. S. Liu, J. H. Tian, TOB1-AS1 suppresses non-small cell lung cancer cell migration and invasion through a ceRNA network. *Exp. Ther. Med.* **18**, 4249–4258 (2019).
117. E. Hatchi, G. Rodier, C. Sardet, L. Le Cam, E4F1 dysfunction results in autophagic cell death in myeloid leukemic cells. *Autophagy* **7**, 1566–1567 (2011).

118. E. Hatchi, G. Rodier, M. Lacroix, J. Caramel, O. Kirsh, C. Jacquet, E. Schrepfer, S. Lagarrigue, L. K. Linares, G. Lledo, S. Tondeur, P. Dubus, C. Sardet, L. Le Cam, E4F1 deficiency results in oxidative stress-mediated cell death of leukemic cells. *J. Exp. Med.* **208**, 1403–1417 (2011).
119. K. Batnini, T. Houles, O. Kirsh, S. Du Manoir, M. Zaroual, H. Delpech, C. Fallet, M. Lacroix, L. Le Cam, C. Theillet, C. Sardet, G. Rodier, Multi-level control of the ATM/ATR-CHK1 axis by the transcription factor E4F1 in triple-negative breast cancer. *Int. J. Mol. Sci.* **23**, 9217 (2022).
120. K. Nakanishi, N. Yoshikawa, Genetic disorders of human congenital anomalies of the kidney and urinary tract (CAKUT). *Pediatr. Int.* **45**, 610–616 (2003).
121. P. Igarashi, X. Shao, B. T. McNally, T. Hiesberger, Roles of HNF-1β in kidney development and congenital cystic diseases. *Kidney Int.* **68**, 1944–1947 (2005).
122. Y. Nakamura, K. Igarashi, T. Suzuki, J. Kanno, T. Inoue, C. Tazawa, M. Saruta, T. Ando, N. Moriyama, T. Furukawa, M. Ono, T. Moriya, K. Ito, H. Saito, T. Ishibashi, S. Takahashi, S. Yamada, H. Sasano, E4F1, a novel estrogen-responsive gene in possible atheroprotection, revealed by microarray analysis. *Am. J. Pathol.* **165**, 2019–2031 (2004).
123. T. Huang, B. Nazir, R. Altaf, B. Zang, H. Zafar, A. C. Paiva-Santos, N. Niaz, M. Imran, Y. Duan, M. Abbas, U. Ilyas, A meta-analysis of genome-wide gene expression differences identifies promising targets for type 2 diabetes mellitus. *Front. Endocrinol. (Lausanne)* **13**, 985857 (2022).
124. M. Lacroix, L. K. Linares, N. Rueda-Rincon, K. Bloch, M. Di Michele, C. De Blasio, C. Fau, L. Gayte, E. Blanchet, A. Mairal, R. Derua, F. Cardona, D. Beuzelin, J. S. Annicotte, N. Pirot, A. Torro, F. J. Tinahones, F. Bernex, J. Bertrand-Michel, D. Langin, L. Fajas, J. V. Swinnen, L. Le Cam, The multifunctional protein E4F1 links P53 to lipid metabolism in adipocytes. *Nat. Commun.* **12**, 7037 (2021).
125. A. Legati, A. Reyes, A. Nasca, F. Invernizzi, E. Lamantea, V. Tiranti, B. Garavaglia, C. Lamperti, A. Ardissone, I. Moroni, A. Robinson, D. Ghezzi, M. Zeviani, New genes and pathomechanisms in mitochondrial disorders unraveled by NGS technologies. *Biochim. Biophys. Acta* **1857**, 1326–1335 (2016).
126. G. Li, R. Luo, W. Zhang, S. He, B. Wang, H. Liang, Y. Song, W. Ke, Y. Shi, X. Feng, K. Zhao, X. Wu, Y. Zhang, K. Wang, C. Yang, m6A hypomethylation of DNMT3B regulated by ALKBH5 promotes intervertebral disc degeneration via E4F1 deficiency. *Clin. Transl. Med.* **12**, e765 (2022).
127. A. Tam, K. S. Lee, S. Lee, W. Burkhalter, L. U. Pascua, T. P. Slavin, Bilateral radial ulnar synostosis and vertebral anomalies in a child with a de novo 16p13.3 interstitial deletion. *Case Rep. Genet.* **2013**, 149085 (2013).
128. M. Lacroix, J. Caramel, P. Goguet-Rubio, L. K. Linares, S. Estrach, E. Hatchi, G. Rodier, G. Lledo, C. de Bettignies, A. Thepot, C. Deraison, K. Chebli, A. Hovnanian, P. Hainaut, P. Dubus, C. Sardet, L. Le Cam, Transcription factor E4F1 is essential for epidermal stem cell maintenance and skin homeostasis. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 21076–21081 (2010).
129. J. R. Nevins, H. S. Ginsberg, J. M. Blanchard, M. C. Wilson, J. E. Darnell Jr., Regulation of the primary expression of the early adenovirus transcription units. *J. Virol.* **32**, 727–733 (1979).
130. J. R. Nevins, Mechanism of activation of early viral transcription by the adenovirus E1A gene product. *Cell* **26**, 213–220 (1981).
131. P. Gilardi, M. Perricaudet, The E4 transcriptional unit of Ad2: Far upstream sequences are required for its transactivation by E1A. *Nucleic Acids Res.* **12**, 7877–7888 (1984).
132. I. Barlat, B. Henglein, A. Plet, N. Lamb, A. Fernandez, F. McKenzie, J. Pouyssegur, A. Vie, J. M. Blanchard, TGF-beta 1 and cAMP attenuate cyclin A gene transcription via a cAMP responsive element through independent pathways. *Oncogene* **11**, 1309–1318 (1995).
133. H. Ishida, K. Ueda, K. Ohkawa, Y. Kanazawa, A. Hosui, F. Nakanishi, E. Mita, A. Kasahara, Y. Sasaki, M. Hori, N. Hayashi, Identification of multiple transcription factors, HLF, FTF, and E4BP4, controlling hepatitis B virus enhancer II. *J. Virol.* **74**, 1241–1251 (2000).

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