Chapter 7

The Key Role of E2F in Tumor Suppression through Specific Regulation of Tumor Suppressor Genes in Response to Oncogenic Changes

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Abstract

E2F, the principal target of the tumor suppressor pRB, plays crucial roles in tumor suppression. Upon dysfunction of pRB, E2F activates tumor suppressor genes such as *ARF*, an upstream activator of the tumor suppressor p53, resulting in the induction of apoptosis and tumor suppression. The E2F activity that activates the tumor suppressor genes is detected only in cancer cells and not in normal growing cells. The E2F activity can drive selective suicide gene expression and induce apoptosis specifically in cancer cells. Thus, the E2F activity provides a beneficial tool to specifically target cancer cells in cancer treatment.

Keywords: E2F, RB, ARF, apoptosis, cancer specific gene expression

1. Introduction

A human body consists of 37 trillion cells and the cell number is maintained by a balance of cell death and cell proliferation. As aged cells are eliminated by cell death, new cells are supplied by cell proliferation to retain appropriate cell numbers. To maintain homeostasis, cell proliferation is strictly regulated by growth signals. Cell proliferation is also induced by abnormal growth stimulation such as overexpression or constitutive activation of oncogenes, which leads to tumorigenesis [1]. To protect cells from tumorigenesis, mammalian cells harbor tumor suppressor pathways, principally mediated by pRB and p53 [2, 3]. The RB pathway consists of pRB and upstream regulators such as cyclin-dependent kinases (CDKs) and CDK inhibitors.

The p53 pathway consists of p53 and upstream regulators such as HDM2 and ARF. The RB pathway and the p53 pathway suppress tumor formation by the induction of cell cycle arrest or apoptosis. The forced inactivation of both pathways in normal cells renders cells tumorigenic and both pathways are disabled in most cancers, indicating that these two pathways play pivotal roles in tumor suppression in normal cells.

The transcription factor E2F, the principal target of the RB pathway, plays central roles in cell proliferation by activating a repertoire of growth-related genes. Consistent with this, overexpression of E2F1, an activator type of E2F family members, in quiescent cells induces progression into S phase [4]. Since E2F plays central roles in cell proliferation, it has generally been thought that defects in the RB pathway upregulate E2F and promote hyperplasia, contributing to tumorigenesis. However, it has also been reported that E2F plays a pivotal role in tumor suppression. E2F1 knockout mice showed increased incidence of tumor formation [5], suggesting a role of E2F1 in tumor suppression. Overexpression of E2F1 also activates p53, the main effector of the p53 pathway, and promotes apoptosis [6], rather than cell proliferation. Knocking out p53 attenuates E2F1-induced apoptosis [7], supporting that the induction of apoptosis is mediated through activation of p53. Of note, the overexpression of E2F1 activates the tumor suppressor gene *ARF*, an upstream activator of p53 [3]. These observations suggest that E2F plays a pivotal role in tumor suppression by activating ARF, and consequently p53. Interestingly, E2F selectively induces the *ARF* gene upon forced inactivation of pRB, which mimics dysfunction of the RB pathway, but not in response to physiological inactivation of pRB by growth stimulation [8, 9]. This observation implies that E2F activates the *ARF* gene specifically in response to oncogenic changes, contributing to tumor suppression. Consistent with this notion, the E2F activation of the *ARF* gene is detected only in cancer cells and is not observed in normal growing cells [8, 9]. Thus, E2F stimulation of *ARF* gene expression can serve as a tool to discriminate cancer cells and normal growing cells. In this chapter, we describe the roles of E2F in cell proliferation and tumor suppression, focusing on the mechanism of E2F dependent, selective regulation of tumor suppressor genes, specifically in response to oncogenic changes.

2. E2F plays central roles in cell proliferation

The proliferation of mammalian cells is dependent on growth stimulation, which promotes cell cycle progression. Once a cell passes through the restriction (R) point, located in late G1 phase, it is programmed to automatically proceed to the end of M phase. Thus, the regulation of the R point is a critical determinant of cell cycle progression and cell proliferation. Key regulators of the R point are the transcription factor E2F, which activates a repertoire of growth-related genes, and the tumor suppressor pRB, which inhibits E2F.

E2F consists of eight family members (E2F1-8), which, based on their function, are divided into transcriptional activators (E2F1–E2F3a) and transcriptional repressors (E2F3b–E2F8). E2F regulates thousands of genes important for cell cycle progression, DNA replication, DNA damage checkpoint, and DNA repair, and plays central roles in cell proliferation [10]. E2F-modulated cell cycle regulatory genes include *Cyclin E* [11], *Cyclin A* [12], and *CDC2* [13, 14].

Cyclin E/CDK2 promotes G1 to S phase transition by inactivating pRB through phosphorylation. Cyclin A/CDK2 promotes progression through S phase. Cyclin A/CDC2 and Cyclin B/CDC2 promote progression through G2 and progression into and through M phase, respectively. E2F-modulated DNA replication genes include *Cdc6* [15], *Cdt1* [16], *Cyclin E* [11], *ASK* [17], and *Cdc45* [18]. Origin recognition complex (ORC) binds to replication origins and marks where DNA replication takes place. Cdc6 and Cdt1 bind to ORC and promote initiation of DNA replication by recruiting the DNA helicase MCM complex to replication origins (**Figure 1**). Cyclin E/CDK2 phosphorylates MCM complex and promotes loading of it onto chromatin. ASK/Cdc7 activates MCM complex by phosphorylation and Cdc45 recruits DNA polymerase α onto chromatin. These E2F targets are essential for DNA replication and G1-S phase transition [19–23]. Accordingly, knocking out all members of activator-type *E2Fs* (*E2F1~E2F3*) abolishes cell proliferation [24]. Precise replication of genomic DNA is important to avoid mutation. E2F also activates genes involved in DNA damage checkpoint, such as *ATM* [25] and *Chk1* [26], and DNA repair, including *Claspin* [27], *BRCA1* [28], and *Rad51* [27]. Thus, E2F plays a pivotal role in cell proliferation by activating a number of genes critical for cell cycle progression and precise DNA replication.

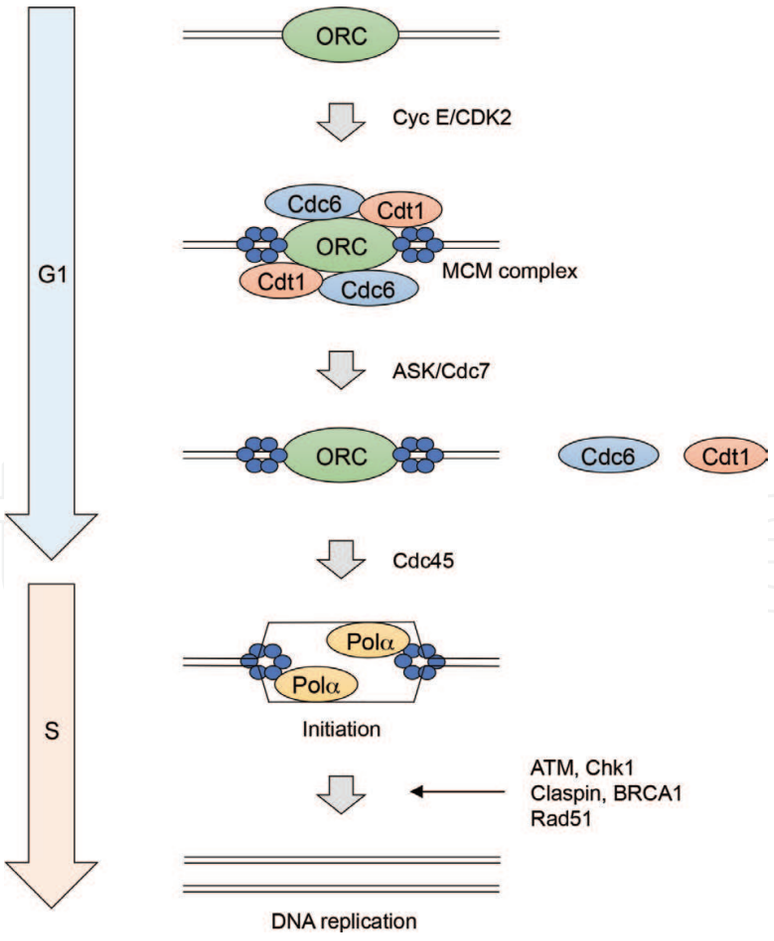

Figure 1. Role of E2F targets in DNA replication. E2F plays central roles in DNA replication by activating genes coding for factors involved in initiation of DNA replication, DNA synthesis, DNA damage checkpoint, and DNA repair.

3. The RB pathway in the control of cell proliferation

pRB is the product of the first identified tumor suppressor gene *retinoblastoma* (*RB1*) [29]. pRB is the principal regulator of G1 to S phase transition by restraining E2F and plays a crucial role in tumor suppression. Based on considerable structural homology, p107 and p130, together with pRB, comprise the RB family. During transition from G1 to S phase upon growth stimulation, RB is inactivated through phosphorylation by CDKs, thereby unleashing E2F and allowing cell cycle progression.

In quiescence, RB family members (pRB and p130) bind to E2F3b-E2F5 on its target promoters and repress their expression (**Figure 2**). The interaction of RB with the transactivation domain of E2F inhibits E2F's transcriptional activity. Furthermore, RB actively represses the expression of E2F target genes by changing chromatin structure through recruitment of histone deacetylase (HDAC) [30], histone methyltransferase (Suv39H1) [31], components of the chromatin remodeling complex (hBrm and BRG1) [32], and DNA methyltransferase (DNMT1) [33] onto their promoters. Upon growth stimulation, D-type cyclin-dependent kinases (CDK4 and 6) are activated, and inactivate p130 and pRB through phosphorylation inhibit binding of RB to E2F3b-5

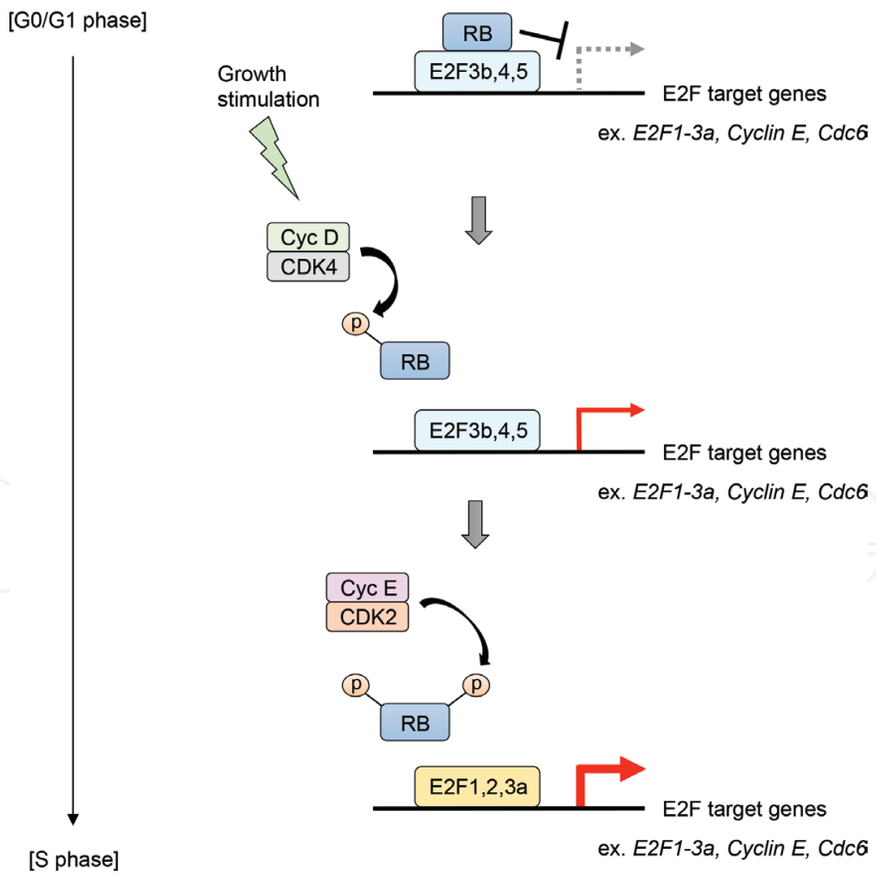

Figure 2. Regulatory mechanism of E2F target genes by E2F and RB. In quiescence, RB family members bind to E2Fs on its target promoters and repress their expression. In response to growth stimulation, Cyclin/CDK inactivates RB family through phosphorylation, activating E2F and its target gene expression.

and its target promoters. This leads to the release of E2F from suppression by RB and induces its target genes including *E2F1-3a* and *Cyclin E* [11, 34]. Cyclin E activates CDK2, which further inactivates RB through phosphorylation. This constitutes a positive feedback loop inactivating RB and activating E2F, resulting in the further induction of E2F targets and initiation of S phase [35]. Thus, regulated functional interactions of E2F and RB play pivotal roles in promoting and restraining cell proliferation, respectively. Given its importance in restraining cell proliferation, RB is often referred to as a "gatekeeper" in the control of cell proliferation.

Consistent with the critical role of RB in restraining cell proliferation, mutation or deletion of the *RB1* gene is responsible for retinoblastoma and various types of cancers including breast cancer [36], osteosarcoma [37], and small cell lung cancer (SCLC) [38]. Since mutation of p107 and p130 are uncommon and considering their function and frequency of inactivation, pRB is thought to be the pivotal tumor suppressor regulating G1-S phase transition [2, 39]. However, although increased tumorigenesis is not detected in $p107^{-/-}$ or $p130^{-/-}$ mice, $RB1^{-/+}p107^{-/-}$ or $RB1^{-/+}p130^{-/-}$ compound mice are more prone to tumor formation than $RB1^{-/+}$ mice [40]. This suggests that, upon loss of pRB function, p107 and p130 can, to some extent, compensate for the tumor suppressor function of pRB [41].

In cancer cells, regulation of G1-S phase transition is lost by the disruption of the RB pathway, which is regarded as a hallmark of cancer [2, 42] (**Figure 3**). Defects in the RB pathway such as deletion or mutation of *RB1* or silencing of its promoter by hypermethylation have been found in breast cancer [36], osteosarcoma [37], and SCLC [38]. Mutation or deletion of the CDK inhibitor

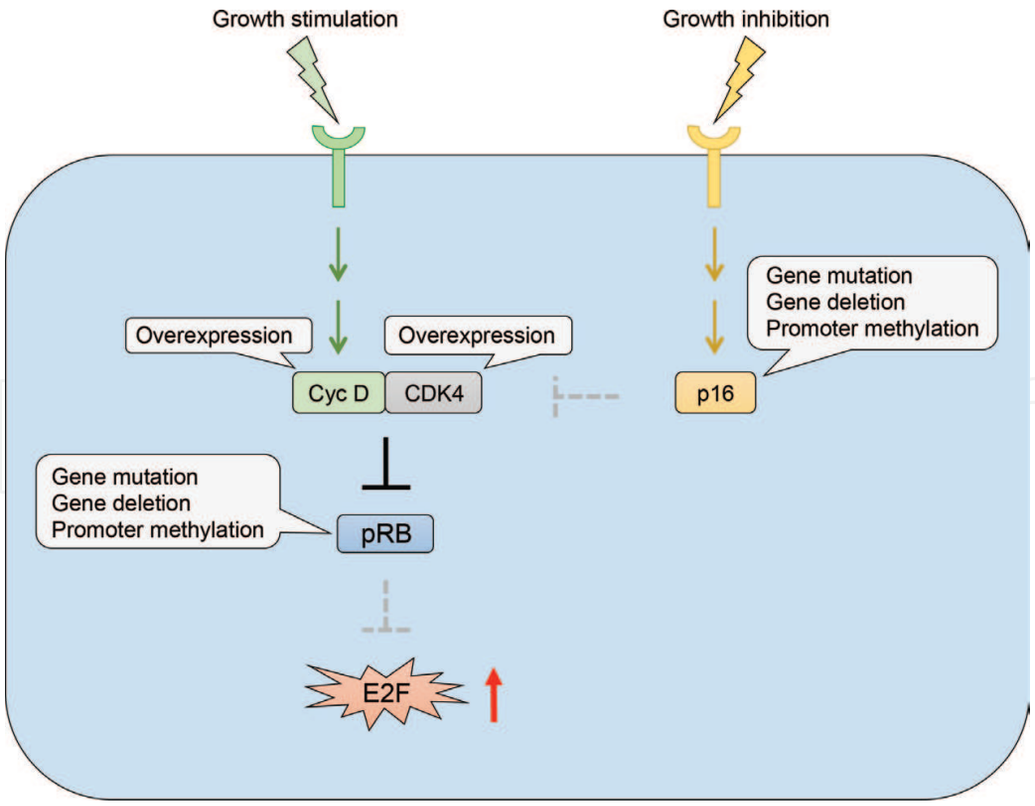

Figure 3. Defects in the RB pathway. pRB, CDKs, or CDK inhibitors are mutated in cancers, resulting in upregulation of E2F activity and its target gene expression.

p16^{INK4a} or silencing of its promoter by hypermethylation was detected at high frequency in a variety of cancers including prostate, renal, and colon cancer [43, 44]. Gene amplification and consequent overexpression of cyclin D1 or CDK4 are also detected in various cancers [45, 46]. Upstream activators of the *Cyclin D1* gene such as c-Myc and Ras are overexpressed or constitutively activated in cancers [47, 48], suggesting that these mutations also contribute to the overexpression of Cyclin D1. Taken together, the RB pathway is, at least at some point, disabled or compromised in almost all cancers. Consequently, pRB is functionally inactivated and E2F activity and its target gene expression are upregulated, leading to the aberrant cell proliferation. This underscores the importance of the RB pathway in tumor suppression.

4. The p53 pathway in the control of cell cycle arrest and apoptosis

p53 plays crucial roles in tumor suppression through the induction of cell cycle arrest or apoptosis (programmed cell death). *TP53*, which codes for p53, is the most frequently mutated gene in a variety of cancers including skin cancer [49], nonsmall cell lung cancer (NSCLC) [50], and breast cancer [51]. *TP53* knockout mice are prone to tumor formation [52, 53] and enhanced expression of p53 induces cell cycle arrest or apoptosis [54]. The target genes involved in cell cycle arrest include CDK inhibitor *p21^{Cip1}*, *14–3-3σ*, and *GADD45* [55] (**Figure 4**). CDK inhibitor p21^{Cip1}, which binds to and inhibits Cyclin D/CDK4, 6, Cyclin E/CDK2, Cyclin A/CDK2, and Cyclin B/CDK1, induces G1 and G2/M arrest [56]. 14–3-3σ binds to the phosphatase Cdc25C, which activates Cyclin B/CDK1, and inhibits its activity by the translocation of the complex from the nucleus into the cytoplasm [57]. GADD45 binds to and inactivates Cdc25C, consequently inhibiting CDK1 to induce G2/M arrest [58]. Activation of these genes by p53 is thought to contribute to tumor suppression through the induction of cell cycle arrest. The target genes involved in apoptosis include *Bax* [59], *Bak* [60], *Noxa* [61], and *Puma* [62] (**Figure 4**). Bax and Bak are Bax family members, whose insertion into mitochondrial membrane induces release of cytochrome *c* and apoptosis. Apoptosis induced by various stimulations is disabled in Bax/Bak-knocked out cells, indicating that Bax and Bak are central players in the induction of programmed cell death [63]. Noxa and Puma directly and indirectly activate Bax and Bak [64]. These observations suggest that p53 contributes to tumor suppression by the induction of apoptosis through activation of Bax and Bak.

The transcriptional activity of p53 is strictly regulated by its binding factors. The oncogene product HDM2, an E3 ubiquitin ligase, induces proteolysis of p53 through ubiquitination and inhibits its activity (**Figure 5**). Under nonstressed conditions, expression of p53 is kept at low levels by binding of HDM2. In response to DNA damage, Chk2 and ATM phosphorylate and activate p53 by inhibiting binding of HDM2 [65]. The tumor suppressor ARF stabilizes p53 by inhibiting HDM2 activity though its sequestration into the nucleolus [66]. Importantly, the expression of ARF is induced by oncogenic changes such as defects in the RB pathway including overexpression of c-myc and Ras [67], and expression of ARF is upregulated in various cancer cells [68]. Based on these observations, ARF is described as a "sensor of oncogenic stresses" and is thought to play crucial roles in tumor suppression, through up-regulation of p53, in response to oncogenic changes. Supporting the importance of its function, mutation, and deletion of *ARF* is detected in various cancers [69] and *ARF$^{-/-}$* mice are prone to tumor formation [70]. The

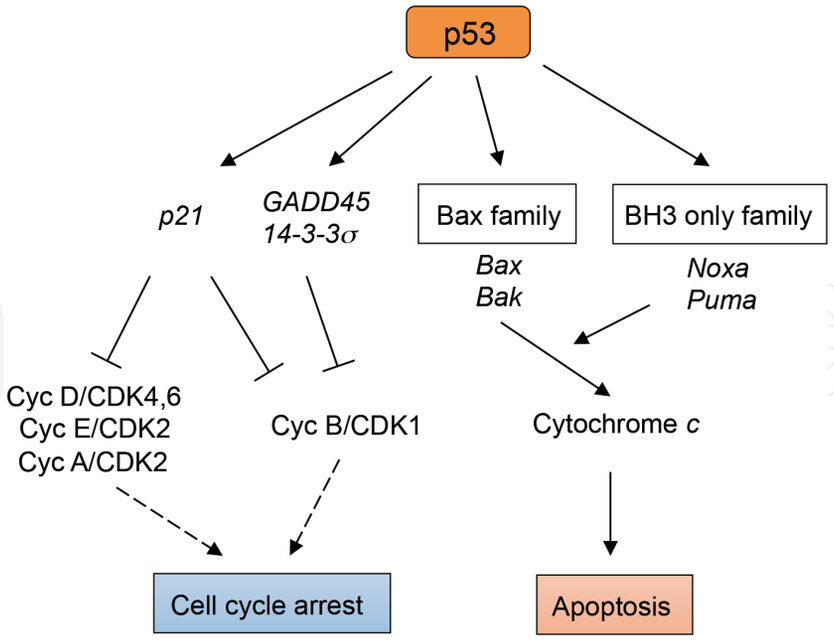

Figure 4. Induction of cell cycle arrest or apoptosis by p53. p53 contributes to cell cycle arrest through the induction of *p21*^{Cip1}, *14–3-3σ*, and *GADD45*, and apoptosis through *Bax*, *Bak*, *Noxa,* and *Puma*.

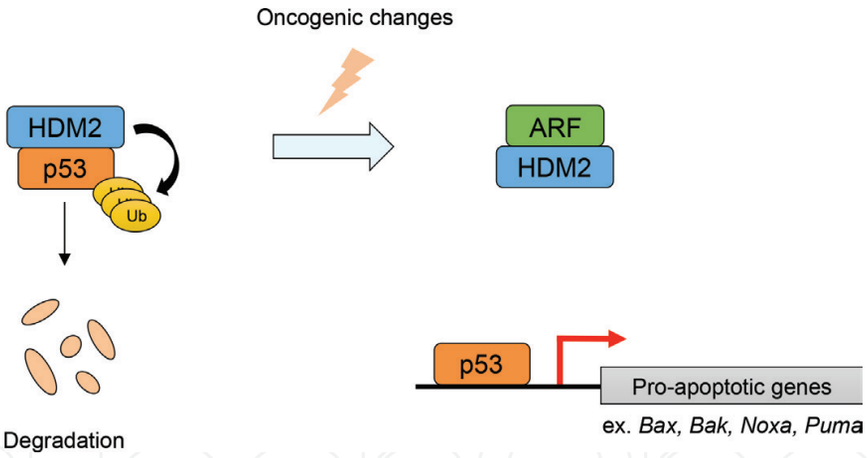

Figure 5. The mechanism of ARF activation of p53. In response to oncogenic changes, ARF stabilizes p53 by inhibiting HDM2 activity.

signal transduction network, from ARF to p53, is referred to as the p53 pathway. Studies using animal models revealed that forced inactivation of the RB and p53 pathways efficiently induce tumorigenesis, suggesting that both play pivotal roles in tumor suppression [71, 72].

5. Pivotal roles of E2F in tumor suppression

E2F plays crucial roles not only in cell proliferation but also in tumor suppression. *E2F1*$^{-/-}$ mice are prone to tumor formation [5] and overexpression of E2F1 induces apoptosis, suggesting that E2F contributes to tumor suppression through the induction of apoptosis.

The target genes involved in apoptosis include *ARF* and *TAp73* [7, 67] (**Figure 6**). ARF is an upstream activator of p53 and plays an important role in transmitting oncogenic signals to p53. The transcription factor TAp73 is a homolog of p53 and induces apoptosis through upregulation of p53 target genes in a p53-independent manner [73]. Apoptosis induced by the overexpression of E2F1 is attenuated in *TP53*$^{-/-}$ cells and *TAp73*$^{-/-}$ cells, and is disabled in *TP53*$^{-/-}$/*TAp73*$^{-/-}$ cells [7]. Moreover, *PPP1R13B* and *JMY*, whose products function as coactivators of p53 and TAp73, are also E2F targets (**Figure 6**) [74], indicating that E2F1 induces apoptosis primarily via p53 and TAp73. Other tumor suppressor genes that are E2F targets include *MOAP1*, *RASSF1*, and *BIM* (**Figure 6**). MOAP1 forms a complex with RASSF1 and activates the proapoptotic protein Bax. BIM is a member of the BH3-only family, which induces apoptosis through direct or indirect activation of Bax [75]. In addition, *Bax* is also a target of p53 and TAp73 [60]. These observations indicate that E2F suppresses tumor formation by the induction of apoptosis through upregulation of p53, TAp73, and their downstream effectors. Importantly, we demonstrated that E2F activates *ARF* and *TAp73* genes upon forced inactivation of pRB, which mimics dysfunction of the RB pathway, but not in response to the physiological inactivation of pRB through growth stimulation [8, 9]. Moreover, a search for genes regulated by E2F in a similar manner to *ARF* and *TAp73* identified *PPP1R13B*, *JMY*, *MOAP1*, *RASSF1*, and *BIM* [76]. These results suggest that E2F contributes to tumor suppression by inducing these genes specifically upon dysfunction of the RB pathway. Consistent with this

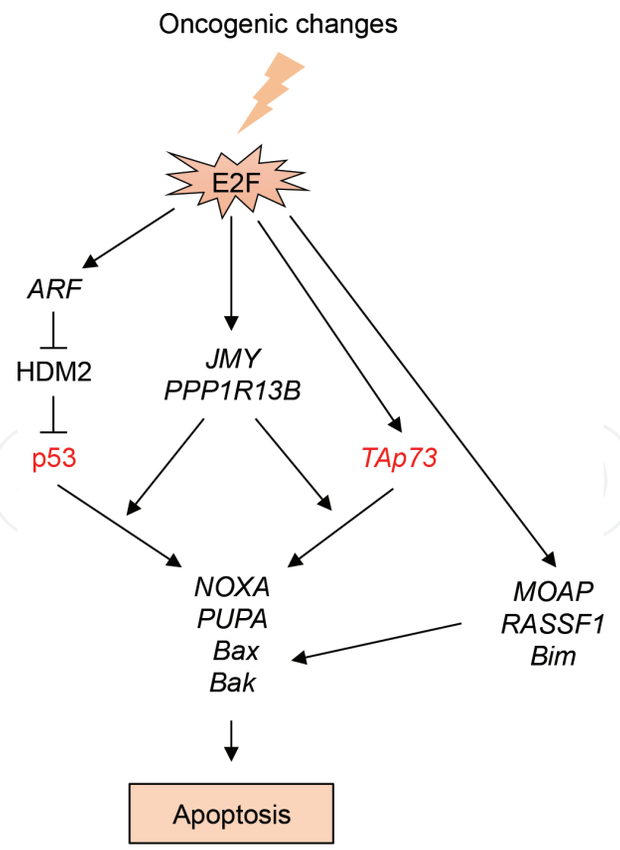

Figure 6. The pathway of E2F-induced apoptosis. In response to oncogenic changes, E2F induces apoptosis through upregulation of p53, TAp73, and their downstream effectors.

notion, E2F activity that activates the *ARF* and *TAp73* genes is detected only in cancer cells and is not present in normal cells [8, 9], underscoring the importance of E2F in tumor suppression. Since E2F selectively activates these tumor suppressor genes in the context of dysfunctional pRB, such E2F activity is referred to as "deregulated E2F activity." This E2F-dependent tumor suppression mechanism implies that disruption of both the p53 and RB pathways is necessary for tumor formation.

6. Regulation of E2F activity to induce apoptosis

Among E2F family members, activator-type E2Fs (E2F1-3) induce tumor suppressor genes such as *ARF* and *TAp73*, with E2F1 exhibiting the highest such activity [9, 76]. Therefore, to understand the regulation of tumor suppression by E2F, elucidation of the mechanism, by which E2F1 activates tumor suppressor genes, is important. Several factors that bind E2F1 and affect its activity are summarized in **Table 1**. TopBP1 is phosphorylated by Akt/PKB upon growth stimulation. The phosphorylated TopBP1 associates with E2F1 [77] and recruits Brg1, a component of chromatin remodeling complex, to E2F1, resulting in the inhibition of E2F1 induction of the *ARF* gene [78]. Jab1, a coactivator of c-Jun [79], binds to E2F1 through the marked box domain and promotes the induction of apoptosis by E2F1 [80, 81]. RIP140 and VHL repress the activation of the ARF promoter by E2F1 [82, 83]. ARF also functions as a transcription cofactor that binds to the transactivation domain of E2F1 to repress E2F1 activation of the ARF promoter [84]. PRMT5 methylates E2F1 on arginine residues 111 and 113, and destabilizes E2F1 [85]. SENP8 deNEDDylates (removes NEDD8) and stabilizes E2F1, resulting in enhancing activation of TAp73 promoter [86]. Sirt1, a histone deacetylase, represses E2F stimulation of the TAp73 promoter [87]. These studies revealed that E2F's ability to activate tumor suppressor genes is regulated by various factors such as transcription cofactors, post-translational modifiers, and histone modifiers. The mechanism of the regulation of E2F activity by these factors is not known in detail and its elucidation is imperative.

Gene name	Function	Monitoring promoter	Effect on E2F activity
Jab1	Transcription cofactor of c-jun	ARF	Upregulation
SENP8	Sentrin-specific protease	TAp73	Upregulation
ARF	Inhibitor of HDM2, transcription cofactor of c-myc	ARF	Repression
PRMT5	Methylase	TAp73	Repression
RIP140	Transcription cofactor of estrogen receptor	ARF	Repression
Sirt1	Histone deacetylase	TAp73	Repression
TopBP1	Transcription cofactor of Miz	ARF	Repression
VHL	E3 ubiquitin ligase, transcription cofactor of p53	ARF	Repression

Table 1. E2F-binding factors and their effects on its activity to activate tumor suppressor genes.

7. Utility of deregulated E2F activity in cancer cell-specific gene expression

In cancer treatment, specifically targeting cancer cells is important for optimal therapeutic efficacy. One strategy is to utilize a cancer-specific promoter to express a cytotoxic gene or a viral gene required for the replication. By regulating a suicide gene such as *HSV-TK* or a proapoptotic gene under the control of cancer-specific promoters, the gene is expressed specifically in cancer cells and causes cell death [88–90]. Alternatively, by regulating a viral gene required for viral replication under the control of these promoters, the gene is expressed specifically in cancer cells, allowing viral replication and cell lysis in a cancer cell-specific manner [91–93]. In this approach, therapeutic effects and side effects are dependent on the promoter activity in cancer cells and normal cells, respectively. Therefore, a promoter with optimal cancer cell-specificity should be used.

For a promoter to be cancer specific, it should have two important characteristics. First, the promoter should have low activity in normal cells to avoid side effects. Second, it should exhibit high activity in a wide variety of cancer cells for maximum therapeutic effects. As promoters thought to exemplify these parameters, hTERT and E2F1 promoters have been utilized. hTERT is a catalytic component of telomerase, which is not expressed in most somatic cells but is present in many types of cancers [94]. Thus, the hTERT promoter exhibits strong promoter activity in many types of cancer cells. However, given that normal stem cells also express hTERT, the hTERT promoter may exhibit strong promoter activity in these cells [95]. The E2F1 promoter is activated by E2F, whose activity is upregulated in cancer cells due to defects in the RB pathway. Thus, E2F1 promoter also exhibits strong promoter activity in many types of cancer cells. However, the E2F1 promoter is also stimulated by physiological E2F activity induced by growth stimulation and thus has a strong promoter activity in normal growing cells [34].

In contrast to the hTERT and E2F1 promoters, which may exhibit strong promoter activity in normal cells, the tumor suppressor ARF promoter, which specifically responds to deregulated E2F activity, is thought to be a better candidate. E2F activity stimulating the ARF promoter, is detected only in cancer cells and not in normal cells [8]. ARF is expressed at high levels in various cancer cells, but not in normally growing cells [68]. Furthermore, the activity of the ARF promoter is detected specifically in tumor tissues and not in normal tissues *in vivo* as revealed using $ARF^{GFP/GFP}$ mice [96]. These observations indicate that the ARF promoter shows optimal cancer cell specificity in a wide variety of cell types and has excellent therapeutic potential.

We showed that the ARF promoter exhibited greater cancer cell specificity than the E2F1 promoter [97]. Adenovirus expressing *HSV-TK*, a suicide gene, under the control of the ARF promoter (Ad-ARF-TK) had more selective cytotoxicity in cancer cells than the analogous E2F1 promoter construct [97]. Moreover, overexpression of the CDK inhibitor $p21^{Cip}$ upregulated deregulated E2F activity specifically in cancer cells and augmented cancer cell-specific cytotoxicity of Ad-ARF-TK [98]. These observations underscore the utility of the ARF promoter and deregulated E2F activity in mediating cancer-specific gene expression (**Figure 7**, upper panel). Furthermore, overexpression $p21^{Cip}$ alone could induce E2F dependent apoptosis specifically in cancer cells [98], suggesting that induction or enhancement of deregulated E2F activity could be a drug target to induce cancer cell-specific apoptosis (**Figure 7**, lower panel). It must be worth testing whether drug-based CDK inhibitors also exhibit similar effects to

p21^{Cip}. Since p21^{Cip} inhibits most of CDKs, identification of responsible CDK, which inhibits deregulated E2F activity, is also important. By using specific inhibitor to the responsible CDK, deregulated E2F activity could be more efficiently enhanced. The combination of deregulated E2F-mediated suicide gene therapy and enhancement of deregulated E2F activity using appropriate CDK inhibitor should also improve deregulated E2F-mediated cancer therapy.

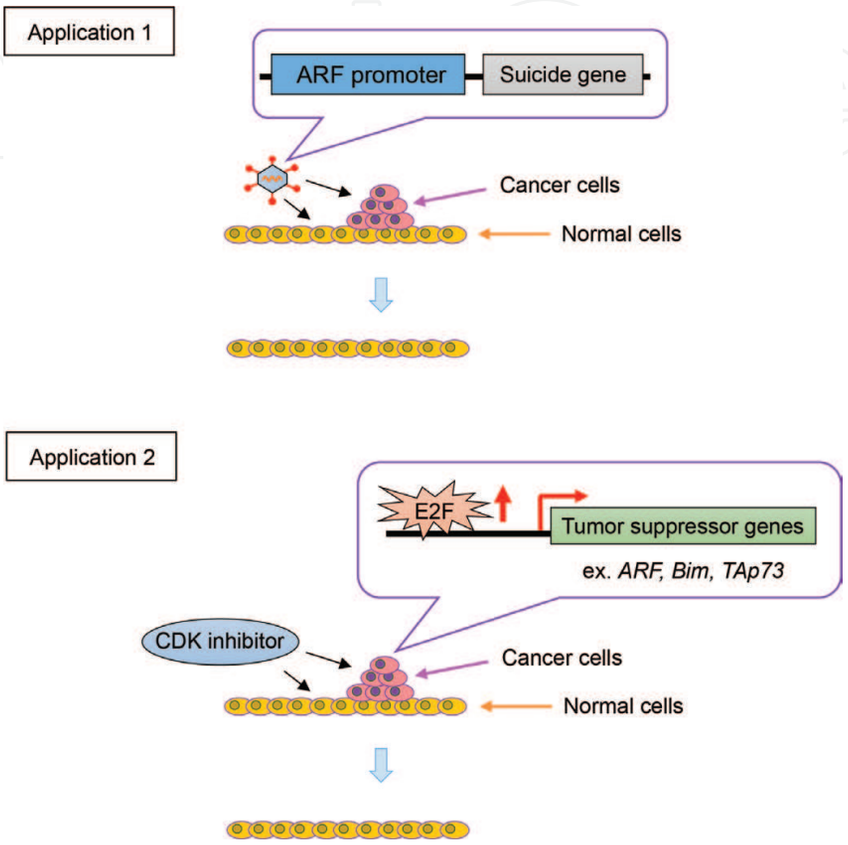

Figure 7. Application of deregulated E2F activity for cancer-specific treatment. Application 1: ARF promoter, which responds to deregulated E2F activity in cancer cells but not to physiological E2F activity in normal cells, drives suicide gene expression, and induces apoptosis specifically in cancer cells. Application 2: Upregulation of deregulated E2F activity by CDK inhibitors activates endogenous tumor suppressor genes and induces apoptosis specifically in cancer cells.

8. Conclusion

E2F is the principal target of the tumor suppressor pRB and defects in the RB pathway are observed in almost all cancers. Upon oncogenic changes, E2F activates *ARF*, an upstream activator of p53 and *TAp73*, resulting in the induction of apoptosis. Importantly, the E2F activity to stimulate *ARF* and *TAp73* expression is not induced by the physiological activation of E2F, such as growth stimulation. Therefore, E2F suppresses tumor formation by inducing apoptosis specifically in response to oncogenic changes through the activation of *ARF* and *TAp73*. Moreover, deregulated E2F-dependent activation of the *ARF* gene is observed only in cancer cells, and not in normal cells, suggesting that deregulated E2F activity represents a beneficial tool to specifically target cancer cells in cancer treatment.

Evidence supporting the potential availability of deregulated E2F activity in cancer therapy is accumulating. The regulation of suicide genes by the ARF promoter has more selective cytotoxicity in cancer cells than the analogous E2F1 promoter construct. Moreover, overexpression of $p21^{Cip}$ upregulates deregulated E2F activity and augments cancer-specific cytotoxicity of the ARF promoter construct. Furthermore, overexpression $p21^{Cip}$ alone can induce E2F-dependent apoptosis specifically in cancer cells. Therefore, deregulated E2F activity can drive selective gene expression and induce apoptosis specifically in cancer cells, supporting its therapeutic potential in a variety of cancers. The development of cancer therapies based upon deregulated E2F activity will require detailed characterization of the components and molecular mechanisms underlying its functional role in oncogenesis and tumor suppression and merits further investigation.

Acknowledgements

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Abbreviations

ARF	Alternative reading frame
ASK	Activator of S-phase kinase
ATM	Ataxia telangiectasia mutated
Bax	Bcl-2-associated X protein
Bak	Bcl-2 homologs antagonist/killer
BIM	BCL-2 interacting mediator of cell death
BRCA1	Breast cancer susceptibility genes 1
BRG1	Brahma-related gene-1
Cdc	Cell division cycle
CDK	Cyclin-dependent kinase
Cdt1	Chromatin licensing and DNA replication factor 1
Chk1	Checkpoint kinase 1
Chk2	Checkpoint kinase 2
DNMT1	DNA methyltransferase 1
E2F	E2 transcription factor
GADD45	Growth arrest and DNA-damage-inducible gene 45
GFP	Green fluorescent protein
hBrm	human Brahma
HDAC	histone deacetylase
HDM2	Human double minute 2

HSV-TK	Herpes simplex virus-1 thymidine kinase
hTERT	Human telomerase reverse transcriptase
Jab1	Jun activation domain-binding protein 1
JMY	Junction-mediating and regulatory protein
MCM	Minichromosome maintenance
MOAP1	Modulator of apoptosis 1
NSCLC	nonsmall cell lung cancer
ORC	Origin recognition complex
PKB	protein kinase B
PPP1R13B	Protein phosphatase 1 regulatory subunit 13B
PRMT5	Protein arginine methyltransferase 5
Puma	p53 upregulated modulator of apoptosis
RASSF1	Ras association domain family member 1
RB	Retinoblastoma
RIP140	Receptor-interacting protein 140
SCLC	Small cell lung cancer
SENP8	Sentrin specific protease family member
Suv39H1	suppressor of variegation 3–9 homolog 1
TopBP1	DNA topoisomerase II-binding protein 1
VHL	Von Hippel–Lindau

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