p53 and E2f: partners in life and death

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Abstract | During tumour development cells sustain mutations that disrupt normal mechanisms controlling proliferation. Remarkably, the Rb–E2f and MDM2–p53 pathways are both defective in most, if not all, human tumours, which underscores the crucial role of these pathways in regulating cell cycle progression and viability. A simple interpretation of the observation that both pathways are deregulated is that they function independently in the control of cell fate. However, a large body of evidence indicates that, in addition to their independent effects on cell fate, there is extensive crosstalk between these two pathways, and specifically between the transcription factors E2F1 and p53, which influences vital cellular decisions. This Review discusses the molecular mechanisms that underlie the intricate interactions between E2f and p53.

Autophagy
A catabolic process involving the degradation of a cell's own components by the lysosomal machinery.

The tumour suppressor p53 is a transcription factor that is activated in response to virtually all cancer-associated stress signals, including DNA damage and oncogene activation. Normally, the levels of p53 protein are low, owing to rapid ubiquitin-dependent degradation largely directed by the E3 ubiquitin ligase MDM2, which is also a target of transcriptional regulation by p53 (REF. 1). Various stresses inhibit MDM2-mediated p53 degradation and/or induce a complex stress-dependent pattern of post-translational p53 modifications that result in p53 stabilization and activation[1]. Once activated, p53 can elicit several different cellular responses, including growth arrest, senescence and apoptosis (FIG. 1a).

The key role of p53 in tumour suppression is dramatically illustrated by the prevalence of *TP53* (the human gene encoding p53) mutations in cancer: it is estimated that 50% of all human tumours carry a *TP53* mutation. Furthermore, in tumours lacking *TP53* mutations, p53 function is often abrogated indirectly, through the overexpression of MDM2 or the inactivation of the cell cycle inhibitor ARF (also known as p14 in humans and as p19 in rodents). ARF interacts with MDM2, inhibiting p53 ubiquitylation and subsequent degradation[2]. Another example of p53 tumour suppressor activity is provided by p53-null mice, which are highly susceptible to early-onset cancer[3,4].

Another family of transcription factors that affect cell fate in general, and in particular cancer development, is the E2f family. Members of the E2f family are downstream effectors of the RB tumour suppressor and have a pivotal role in controlling cell cycle progression (FIG. 1b). Initially, studies revealed that E2fs determine the timely expression of many genes that are required for entry into and progression through S phase of the cell cycle. However, it has become clear that transcriptional activation of S phase-associated genes is only one facet of E2f activity: we now know that E2fs both transactivate and repress gene expression to regulate a wide range of biological processes, including DNA replication, mitosis, the function of DNA damage checkpoints, DNA repair, differentiation and autophagy[5,6]. Moreover, at least one member of the family — E2F1 — can also induce apoptosis[7] (FIG. 1b). E2F1-induced apoptosis is mediated by both p53-dependent and p53-independent pathways[6].

In mammals, the E2f family comprises eight genes (*E2F1–8*), which give rise to nine distinct proteins[8]. E2f family members have been categorized into subfamilies on the basis of their transcriptional activity, structure and interaction with Rb family members. E2F1, E2F2 and E2F3A, which interact only with RB, constitute one subfamily and are often referred to as the 'activator E2fs', as they are believed to function mainly in activating gene expression. E2F4–8 largely function in the repression of gene expression and are generally referred to as the 'repressor E2fs'. The activator E2fs and repressor E2fs affect the expression of mostly overlapping subsets of target genes. In the subfamily of repressor E2fs, E2F4 and E2F5 repress gene expression in an Rb family-dependent manner, whereas E2F6–8 exert transcriptional repression through distinct, Rb-independent, mechanisms.

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At a glance

- There is extensive crosstalk between the Rb–E2f and MDM2–p53 pathways, and specifically between the transcription factors E2F1 and p53, which influences vital cellular decisions.
- The abundance and activity of both p53 and E2f are often controlled by the same cancer-associated stimuli. Their common regulators include checkpoint kinases and acetyltransferases, MDM2 and the *CDKN2A* locus.
- Deregulated E2f, which is often present in human tumours, constitutes an oncogenic stress that activates p53. Specifically, E2f indirectly affects the level and activity of p53 by upregulating the expression of many proteins that stabilize and activate p53. Examples include ARF, ataxia telangiectasia mutated and PIN1.
- E2f and p53 cooperate in restricting tumorigenesis by inducing cell death. Their cooperation in apoptosis is attributed to the ability of E2F1 to activate p53. In addition, they activate many pro-apoptotic genes that may cooperate in apoptosis.
- Protein complexes that contain Rb family members and repressor E2fs mediate p53-induced growth arrest and senescence; the latter is an important *in vivo* mechanism that contributes to protection against cancer.

Generally, activity of the activator E2fs is required for cell proliferation, whereas the repressor E2fs function in cell cycle exit and differentiation.

A puzzling feature of activator E2fs, in particular E2F1 but in some settings also E2F2 and E2F3 (REF. 8), is the ability to induce seemingly contradictory processes, such as proliferation and apoptosis[9]. Given this functional dichotomy it is perhaps not surprising that E2F1 has both oncogenic and tumour suppressive activities, and in mouse models there are examples of both positive and negative effects on tumorigenesis when *E2f1* is either deleted or overexpressed[10]. One hypothesis is that the apoptosis induced by deregulated E2f activity serves as a fail-safe mechanism to counter concomitant hyperproliferative signals. The apoptotic response to deregulated E2f is best demonstrated by the observation that *Rb1*-deficient mouse embryos have increased apoptosis, which is suppressed by the loss of either *E2f1* or *E2f3* (REFS 11–13). Notably, however, in *Rb1*-heterozygous mice, loss of *E2f1* impairs the development of pituitary and thyroid tumours but promotes tumour incidence in other tissues[14], indicating that the role of E2F1 in tumorigenesis is context dependent and tissue specific.

Deregulated E2f activity is observed in the vast majority of human tumours and occurs through several different mechanisms. These include the functional loss of RB; amplification of *CCND1*, which encodes cyclin D1 and promotes the phosphorylation of RB; loss of INK4A (also known as p16), a cyclin-dependent kinase (CDK) inhibitor that inhibits the phosphorylation of RB; and expression of the human papillomavirus oncoprotein E7, which disrupts Rb–E2f complexes[15]. For reasons that are not fully understood alterations in E2fs do not occur frequently in human tumours, although in certain tumours amplification and overexpression of *E2F3* has been observed[16–18].

In many human tumours both the INK4A–Rb–E2f and the ARF–MDM2–p53 pathways sustain defects resulting in the functional inactivation of p53 and the deregulation or hyperactivation of E2f. These concurrent defects are observed in a wide range of human tumours, emphasizing the crucial role of these pathways in oncogenesis in general[15]. A simple interpretation of this observation is that these pathways function independently in the control of cell fate. In line with this premise, small DNA tumour viruses have evolved viral proteins that inactivate both Rb and p53 (REF. 19) (BOX 1). However, data accumulated over the past decade reveal that there is extensive crosstalk between the INK4A–Rb–E2f and the ARF–MDM2–p53 pathways and in particular between the transcription factors E2F1 and p53. Therefore, the apparent requirement to abrogate both pathways during malignant transformation reflects not the autonomy of each pathway but rather the vital nature of their participation in controlling cell cycle progression and viability.

This Review discusses the molecular mechanisms that underlie the intricate interactions between E2fs and p53, focusing on four distinct but related functional aspects of the interactions. First, the abundance and activity of both p53 and E2f are often regulated by the same cancer-associated stimuli. Second, deregulated E2f (present in most human tumours) constitutes an oncogenic stress that increases the level and activity of p53. Third, E2f and p53 cooperate in restricting tumorigenesis by inducing cell death. Last, protein complexes that contain E2f mediate p53-induced growth arrest and senescence, and the latter is an important *in vivo* mechanism that contributes to protection against cancer.

Common upstream regulators of p53 and E2F1

As p53 and E2f are pivotal regulators of cell proliferation and viability, their abundance and activity are tightly regulated. The most well-known regulators of E2f and p53 are RB and MDM2, respectively. In addition, p53 is extensively regulated through a plethora of post-translational modifications that affect its levels, subcellular localization, DNA binding and transactivation potential, as well as the subset of target genes that it activates. Similarly, post-translational modifications regulate the levels and activity of E2f. Notably, emerging evidence supports the idea that some regulators affect both p53 and E2F1 (FIG. 2).

Checkpoint kinases and acetylation. p53 and E2F1 are stabilized in response to various stresses, in particular DNA damage[20–22], and both are phosphorylated by ataxia telangiectasia mutated (ATM), as well as the checkpoint kinases CHK1 and CHK2, and this phosphorylation contributes to their stabilization[23–28] (FIG. 2). Additionally, phosphorylation by these kinases activates p53 and fine-tunes its response to DNA damage. Similarly, DNA damage-induced phosphorylation modulates E2F1 activity. Specifically, CHK1 and CHK2 were shown to promote E2F1 stabilization and activity after genotoxic stress and thereby contribute to E2F1-induced upregulation of p73 (a member of the p53 family) and consequently apoptosis[28]. It has been proposed that this CHK1/CHK2–E2F1–p73 pathway functions as a backup when p53 is defective to ensure that damaged cells can undergo apoptosis. In many human tumours

that sustain an inactivating mutation of *TP53* the CHK1/CHK2–E2F1–p73 pathway is still intact. Therefore, this pathway has potential therapeutic importance as it can be activated to induce apoptosis in tumours lacking functional p53.

In addition to phosphorylation, both p53 and E2F1 undergo specific acetylations. Acetylation of p53 by p300 and PCAF in response to DNA damage is associated with DNA binding and transactivation by p53 (REF. 1). Likewise, E2F1 is acetylated by p300 and PCAF[29–31]. DNA damage-induced acetylation by PCAF stabilizes E2F1 (REF. 30) and biases it towards transactivation of pro-apoptotic targets, such as p73 (REF. 29). Overall, DNA damage-induced stabilization and activation of both E2F1 and p53, often by the same regulators, contributes to their apoptotic activity.

Another regulator of protein acetylation, the mammalian NAD-dependent deacetylase SIRT1, affects both p53 and E2F1. SIRT1 is an important regulator of metabolism, senescence, longevity and cancer, and it targets several transcriptional regulators, thereby affecting pivotal stress-responsive signal transduction pathways[32]. In particular, SIRT1 interacts with and inhibits the transcriptional and apoptotic functions of both p53 and E2F1 (REFS 33–35). Furthermore, SIRT1 was reported to repress both E2F1-dependent and p53-dependent apoptosis in response to DNA damage[33–35] (FIG. 2). Therefore, SIRT1 is a potentially crucial regulator of p53 and E2F1, particularly in the context of DNA damage. The existing data suggest that combining DNA-damaging drugs with inhibitors of SIRT1 could have synergistic effects in cancer therapy by maximally activating p53- and E2f-induced apoptosis.

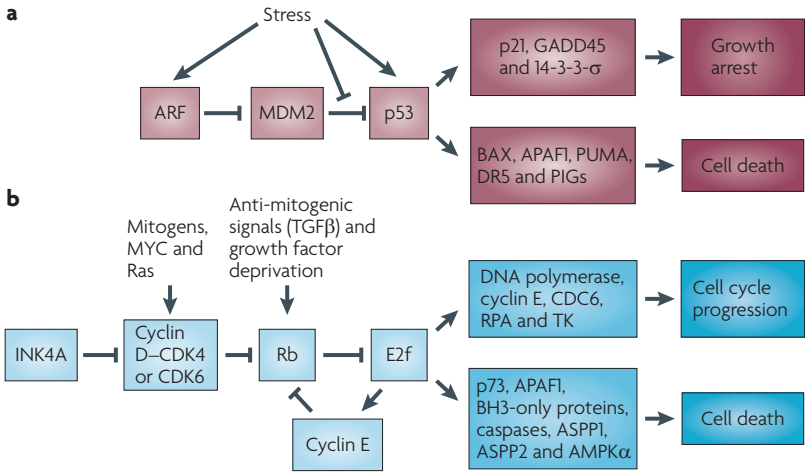


Figure 1 | **Regulation and activities of p53 and E2f. a** | The p53 transcription factor is negatively regulated by the E3 ubiquitin ligase MDM2. In response to various stresses the p53–MDM2 interaction is disrupted, either by post-translational modifications of p53 and/or MDM2 or by an interaction between ARF and MDM2, which enables p53 accumulation and activation. When p53 is activated it can induce several biological responses, including growth arrest, cell death, senescence and differentiation. p53-induced growth arrest is mediated by transactivated genes that encode inhibitors of cell cycle progression, such as the cyclin-dependent kinase (CDK) inhibitor p21. p53-induced apoptosis involves transactivation of numerous pro-apoptotic genes, as well as transcription-independent mechanisms, the latter typically involving the mitochondria[132]. **b** | E2f transcriptional activity is modulated by multiple mechanisms, the best known being interaction with members of the Rb family, namely RB, p107 and p130 (REF. 8). The presence of E2f–Rb complexes at the promoter of an E2f target gene not only inhibits the ability of E2f to transactivate but also actively represses transcription through the recruitment of various chromatin modifiers and remodelling factors, including histone deacetylases (not shown)[5]. Under conditions that trigger anti-proliferative signals, such as administration of transforming growth factor-β (TGFβ), Rb family members restrict cell proliferation largely through association with E2fs. These repressive E2f–Rb complexes mediate cell cycle exit and differentiation. Formation of Rb–E2f complexes is cell cycle regulated. Specifically, cyclins expressed at the G1 phase of the cell cycle activate their associated CDKs, which in turn phosphorylate Rb family members[133], resulting in the dissociation of Rb–E2f complexes. This leads to derepression and activation of E2f-regulated genes. Many E2f-regulated genes have a crucial role in S phase entry and cell cycle progression. In addition, E2F1 regulates the expression of pro-apoptotic genes and can induce apoptosis. APAF1, apoptotic protease-activating factor 1; ASPP1, apoptosis-stimulating protein of p53 1; DR5, death receptor 5; PIG, p53-induced gene; RPA, replication protein A; TK, thymidine kinase.

MDM2. The E3 ubiquitin ligase MDM2 seems to regulate both p53 and the Rb–E2f pathway. As alluded to above, MDM2 is a key regulator of p53. First, MDM2 directly binds to p53 and inhibits its transcriptional activity[36,37]. Second, as a p53-selective E3 ubiquitin ligase, MDM2 promotes p53 ubiquitylation and targets it for proteasomal degradation[36,37]. Third, recent studies show that MDM2 inhibits *TP53* mRNA translation[38]. Both extrinsic stimuli, such as DNA damage, and intrinsic stimuli, such as oncogene activation, impinge on the MDM2–p53 interaction to regulate the levels and transcriptional activity of p53.

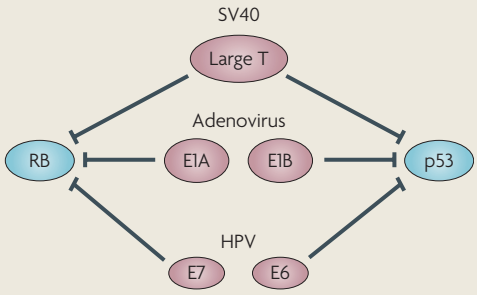
Although negatively regulating p53 is probably the primary function of MDM2, emerging evidence indicates that MDM2 possesses p53-independent tumorigenic activity, at least partly mediated by the Rb–E2f pathway. In line with a positive effect of MDM2 on the E2f pathway, MDM2 can physically interact with RB, E2F1 and the heterodimeric partner of E2F1, DP1, to promote the G1/S cell cycle transition[39–43]. Accordingly, MDM2 interaction with E2F1 or DP1 can stimulate transcription of E2F1 target genes that are involved in cell cycle progression[40]. Moreover, targeting of E2F1 for degradation by the F-box protein SKP2 is antagonized by the binding of MDM2 (REF. 44). Furthermore, MDM2 can promote RB degradation in a proteasome-dependent and ubiquitin-independent manner[43]. Indeed, in human cells *MDM2* ablation can result in RB accumulation and the inhibition of DNA synthesis, and MDM2 overexpression can inhibit RB-mediated growth suppression and can stimulate E2f transactivation activity[42,43].

However, an opposing outcome of interactions between MDM2 and the E2f pathway is suggested by the finding that MDM2 can downregulate the levels of E2F1 and DP1 subunits by inducing the degradation of E2F1–DP1 heterodimers[45]. Nevertheless, this apparently contradictory interaction could in fact promote tumorigenesis, as it has been suggested that such E2F1 downregulation could specifically antagonize E2F1-mediated apoptosis in p53-null cells[45]. Clearly, further studies are needed to characterize the role that MDM2 has in regulating E2F1 levels and activity. Nevertheless, studies to date suggest that overexpression of MDM2, which frequently occurs in numerous types of human tumours, probably modulates cell fate not only through p53 but also through the Rb–E2f pathway.

Box 1 | **Small DNA tumour viruses, p53, E2f and cancer**

The small DNA tumour viruses, simian virus 40 (SV40), human papillomaviruses (HPVs) and human adenoviruses can cause tumours in animals. Also, some HPVs are associated with cervical cancer in humans. The transforming potential of these viruses is mediated by viral oncoproteins that are essential both to establish tumours and to maintain the transformed cell phenotype.

The small DNA tumour viruses need to stimulate cellular pathways required for S phase entry and progression. Then the viruses use the cellular DNA precursors and enzymatic activities to replicate their own DNA. They effect cellular stimulation by releasing E2f from the inhibitory control of RB (see the figure). Accordingly, large T antigen of SV40, E1A protein of adenovirus and E7 protein of HPV each bind RB and release E2f. The uninhibited E2f transactivates many genes required for DNA replication. In addition, as part of a cellular fail-safe mechanism, the deregulated E2f activates p53 and thereby induces p53-mediated apoptosis. To circumvent this p53-mediated cell death (which would limit viral replication) the small DNA tumour viruses have evolved proteins that bind and inactivate p53. Large T antigen of SV40, E1B of adenovirus and E6 of HPV each bind and inactivate p53. In summary, to ensure their successful replication, the small DNA tumour viruses have evolved viral proteins that inactivate both RB and p53 (REF. 19). The transforming effect of these viruses is simply a by-product of their ability to initiate DNA replication and bypass the self-destruct mechanism triggered by inappropriate S phase entry. Both RB and p53 must be inactivated for virus-induced transformation. Initially, these data were interpreted as supporting the notion that the Rb–E2f and p53 pathways function independently in determining cell fate. However, subsequent analyses have unveiled intricate crosstalk between these pathways.



In agreement with this idea, the small-molecule MDM2 antagonist Nutlin 3 (originally developed to inhibit the p53–MDM2 interaction and activate p53 signalling) increases chemotherapy-induced apoptosis in cancer cells lacking functional p53 by activating E2F1 (REFS 46,47). Moreover, E2F1 transcriptional activity was found to be a crucial determinant of Nutlin 3-induced apoptosis in human tumour cell lines[48], highlighting the clinical relevance and potential of interactions between MDM2 and the E2f pathway, which could be exploited to treat tumours without functional p53.

The CDKN2A locus. The *CDKN2A* locus encodes two functionally unrelated inhibitors of cell cycle progression, INK4A and ARF, which activate RB and p53, respectively[49,50] (FIG. 2). INK4A inhibits phosphorylation of RB by CDK4 and CDK6, and thereby maintains RB in its growth-suppressive mode that arrests cells at the G1 phase of the cell cycle. ARF, as discussed below, stabilizes and activates p53. The organization of the *CDKN2A* locus is unusual, with the DNA sequences encoding the two proteins partially overlapping, although they are translated using alternative reading frames. Despite the partial overlap, ARF and INK4A are independently regulated, respond differentially to various signals and are separately silenced or mutated in some human tumours. Nevertheless, in many human tumours mutations or deletions in the overlapping part of these genes inactivate INK4A and ARF simultaneously, thereby compromising the functions of both RB and p53. Consequently, the *CDKN2A* locus is often considered as a regulator of both p53 and E2f.

The division of labour between the two products of the *CDKN2A* locus is probably not so strict. Cells devoid of all three Rb family members are resistant to ARF-induced growth arrest[51,52]. Similarly, cells overexpressing a dominant-negative E2f do not arrest in response to ARF[53]. These observations indicate that Rb family–repressor E2f complexes function downstream of ARF. In addition, cells lacking p53 exhibit marked resistance to INK4A-induced growth arrest[54]. Such findings corroborate and highlight the complex nature of crosstalk between the E2f and p53 pathways.

E2f activates p53

As mentioned above, most human tumours lack functional RB owing to one of several possible defects in the INK4A–RB cascade that lead to the activation of E2f. This deregulated and hyperactive E2f, which is present in most human tumours, constitutes an oncogenic stress that activates p53. One molecular mechanism that underlies the activation of p53 involves the direct transcriptional regulation of $CDKN2A^{ARF}$ by E2f[55]. ARF serves as a sensor of hyperproliferative signals that are generated by deregulated oncogenes such as *E2f*. An E2f-induced increase in ARF expression leads to p53 stabilization and activation[56] (FIG. 3). Notably, ARF also has p53-independent anti-proliferative activities[49] and it might therefore mediate p53-independent effects of E2F1 and should not be seen strictly as a linker between E2F1 and p53. Interestingly, E2f-regulated ARF expression is mediated through a non-consensus E2f binding site and, therefore, ARF is not upregulated by E2f during normal cell cycle progression. Only deregulated E2f activity leads to ARF expression and ensuing p53 activation[57]. In summary, deregulated E2f directly transactivates the expression of $CDKN2A^{ARF}$, which inhibits MDM2 function and leads to the stabilization and activation of p53.

Transcriptional regulation of $CDKN2A^{ARF}$ by E2f is only part of the E2f–ARF relationship. ARF interacts with E2F1 *in vivo*[58,59], inhibits its transcriptional activity in a p53-independent manner[59,60] and targets it for degradation[58]. Notably, ARF affects the biological outcome of E2F1 activity as E2F1-induced apoptosis is inhibited by ectopic expression of ARF[60] and augmented by the loss of $CDKN2A^{ARF}$ (REFS 61,62). Therefore, a negative feedback loop exists between E2f and ARF, whereby ARF inhibits its transactivator E2f. Similarly, and in addition, the relationship between p53 and ARF constitutes another negative feedback loop, as p53 restricts its own stabilization by downregulating transactivation of the $CDKN2A^{ARF}$ promoter[63]. Therefore, ARF represents a pivotal node in E2f–p53 crosstalk, and the p53–ARF–E2f module consists of two negative feedback loops: ARF stabilizes and activates p53 and is transcriptionally downregulated by p53; in addition, $CDKN2A^{ARF}$ is transcriptionally

upregulated by E2f (and/or transcriptionally downregulated by Rb–E2f complexes) and destabilizes and inhibits E2f (FIG. 3).

In some settings, E2F1-induced activation of p53 can be mediated by mechanisms that are independent of ARF, as indicated by the findings that RB inactivation or E2F1 overexpression can induce p53-dependent apoptosis in *Cdkn2a*^{ARF}-deficient mice and cells[61,62,64]. E2f-induced apoptosis in the absence of ARF correlates with p53 phosphorylation on residues that are also phosphorylated in response to DNA damage by the protein kinases ATM and ataxia telangiectasia and Rad3-related (ATR) and their downstream effectors, CHK1 and CHK2 (REFS 61,65,66). Accordingly, E2F1 has been found to influence the expression and/or activity of ATM and CHK2, as well as the stress-related kinase p38, all of which phosphorylate and activate p53 (REFS 66–69) (FIG. 3). Furthermore, in cells lacking functional ATM, the ability of E2F1 to induce the phosphorylation of p53 and, therefore, apoptosis is impaired[67,68]. Also, cells with mutated Nijmegen breakage syndrome protein 1 (NBS1, a component of the MRE11–RAD50–NBS1 DNA damage response complex) exhibit attenuated p53 phosphorylation and apoptosis in response to E2F1 expression[67,68]. In summary, several DNA damage response factors participate in ARF-independent crosstalk between E2F1 and p53 (REFS 67,68). Notably, cancer-related aberrations in the Rb pathway seem to induce (to variable degrees) the p53-dependent DNA damage response. This, therefore, creates dissimilar selection pressures to inactivate p53 in tumour cells. For example, inactivation of RB, but not overexpression of cyclin D or inactivation of INK4A, induces p53 phosphorylation[70].

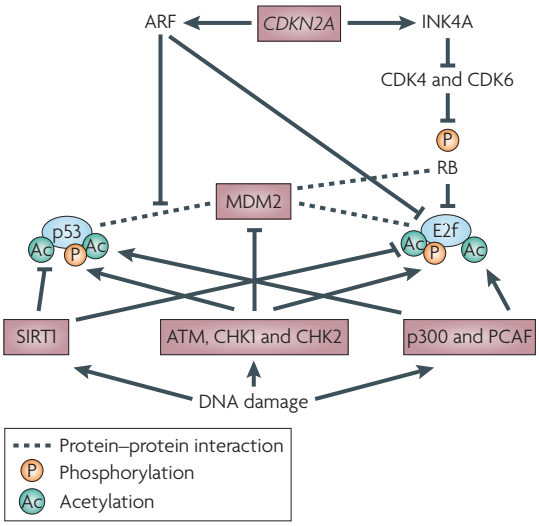


Figure 2 | **Common regulators of E2f and p53.** One common regulator of E2fs and p53 is the *CDKN2A* locus, which encodes INK4A and ARF. ARF positively regulates p53 by inhibiting MDM2, and negatively regulates E2f. INK4A negatively regulates E2f activity by inhibiting RB phosphorylation. Another regulator of p53 and E2f is MDM2, which negatively regulates p53 and also affects the Rb–E2f pathway. E2f and p53 both function in the DNA damage response. Some common regulators also function in this setting. The DNA damage-induced kinases ataxia telangiectasia mutated (ATM), CHK1 and CHK2 phosphorylate and stabilize both proteins. In addition, both proteins are positively regulated by damage-induced acetylation by p300 and PCAF and are negatively regulated by the deacetylase SIRT1. Therefore, a complex, and partially common, set of regulators modulates the activity of p53 and E2f.

E2F1, E2F2 and E2F3 can also influence p53 activity by binding directly to p53. This mechanism of crosstalk has been best studied for E2F1. Binding of E2F1 requires p53 phosphorylation at Ser315 and increases p53 nuclear retention, perhaps by masking a nuclear export signal in p53, and therefore improves p53 DNA binding, as well as transactivation and apoptotic functions[71,72]. Other putative mechanisms underlying crosstalk between E2f and p53 involve certain E2f-regulated genes, such as *PIN1* (REF. 73), which encodes a prolyl isomerase; *SIRT1*, which encodes a deacetylase[33]; and *SKP2* (REF. 74), which encodes an F-box protein that targets several cell cycle proteins, including p27, for ubiquitylation and subsequent degradation.

PIN1 interacts with p53 during stress[75,76] and orchestrates p53 acetylation and dissociation from inhibitor of apoptosis-stimulating protein of p53 (iASPP); therefore, PIN1 increases the pro-apoptotic activity of p53 (REF. 77). In contrast to this E2f–PIN1-mediated activation of p53, SKP2 and SIRT1 probably inhibit p53 activity. Specifically, SKP2 antagonizes the interaction between p300 and p53, thereby suppressing p300-mediated acetylation of p53 and accordingly the transactivation ability of p53 (REF. 78). Similarly, SIRT1 binds and deacetylates p53 thereby repressing p53-induced apoptosis[34].

In summary, current data indicate that E2f can affect, both positively and negatively, the level and activity of p53 through the regulation of numerous genes (FIG. 3). Activation of p53 by E2F1 is well established, whereas suppression of p53 activity by E2f, for example through SKP2 or SIRT1, remains enigmatic. In particular, the physiological context in which such inhibitory crosstalk can determine cell fate needs to be identified.

Considering the many E2f–p53 functional interactions and feedback mechanisms described above, a puzzling question is raised: what determines the path a cell takes in response to a given stress signal? Clearly, not all the interactions discussed here operate simultaneously. Deciphering the contextual relevance of each E2f–p53 interaction and the effects of external stimuli on these interactions are goals of ongoing research. The abundance of proteins and pathways that link E2f activity to p53 activation probably reflects the importance of p53 in the cellular response to deregulated E2f. In most cases, the biological outcome of E2f-mediated p53 activation is p53-induced apoptosis. As such, this activation of p53 constitutes the fail-safe switch that directs pre-malignant cells with deregulated E2F1 to elimination before they become fully transformed.

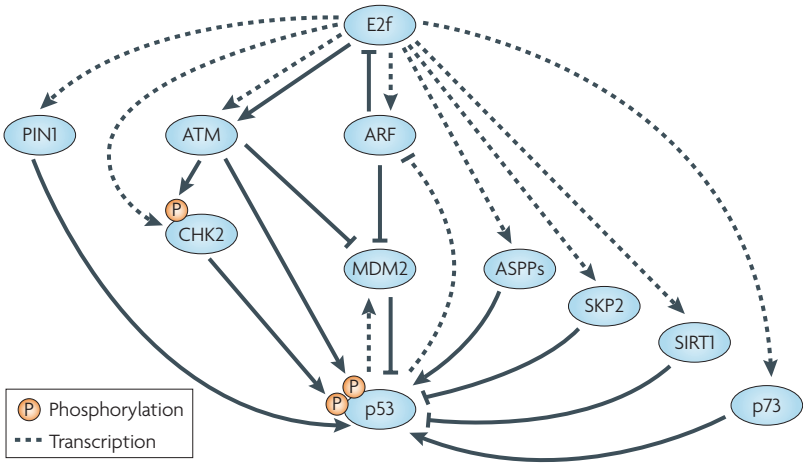


Figure 3 | **E2f regulates p53 level and activity.** E2f affects the levels and activity of p53 indirectly, by regulating the expression of genes that encode proteins that impinge on p53. Among these genes are positive and negative regulators of p53 that affect p53 through distinct mechanisms. ARF increases p53 protein levels by inhibiting MDM2. Ataxia telangiectasia mutated (ATM) and CHK2 phosphorylate and activate p53 in response to DNA damage. PIN1 interacts with p53 in response to genotoxic stress or the activation of oncogenes and increases the pro-apoptotic activity of p53. Apoptosis-stimulating of p53 protein 1 (ASPP1) and ASPP2 are pro-apoptotic cofactors of p53. p73 is a p53 family member that also serves as a pro-apoptotic cofactor of p53 (REF. 134). E2f targets that negatively regulate p53 include SKP2 and SIRT1.

A set of experiments support this model; specifically, mice directed to express a human *E2F1* transgene in the epidermis exhibit hyperproliferation, as well as p53-dependent apoptosis, and develop skin tumours at the age of around 1 year[79]. Importantly, crossing these *E2F1*-transgenic mice with *Trp53*-deficient mice (null or heterozygous) results in mice that show reduced epidermal E2F1-induced apoptosis, but E2F1-induced hyperproliferation is unaffected. Moreover, the resulting *Trp53*-deficient *E2F1*-transgenic mice develop E2F1-induced skin tumours at an earlier age, indicating that p53 has an important role in eliminating cells with deregulated E2F1 activity[80].

E2f and p53 cooperate in apoptosis

Studies performed 15 years ago, soon after the cloning of *E2F1*, demonstrated that E2F1 and p53 cooperate to induce apoptosis[81]. Intensive research has identified several molecular mechanisms underlying this cooperation. First, as described above, E2F1 induces stabilization and activation of p53. Second, E2F1 and p53 separately transactivate a plethora of crucial pro-apoptotic genes, raising the possibility that one or more of their respective targets cooperate to induce apoptosis. For example, E2F1 transcriptionally regulates the expression of several caspases[82], whereas p53 upregulates expression of BAX[83], which affects the release of pro-apoptotic molecules, such as cytochrome *c* and DIABLO, from the mitochondria, thereby indirectly contributing to the activation of caspases (FIG. 4). Third, several pro-apoptotic genes, including *APAF1*, *SIVA* and the BH3-only protein-encoding genes *NOXA* and *PUMA*, seem to be transcriptionally regulated by both E2F1 and p53 (REFS 84–89) (FIG. 4). Taken together with the activation of p53 by E2F1, this pattern of regulation can be considered a feedforward loop (BOX 2). Also, both p53 and E2F1 were shown to negatively regulate the anti-apoptotic members of the Bcl-2 family BCL2 and MCL1 through various mechanisms[90–93] (FIG. 4). Therefore, in cells in which RB is functionally inactive, leading to hyperactive E2F1 (although the p53 pathway is intact), p53 and E2F1 can cooperate in the regulation of apoptotic genes, thereby increasing the likelihood that such pre-malignant cells are eliminated. Loss of p53 in this context decreases the apoptotic potential of the cells.

E2F1 activity biases p53 towards apoptosis. p53 functions as a key signal integrator that translates diverse stress signals into distinct cellular outcomes, including cell cycle arrest and apoptosis. The p53-mediated response depends not only on the incoming stress signal but also on the intracellular environment. Typically, the assorted intracellular and extracellular signals are transduced by specific cofactors that are associated with p53 and certain p53 post-translational modifications, which in turn to a large extent dictate the subgroup of p53 target genes that are induced or repressed[1]. E2F1-dependent activation of p53 results specifically in apoptosis. The previously mentioned crosstalk between E2F1 and p53, which is mediated through ARF, ATM, CHK2 and PIN1, does not fully explain this phenomenon. The bias of E2F1-activated p53 towards apoptosis, as opposed to growth arrest, is largely attributed to the ability of E2F1 to upregulate the expression of two pro-apoptotic p53 cofactors, ASPP1 and ASPP2 (REFS 94–96). The Aspp family comprises three members, ASPP1, ASPP2 and iASPP, all of which bind the DNA-binding domain of p53. iASPP inhibits p53-mediated apoptosis, whereas ASPP1 and ASPP2 increase p53-dependent apoptosis by stimulating the binding of p53 to pro-apoptotic gene promoters, such as those of *BAX* and *PIG3* (also known as *TP53I3*)[97].

In addition, E2F1 upregulates the expression of tumour protein p53-inducible nuclear protein 1 (*TP53INP1*)[94], which mediates p53 phosphorylation on Ser46, a modification shown to trigger the dissociation of p53 from iASPP[77] and to promote the induction of p53 apoptotic targets, such as *P53AIP1* (REF. 98). In summary, through the transcriptional regulation of *ASPP1*, *ASPP2*, *PIN1* and *TP53INP1*, E2F1 activity favours the formation of p53–ASPP1 and p53–ASPP2 complexes rather than p53–iASPP complexes and so biases p53 activity towards apoptosis.

Interestingly, at least in some organisms the crosstalk between E2f1 and p53 in the induction of apoptosis seems to be context dependent. For example, in the development of *Drosophila melanogaster* it seems that the pro-apoptotic activities of E2f and p53 are independent of one another[99]. However, E2f- and p53-induced apoptosis converge in the context of a DNA damage response[99]. This convergence, which is evolutionarily conserved and present in humans, is relevant to tumour progression and treatment as

p53 status and levels of E2f activity influence the extent of the apoptotic response of the cells to both tumorigenesis-associated and chemotherapy-induced DNA damage.

E2f and p53 in cell cycle arrest and senescence

Studies of crosstalk between E2F1 and p53 in the context of apoptosis largely indicate that E2F1 functions upstream of p53. However, examination of the functional links between p53 and E2fs in cell cycle progression reveals a different order of events. In cell cycle arrest the most well-documented link between p53 and E2fs is the CDK inhibitor p21, a classic transcriptional target of p53 that impinges on the Cdk–Rb–E2f pathway leading to the repression of E2f activity and cell cycle arrest. Furthermore, recent studies have provided evidence for additional p53–E2f crosstalk in cell cycle arrest, whereby activator E2fs repress p53 activity and repressor E2fs function downstream of p53.

Activator E2fs can function as suppressors of p53. Recent studies suggest that transcriptional activation and repression by E2fs are mechanistically linked by p53. Specifically, targeted disruption of activator E2fs (E2F1, E2F2 and E2F3) leads to p53 activation and the sequential induction of p53 target genes, including *CDKN1A* (which encodes p21); hypophosphorylation of RB, p107 and p130; recruitment of E2F4–p130 complexes to E2f-regulated promoters; repression of E2f target genes; and ultimately cell cycle arrest at the G1/S and/or G2/M checkpoints[100–102]. Surprisingly, ablation of *TP53* in cells also deficient in *E2F1–3* was found to restore the expression of E2f target genes, as well as the capacity of such cells to proliferate[100,101]. These data indicate that negative regulation of p53 by the activator E2fs is required for normal cell cycle progression (FIG. 5). Notably, assuming that this functional link between E2f and p53 is conserved between mice and humans there are potentially important clinical implications as it suggests that anticancer therapeutic strategies targeting E2F1–3 will be effective only in tumour cells that have functional p53. Also, these data indicate that E2F1–3 control p53-dependent mechanisms that in turn control E2f-mediated repression and that this repression is crucial for normal cellular proliferation (FIG. 5). Therefore, anticancer therapeutic strategies that induce p53-mediated growth arrest of tumour cells will succeed only in cells in which the repressor E2fs remain functional.

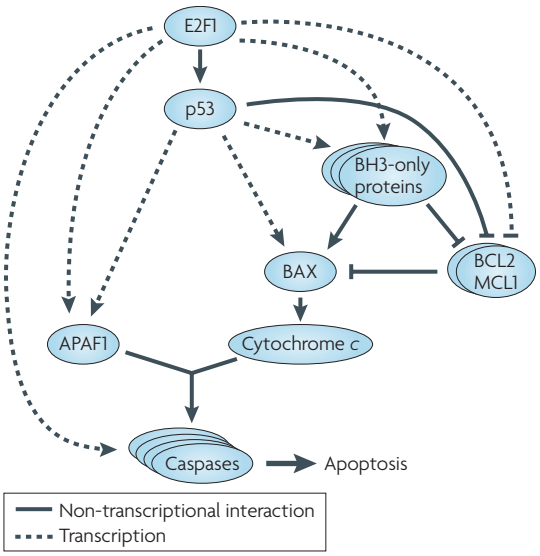


Figure 4 | **E2F1 and p53 cooperate in apoptosis.** Several distinct molecular mechanisms underlie the p53–E2F1 cooperation in apoptosis: E2F1 activates p53; E2F1 and p53 activate distinct pro-apoptotic genes that can cooperate in inducing apoptosis; p53 and E2F1 co-regulate pro-apoptotic genes, for example the BH3-only protein-encoding genes *PUMA* and *NOXA*; and p53 and E2F1 negatively regulate anti-apoptotic genes such as *BCL2* and *MCL1*.

Certain aspects of this E2f–p53 crosstalk remain enigmatic. Notably, ablation of *CDKN1A* in *E2F1–3*-deficient cells rescues the G1/S arrest but not the G2/M arrest. These observations indicate, on the one hand, that p21 has an important role in mediating E2f-dependent, p53-induced growth inhibitory effects at the G1/S boundary and, on the other hand, that p53 directs E2f-mediated growth arrest at the G2/M boundary through p21-independent mechanisms that are as yet unidentified[101]. This p53-induced E2f-mediated arrest at the G2/M boundary may involve the p53 targets GADD45 and 14-3-3-σ, which affect the activity of the CDC2 (also known as CDK1) kinase. The identity of the E2f target genes that mediate this p53-induced arrest at the G2/M boundary remains to be determined. Also, it is currently unclear how activator E2fs downregulate p53 activity. Loss of *E2F3* results in the derepression of $CDKN2A^{ARF}$, concomitant p53 activation and increased p21 levels, suggesting that the repression of $CDKN2A^{ARF}$ by E2F3 mediates the repression of p53 by activator E2fs[103]. However, other studies show that the loss of *E2F1–3* leads to the activation of p53 target genes independently of $CDKN2A^{ARF}$ transcriptional induction[100]. Therefore, additional studies are required to elucidate the mechanism underlying E2f-induced suppression of p53.

Repressor E2fs are effectors of p53-induced growth arrest and senescence. Several studies indicate that the Rb family–repressor E2f complexes that function downstream of p53 mediate growth arrest at G1 and G2 cell cycle checkpoints. First, loss of Rb abrogates DNA damage-induced p53-mediated G1 growth arrest[104]. Second, in response to DNA damage or ectopic p53 expression, p130–E2F4 complexes bind the promoters of genes required for the G2/M transition and repress their expression, thereby contributing to damage-induced arrest at G2 (REFS 105,106). Last, expression of either a dominant-negative E2f (which neither transactivates target genes nor interacts with Rb family members but displaces Rb family–E2f complexes from DNA) or the human papillomavirus E7 protein (which disrupts Rb–E2f complexes) relieves DNA damage-induced G2 arrest[105,107].

Replicative senescence
A largely irreversible spontaneous proliferative arrest of normal untransformed cells after a limited number of cell divisions. It is often caused by progressive shortening of the telomeres at each round of cell division.

Premature senescence
Senescence that occurs before telomeric shortening. Such premature senescence is often associated with the activation of the tumour suppressors INK4A, ARF, p53 and RB.

Similarly, Rb family–repressor E2f complexes are essential downstream effectors of p53-mediated senescence, whether it is spontaneous replicative senescence or premature senescence. Accordingly, mouse embryonic fibroblasts (MEFs) deficient in ARF or p53 do not undergo spontaneous replicative senescence[108,109]. Also, MEFs lacking all three Rb family members (from mice with triple knockout of *Rb1*, *Rbl1* (which encodes p107) and *Rbl2* (which encodes p130)) do not senesce and are resistant to ARF-induced growth arrest[51,52]. Finally, MEFs expressing dominant-negative E2f are immortal and resistant to either ARF- or p53-induced senescence[53]. In summary, a large body of evidence indicates that protein complexes containing members of the Rb family and repressor E2fs are essential downstream mediators of p53-induced growth arrest — be it a transient arrest, at either the G1 or G2 checkpoint, or the irreversible arrest exhibited by senescent cells.

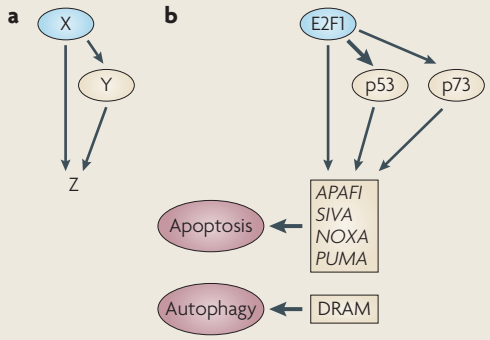
Box 2 | **E2f and the p53 family: a modified feedforward loop**

Expression of the pro-apoptotic genes *APAFI*, *SIVA*, *NOXA* and *PUMA* is regulated by both E2F1 and p53 (REFS 84–89). Taken together with the activation of p53 by E2F1, this pattern of regulation constitutes a modified feedforward loop.

A feedforward loop is a common network motif that consists of three genes: a regulator, X, that regulates Y, and a gene, Z, that is regulated by both X and Y (see the figure, part **a**). In transcriptional networks the functions of the transcription factors X and Y can be integrated to regulate the Z promoter through several 'gates': an 'AND gate', in which both X and Y are needed to activate Z, an 'OR gate', in which binding of either regulator is sufficient, or a 'SUM gate' that exhibits additive input function[124].

Notably, the E2F1–p53 feedforward loop (see the figure, part **b**) is not a classic one as E2F1 does not activate p53 transcriptionally. Instead, E2F1 transcriptionally regulates several genes that affect the stability and activity of p53. The mode of action of this modified E2f–p53 feedforward loop has not been determined. Nevertheless, as ectopic expression of E2F1 upregulates the apoptotic genes even in cells lacking p53, an AND gate mode of action can be excluded. It is most likely that such a feedforward loop functions through an OR gate or a SUM gate at the level of the pro-apoptotic genes. As E2F1 and p53 are both activated in response to various stresses, in particular DNA damage, it is possible that, at least in this context, they cooperate in upregulating expression of their common target genes (*APAFI*, *SIVA*, *NOXA* and *PUMA*) above a threshold to ensure the stressed cell attains an apoptotic fate.

E2F1 and p53 also potentially regulate non-apoptotic genes through a feedforward loop. The genes encoding the NAD-dependent deacetylase SIRT1 and DRAM (which is associated with autophagy) are regulated by both p53 and E2F1 (REFS 33,119,125,126). In addition, *CDKN1A*, a key p53 target gene that encodes the cell cycle inhibitor p21, has been proposed to also be regulated by E2F1 (REF. 127). Further study is required to understand the role of feedforward loop motifs in the regulation of biological processes by E2F1 and p53. Noteworthy is the finding that E2F1 directly regulates the expression of the p53 family member p73 (REFS 128–130). Like p53, p73 can regulate the expression of many pro-apoptotic genes and therefore has been suggested to have a pivotal role in E2F1-induced p53-independent apoptosis. Also, p73 directly transactivates *DRAM*[131]. Therefore, E2F1 and p73 and their common target genes constitute another feedforward loop, in this case a more classic one that directs cells towards cell death.



Potential inhibition of activator E2fs by p53-regulated genes. Another potential link between p53 and E2fs that could play a part in regulating cell proliferation is suggested by the recent identification of p53-regulated genes that inhibit activator E2fs. For example *BTG3*, a candidate tumour suppressor induced by p53 after DNA damage, was recently shown to modulate the G2/M checkpoint by blocking E2F1 DNA-binding activity[110] (FIG. 5). Another example is *miR-34a*, which encodes a microRNA upregulated by p53 that partially mediates p53-dependent antiproliferative and pro-apoptotic effects[111,112]. The genes targeted, and therefore downregulated, by *miR-34a* have not been fully characterized, although data indicate that *miR-34a* inhibits the E2f pathway in human colon cancer cells[113] and specifically the expression of E2F3 (REF. 114). Notably, E2F3 has a crucial role in cell proliferation[102,115] and is amplified or overexpressed in certain human cancers, such as bladder and prostate cancer, suggesting an important role for this particular family member in some human malignancies[16,17,116]. Therefore, it is conceivable that a p53–*miR-34a*–E2F3 axis substantially affects cell proliferation and transformation.

Future directions

Data accumulated over the past 15 years clearly indicate that although p53 and E2f are each typically deregulated in human cancer and affect the fate of cancer cells, they do not function independently. Indeed, there is extensive crosstalk between these two transcription factors and, more generally, between the pathways in which they function.

Notably, the mechanisms of crosstalk between E2Fs and p53 have generally been studied in tissue culture. Therefore, determining the physiological relevance of such crosstalk is of great importance. In particular, given the many signals that co-regulate E2fs and p53, and the multiple mechanisms of crosstalk between E2fs and p53, it is imperative to identify *in vivo* the specific interactions that influence cancer development and treatment.

It is likely that other functional interactions between these two pivotal regulators of cell proliferation and viability remain to be discovered. For example, in addition to the documented effects of MDM2 (a p53 transcriptional target) on E2f activity, other p53 effectors could affect E2f levels and activity. For example, p53 target genes that affect signal transduction pathways, such as the genes encoding the phosphatases PTEN and WIP1, could indirectly affect phosphorylation of Rb and so influence E2f activity.

Notably, the regulation of crucial apoptotic genes by E2F1 and p53 (and/or p73) in a feedforward loop requires additional study. In particular, it remains to be confirmed whether this feedforward loop has a significant role in E2F1–p53 cooperation during apoptosis. Also, although the effects of E2f–p53 functional connections in cell proliferation, senescence and apoptosis are well documented, further study is necessary to develop a comprehensive understanding of the outcomes of such crosstalk on other biological processes. For example,

Gain-of-function mutations
Mutations that change the gene such that the protein gains a new and abnormal function.

both p53 and E2F1 have recently been shown to have a role in autophagy[117–120], which raises the question: do they cooperate in this and other types of non-apoptotic programmed cell death? Similarly, do p53 and E2F1 cooperate in DNA damage responses other than apoptosis? Both p53 and E2F1 were shown to affect DNA repair and activation of checkpoints, primarily through the transcriptional regulation of genes that function in these processes. However, it remains to be more rigorously investigated whether they cooperate in the regulation of the DNA damage responses.

We suspect that more E2f–p53 crosstalk remains to be discovered. For example, many studies demonstrate that certain p53 mutants are not simply inactive but have, in fact, gain-of-function mutations[121]. It is not known whether E2fs contribute to mutant p53 functions. Also, there could be crosstalk between p53 and the most recently identified members of the E2f family: E2F7 and E2F8. These E2fs mainly function as transcriptional repressors in an Rb-independent manner and seem to repress the expression of a subset of E2f-regulated genes (including *E2F1*) and thereby influence cell proliferation and viability. Initial studies support such crosstalk, as levels of apoptosis detected in *E2f7*$^{-/-}$;*E2f8*$^{-/-}$ mice are significantly reduced when p53 is inhibited[122].

In addition, the emerging field of onco-miRs is expected to reveal new aspects of the crosstalk between p53 and E2fs. For example, there could be miRs that are co-regulated by E2f and p53, as suggested by a recent study identifying miRs that are repressed by p53 in an E2f-dependent manner[123]. In addition, there could be miRs that are regulated by either E2f or p53, and target the other. Indeed, the aforementioned *miR-34a* may represent the first example of this situation[111,112,114].

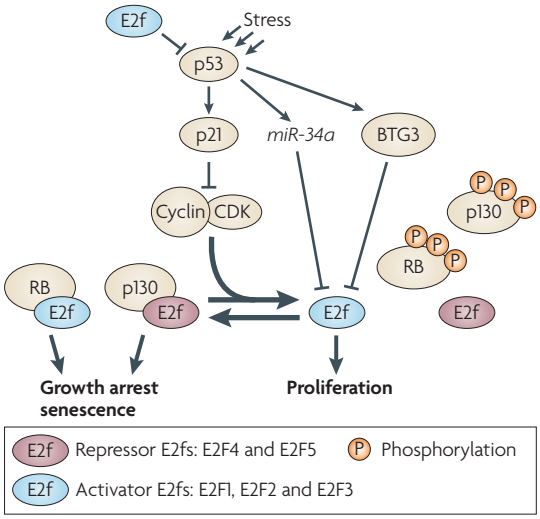


Figure 5 | **E2f in growth control.** Crosstalk between E2fs and p53 also exists in the context of cell cycle exit and senescence. First, activator E2fs function as suppressors of p53. Second, p53-induced growth suppression is mediated by Rb family–repressor E2f complexes, partially through the cyclin-dependent kinase (CDK) inhibitor p21. In addition, p53-regulated genes, such as *BTG3* and *miR-34a* can inhibit activator E2fs, resulting in the inhibition of proliferation.

Clearly the Rb–E2f and MDM2–p53 pathways, along with the multifaceted crosstalk between them, are crucial regulators of cell cycle progression and viability. As the pieces of the p53–E2f crosstalk puzzle fall into place, the big challenge lying ahead is to translate this knowledge into combined therapeutic approaches that improve the diagnosis and treatment of cancer patients.

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DATABASES

Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
APAF1 | *BTG3* | *CCND1* | *NOXA* | *P53AIP1* | *PIG3* | *PIN1* | *PUMA* | *Rb1* | *SIVA* | *TP53INP1*
Pathway Interaction Database: http://pid.nci.nih.gov/
ARF | E2f | p38
UniProtKB: http://www.uniprot.org
ASPP1 | ASPP2 | ATM | ATR | BAX | BCL2 | CDC2 | CHK1 | CHK2 | DP1 | E2F1 | E2F2 | E2F3 | E2F4 | E2F5 | E2F7 | E2F8 | iASPP | INK4A | MCL1 | MDM2 | MRE11 | NBS1 | p21 | p300 | p53 | p73 | p107 | p130 | PCAF | PTEN | RAD50 | RB | SIRT1 | SKP2 | WIP1

FURTHER INFORMATION

Doron Ginsberg's homepage:
http://www.biu.ac.il/faculty/ginsbed

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