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## Emerging roles of E2Fs in cancer: an exit from cell cycle control

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### Abstract

Mutations of the retinoblastoma tumour suppressor gene (*RB1*) or components regulating the RB pathway have been identified in almost every human malignancy. The E2F transcription factors function in cell cycle control and are intimately regulated by RB. Studies of model organisms have revealed conserved functions for E2Fs during development, suggesting that the cancer-related proliferative roles of E2F family members represent a recent evolutionary adaptation. However, given that some human tumours have concurrent *RB1* inactivation and E2F amplification and overexpression, we propose that there are alternative tumour-promoting activities for the E2F family, which are independent of cell cycle regulation.

Two decades of experimentation link E2F activity to cell cycle control<sup>1–4</sup>. The E2Fs are a large family of transcription factors containing one or more conserved DNA binding domains (DBDs) that bind target promoters and regulate their expression<sup>1,2</sup>. The RB tumour suppressor and the RB-related pocket proteins p107 and p130 directly associate with E2Fs and can be co-recruited to E2F-responsive promoters to modulate gene expression<sup>5</sup>. Functional inactivation of *RB1* in various human cancers and transgenic animal models leads to deregulated E2F activity, which has been correlated with aberrant cell proliferation and in some instances cell death<sup>6–8</sup>. Consistent with these observations, global gene expression profiling and promoter occupancy (chromatin immunoprecipitation (ChIP)-on-chip and ChIP-sequencing) arrays have confirmed that many genes that are crucial for proper cell cycle progression are bona fide E2F targets<sup>9–15</sup>, therefore establishing a direct role for E2Fs in governing cell proliferation. In recent years, more members and isoforms of the E2F family have been described and their functions explored in different physiological settings unrelated to the control of cell proliferation<sup>1,2,8,10,16–21</sup>. These studies have yielded results inconsistent with the central dogma of the RB–E2F pathway that has been defined by *in vitro* and cell culture assays. The aim of this Review is to present emerging data from mouse models of development and tumorigenesis that challenge the principles of RB–E2F function in coordinating cell cycle progression. We present the view that *in vivo* E2F functions extend beyond the control of cell proliferation, and discuss how these functions

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**DATABASES** **Entrez Gene:** <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>

*CDK8* | *E2F1* | *ErbB2* | *Hras*

**UniProtKB:** <http://www.uniprot.org>

*CDK1* | *CDK2* | *CDK4* | *CDK6* | *CEBPα* | *E2F2* | *E2F4* | *E2F5* | *E2F6* | *E2F7* | *E2F8* | *MYC* | *p107* | *p130* | *RB*

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**SUPPLEMENTARY INFORMATION** See online article: [S1](#) (box)

may be implicated in the pathogenesis of multiple human cancers independent of cell cycle control.

## E2Fs: the current paradigm

### E2F function is regulated by cyclin-dependent kinases (CDKs) and RB

It was discovered nearly two decades ago that infection by DNA tumour viruses caused excessive host cell proliferation, which was associated with the binding of specific cellular factors – later named E2Fs – to viral gene promoters<sup>22,23</sup>. These initial observations paved the way for the identification of an intricate signalling pathway that has been shown to culminate in the control of E2F transcription activity (BOX 1). Since the identification of the founding E2F family member *E2F1* (Refs 24,25) two distinct genes in *Drosophila melanogaster* and seven additional genes in mammals have been found to encode the signature DBDs that give these transcription factors their DNA binding specificity<sup>1,2,8</sup> (FIG. 1; see Supplementary information S1 (box)). The key finding that overexpression of E2F activators could trigger quiescent cells to enter G1 phase, independent of growth factor stimulation, linked this transcriptional module to cell cycle control<sup>26</sup> and fuelled the initial identification of important E2F target genes that are involved in DNA replication. Thereafter, numerous studies focusing on how E2Fs contribute to cell proliferation elucidated a molecular signalling network that converged on the post-translational modifications of RB and RB-related proteins (which are mediated by cyclin-CDK complexes) and the subsequent waves of E2F-dependent transcription activation and repression that guarantee the timely movement of cells through all four phases of the cell cycle<sup>27s-30</sup> (FIG. 2). It should be emphasized that the simple classification of E2Fs as activators and repressors is mostly based on the analysis of cells cultured *in vitro* and lacks *in vivo* validation.

### Redundancy, antagonism and feedback: the complexities of E2F function

Three obstacles have precluded a thorough analysis of the role of E2Fs *in vivo*. First, the high degree of functional redundancy among activators (E2F1, E2F2, E2F3a and E2F3b)<sup>1,2,6-8,31,32</sup> and repressors (E2F4, E2F5, E2F6, E2F7 and E2F8)<sup>17,33</sup> has made the study of these proteins technically challenging and experimental results difficult to interpret. The systematic analyses of mice with single and compound E2F mutations initially revealed the redundant nature of E2F activity. Moreover, recent gene knock-in strategies in mice have demonstrated that expression from the endogenous *E2f3a* locus of the highly related *E2f3b* isoform or the more distantly related *E2f1* family member suppressed most of the early postnatal developmental phenotypes associated with the inactivation of *E2f3a*<sup>31</sup>.

The second obstacle impeding a complete understanding of E2F functions *in vivo* is the apparent functional antagonism between E2F-mediated activation and repression in the control of normal cell proliferation. Results from initial studies in *D. melanogaster* seemed to validate the straightforward model of E2F function as an activity that promoted cell cycle progression. Unlike in mammals, the *D. melanogaster* E2F family consists of a single transcription activator (E2F) and repressor (E2F2). Both proteins require dimerization with the transcription factor DP, which is encoded by a single gene. Loss-of-function mutations in *E2f* lead to a decrease in the expression of classic E2F-regulated genes, DNA synthesis and larval survival<sup>34,35</sup>. Surprisingly, loss of *E2f2* restored cell proliferation in *E2f*-mutant larvae and significantly delayed lethality until mid or late pupal stages<sup>35</sup>. These results suggest that the net effect of E2Fs on cell proliferation is dependent on the balanced interplay between activation and repression on a shared set of E2F-regulated genes. However, subsequent analysis of gene expression in S2 cells lacking *E2f*, *E2f2* or *Dp* (the latter of which abrogates total E2F activity in *D. melanogaster*) revealed surprisingly little

overlap in genes regulated by *E2f* and *E2f2* (REF. 11). Consistent with this gene expression data, a recent genetic mosaic screen found that the cell cycle defects induced by loss of E2F could be rescued independently of functionally inactivating *E2f2* and abrogating E2F2-mediated repression<sup>36</sup>. Therefore, in addition to regulating a common set of cell cycle genes, E2F and E2F2 seem to contribute independently to the normal proliferative potential of a cell.

The third reason why the study of E2Fs remains a challenge is their ability to regulate the expression of other members of the family, forming complex feedback loops to ensure a balanced level of activators and repressors in each phase of the cell cycle. A clear example of this was revealed by the recent molecular analysis of *E2f7*<sup>-/-</sup>;*E2f8*<sup>-/-</sup> mouse embryos<sup>17</sup>. E2F7 and E2F8 directly repress *E2f1* expression and double-knockout embryos have increased levels of E2F1 and widespread apoptosis. The removal of *E2f1* from these mutant embryos significantly reduced the level of apoptosis. E2F7 and E2F8 are the most recently discovered E2F family members and represent a structurally distinct arm of the repressor subclass that interestingly is not unique to mammals. The model plant *Arabidopsis thaliana* has three such 'atypical' E2Fs, E2FE, E2FD and E2FF<sup>37</sup> (Supplementary information S1 (box)). The general functions of these plant E2F repressors involve the control of endocycle onset and progression, as well as the regulation of cell size<sup>38,39</sup>. Similar to their plant counterparts, the expression of mammalian *E2F7* and *E2F8* is E2F-regulated – *in silico* analysis of their promoters identified consensus E2F binding motifs to which E2F1, E2F3, E2F4 and E2F7 were shown to bind using ChIP assays<sup>40,41</sup>.

### RB–E2F developmental mouse models support the canonical pathway

A considerable number of developmental studies using RB and RB–E2F compoundmutant mice seem to reinforce the role of E2Fs in cell proliferation and apoptosis that was originally identified *in vitro*<sup>42–44</sup>. Although homozygous inactivation of *Rb1* in mice leads to embryonic lethality that is accompanied by massive ectopic proliferation and apoptosis in multiple tissues<sup>45–47</sup>, the deletion of E2F activators has been shown to partially rescue the proliferative and/or apoptotic phenotypes. Specifically, the loss of *E2f1* or *E2f3a* most robustly suppressed *Rb1*-mutant phenotypes and extended the viability of *Rb1*<sup>-/-</sup> embryos to late stages of embryogenesis<sup>42,48</sup>. The loss of *E2f3b*, which encodes an E2F3 isoform thought to repress gene expression, also alleviated a subset of phenotypes that are associated with the complete loss of *Rb1* (REF.48–51). The observation that loss of the repressor *E2f4* or *E2f5* did not rescue *Rb1*-mutant phenotypes in the mouse embryo is consistent with their role as binding partners of RB that mediate repression<sup>44</sup>. Taken together, these observations generally support the canonical role of E2F1–E2F3 activators and E2F4 and E2F5 repressors as effectors of RB that control cell proliferation and apoptosis.

In summary, the prevailing view has been that phenotypes associated with the inactivation of specific E2F family members result from the loss of E2F-mediated activation or repression of genes directly involved in DNA synthesis, mitotic progression and apoptosis. Although the above analyses of RB–E2F compoundmutant mice support the function of E2F activators in regulating proliferation and apoptosis, the detailed analysis of E2F-knock-in mice<sup>31</sup> suggests that members of this gene family have overlapping functions in the regulation of many essential processes in addition to the cell cycle. Evidence from the model organism *D. melanogaster* also contradicts the simple antagonistic nature of E2F-mediated activation and repression, and recent knockout models in mice illustrate the existence of an extensive E2F cross-regulatory network. Clearly, these exciting new findings reveal that we are only beginning to understand the immense complexity of how the E2F family participates in the control of cell proliferation and in other, as yet uncharted, biological processes.

## Regulation of E2F target genes

E2F family members regulate overlapping and distinct subsets of genes. The specificity of E2F-dependent activation and repression is dictated by interactions between individual family members and various cofactors. For instance, *in vitro* pull-down assays showed that E2Fs can associate with different histonemodifying enzymes that direct specific chromatin configurations<sup>13,52</sup> (FIG. 2b).

As changes in gene expression reflect direct and indirect functions of transcription factors, several laboratories have expended considerable effort on ChIP-based technologies to identify direct E2F-regulated targets<sup>10,12–15,20,53</sup>. This approach has led to the identification of many E2F-responsive genes beyond those involved in proliferation and apoptosis, including genes that participate in biological processes as diverse as cell differentiation, metabolism and animal development<sup>9–12,54</sup>. Furthermore, these studies have distinguished targets that are uniquely regulated by individual E2F family members from targets that are co-regulated by multiple members. Work from the Farnham laboratory has also demonstrated the recruitment of E2F1 to an unprecedented number of target promoters that are not always restricted to consensus E2F binding sites<sup>15,20</sup>. This level of promiscuity in E2F–chromatin associations could be due to the recruitment of E2Fs by other transcription factors, such as nuclear factor- $\kappa$ B (NF- $\kappa$ B)<sup>55</sup>, MYC<sup>56</sup> and CAAT enhancer/binding protein- $\alpha$  (CEBP $\alpha$ )<sup>57,58</sup>, or by components of the transcriptional machinery. Although they are undoubtedly intriguing, most of these results were obtained using cell culture systems. Whether the newly identified transcriptional networks involving E2Fs reflect their true physiological roles *in vivo* (such as the regulation of inflammatory response together with NF- $\kappa$ B<sup>55</sup>) remains to be determined.

Revealing the full range of E2F functions will require the identification of direct targets by two synergistic approaches, global gene (including microRNA gene) expression profiling and ChIP–sequencing. The first approach is unbiased and identifies all genes that have deregulated expression in the absence of E2F family members. The second approach validates these genes as direct E2F targets. The fact that both types of analysis can be carried out with materials extracted from control and E2F knockout mouse tissues allows for a rigorous examination of tissue-specific compared with general effects of E2F functions throughout multiple stages of metazoan development. The ability to carry out these analyses in normal tissue and tumour tissue ranging from pre-malignant to metastatic will also distinguish physiological E2F functions from those that may have evolved during tumour development. For example, a requirement for different CDKs in normal and oncogenic cell proliferation has been reported<sup>59</sup>. This idea is supported by elegant work from the Sicinski group that clearly demonstrated that *Cdk4*<sup>-/-</sup> mice develop normal mammary glands but are resistant to *ErbB2*- or *Hras*-driven mammary tumorigenesis<sup>60,61</sup>.

## Complexity of E2F functions *in vivo*

Studies using germline *Rb1*<sup>+/-</sup> and chimeric *Rb1*<sup>-/-</sup> mice that develop various neuroendocrine tumours have produced unexpected phenotypes when E2Fs are inactivated. The logic behind these studies was based on developmental studies showing that the inactivation of *Rb1* led to the deregulated function of activator E2Fs and that the subsequent removal of these E2Fs resulted in a reversion of *Rb1* mutant phenotypes. Although the results from these studies do not dismiss a role for E2Fs in cell proliferation and apoptosis, they have not truly furthered our understanding of the mechanisms, molecular partners and cellular contexts that contribute to E2F-mediated activation and repression. In brief, the following studies in mice contradict a uniform role for E2Fs in cancer.

## The role of E2F activators in cancer, as defined in the context of Rb1 mutation

Unlike humans inheriting one mutated or deleted *RB1* allele who develop retinoblastomas, germline *Rb1*<sup>+/-</sup> mice develop intermediate lobe pituitary and C cell thyroid tumours<sup>62,63</sup>. The initial studies by Jacks and colleagues exploring the role of E2F activators in driving tumour initiation showed that ablation of *E2f1* in *Rb1*<sup>+/-</sup> mice significantly reduced the incidence of both pituitary and thyroid tumours, prolonging the tumour-free lifespan of *Rb1*<sup>+/-</sup> mice<sup>64</sup>. This was the first genetic evidence to support an oncogenic role for an E2F family member in the context of *Rb1* inactivation. The interpretation that E2F1 has a uniform oncogenic role, however, is clouded by the observation that *E2f1*<sup>-/-</sup> mice develop lymphoma or occasionally tumours of mesenchymal origin (TABLE 1). The fact that the loss of *Rb1* in T cells does not result in lymphomagenesis<sup>65</sup> suggests that the tumour-suppressive role of *E2f1* in this context may be RB independent. The role of *E2f3* as an effector of RB action seems to be slightly more complex. Loss of *E2f3* in germline *Rb1*<sup>+/-</sup> mice, similar to the loss of *E2f1*, suppressed the development of pituitary tumours<sup>66</sup>. This is consistent with *E2f3* functioning as an oncogene downstream of *Rb1*. However, its loss also promoted the development and metastasis of medullary thyroid tumours in *Rb1*<sup>+/-</sup> mice, suggesting that *E2f3*, like *E2f1*, may function as a tumour suppressor<sup>66</sup>. It is possible that E2F3 might promote proliferation in the pituitary but have additional RB-independent roles in the thyroid. As the direct functions of E2F1 and E2F3 have not been extensively explored in the pituitary and thyroid, the precise molecular basis for their apparent pro-tumorigenic and anti-tumorigenic actions in these tissues remains unclear.

Further attempts to inactivate E2F function in chimeric *Rb1*<sup>-/-</sup> mice produced intriguing but inconclusive results. *Rb1*<sup>-/-</sup> chimeras were previously shown to be fully viable and develop pituitary tumours at a young age<sup>67,68</sup>. In more recent studies by Parisi and colleagues, thyroid tumours and pulmonary neuroendocrine cell (PNEC) hyperplasia were also observed in *Rb1*<sup>-/-</sup> chimeras<sup>69</sup>. Ablation of *E2f3* in *Rb1*<sup>-/-</sup> chimeras suppressed PNEC, which is thought to precede the development of small cell lung carcinoma (SCLC), but failed to suppress the onset or development of either pituitary or thyroid tumours<sup>69</sup>. Why deletion of *E2f3* can mediate different pituitary and thyroid tumour outcomes in *Rb1*<sup>+/-</sup> compared with *Rb1*<sup>-/-</sup> chimeric mice is not clear. It is entirely possible that the role of E2F3 in these contexts is tumour cell non-autonomous and that loss of E2F3 in these two different microenvironments leads to different or even opposing outcomes. Given that several cell non-autonomous functions have been described for RB and E2F in *Caenorhabditis elegans* and mice<sup>70-73</sup>, it would be prudent to caution against the over-interpretation of the above analyses until more definitive molecular mechanisms have been provided.

Finally, loss of *E2f2*, but not *E2f1* or *E2f3*, in mice increased *Myc*-induced T cell lymphomagenesis, and the reintroduction of E2F2 into these tumours stimulated apoptosis of the tumour cells<sup>74</sup>. This result implicates E2F2 as a tumour suppressor. By contrast, loss-of-function models have also revealed that *E2f2* seems to behave as an oncogene in simian virus 40 (SV40) large T antigen-induced intestinal hyperplasia, as its ablation reduced epithelial cell proliferation<sup>75</sup>. These findings provide another example of an E2F activator, functioning as an oncogene or as a tumour suppressor in a tissue-specific manner. Consistent with this, the disruption of RB and components of the RB pathway has been shown to affect gastrointestinal but not haematopoietic malignancies, suggesting that the role of E2F2 in T cells may be RB independent. It should be noted that the inability to identify the exact site of gene action is a confounding characteristic of the analysis of germline knockout mice and may underlie the observed discrepancies that mitigate a unified view of how E2F1–E2F3 activity influences cancer.



## The role of E2F repressors in cancer, as defined in the context of Rb1 mutation

The analysis of *E2f4* inactivation and its effects on tumour formation in germline *Rb1*<sup>+/-</sup> mice revealed tissue-specific roles for this abundantly expressed E2F repressor. The loss of E2F4, a classic repressor known to physically bind all pocket protein family members, extended the lifespan of *Rb1*<sup>+/-</sup> mice by suppressing pituitary and thyroid tumorigenesis<sup>76</sup>, suggesting an oncogenic role for E2F4 in these tissues. This result was partially recapitulated in chimeric *Rb1*<sup>-/-</sup>;*E2f4*<sup>-/-</sup> mice, which had a reduced incidence and delayed development of pituitary tumours<sup>77</sup>. Interestingly, as in compound *Rb1*<sup>-/-</sup>;*E2f3*<sup>-/-</sup> chimeras, the *Rb1*<sup>-/-</sup>;*E2f4*<sup>-/-</sup> chimeras also had reduced PNEC hyperplasia. It is intriguing that the loss of an activator (for example, E2F3) or repressor (for example, E2F4) leads to similar outcomes in *Rb1*-deficient tumours. To reconcile this paradox, the authors suggested that in the absence of E2F4, the E2F1 and E2F3 activators, which normally do not bind p107 and p130, could now be recruited into a complex with these pocket proteins. The consequence of this pocket protein–E2F rearrangement is the sequestration of E2F activators into a protein complex that has a repressor function, raising the possibility that activators could compensate for repressors and vice versa in a context-dependent manner. By contrast, the development of ganglionic neuroendocrine neoplasms and urothelial transitional carcinomas in *Rb1*<sup>-/-</sup>;*E2f4*<sup>-/-</sup> double chimeras, which was not observed in chimeric *Rb1*<sup>-/-</sup> or *E2f4*<sup>-/-</sup> mice, suggests that E2F4 has tumour suppressive properties. It therefore seems that by regulating alternative sets of genes in different tissues E2F4 can function as a tumour suppressor or an oncogene. A full understanding of how this potential switch in target gene specificity might occur in multiple cellular contexts is lacking.

Given the lack of clearcut *in vivo* data to support the classification of E2Fs as repressors or activators, it is conceivable that these two subclasses function similarly in the animal. Therefore, a simple explanation for why E2F3 and E2F4 can function as either oncogenes or tumour suppressors is that both may behave as transcription activators or repressors *in vivo*. Additional supporting evidence of this has been published in several recent studies. Work from the Zubiaga group has shown that E2F2 suppresses cell cycle gene expression in T cells and that the targeted disruption of *E2f2* accelerated S phase entry and cell division<sup>78</sup>. E2F3b, which is constitutively expressed in all phases of the cell cycle, was initially shown to bind to the *Arf* promoter and to directly repress *Arf* transcription<sup>49</sup>. Knockin and *E2f3* isoform-specific knockout studies in mice have revealed overlapping functions for *E2f3a* and *E2f3b* in early mammalian development<sup>31,32</sup> and indicate that both isoforms could function as activators or repressors.

Currently, the role of the new repressor arm of the E2F family, E2F7 and E2F8, in cancer is virtually unknown. However, given their crucial role in the control of proliferation and apoptosis in embryonic development, it would not be surprising to find that these E2Fs are involved in cancer. The expression of these E2Fs needs to be evaluated in clinical samples and in the context of tumour models.

## Challenges to the current RB–E2F paradigm

**CDKs and E2Fs: a role in normal cell proliferation?**—A seminal series of mouse knockout studies recently revealed that most cells in the animal continue to proliferate in the absence of CDK2, CDK4 or CDK6 (REFS 79,80). Mice individually lacking *Cdk2*, *Cdk4* or *Cdk6* are fully viable and have developmental phenotypes that pinpoint specific roles for these Cdk's in the control of homeostatic proliferation of specialized cell types<sup>59</sup>. Strikingly, *Cdk2*<sup>-/-</sup>;*Cdk4*<sup>-/-</sup>;*Cdk6*<sup>-/-</sup> triple-knockout mouse embryos develop to mid gestation and die around embryonic day 12.5 (E12.5) from defective haematopoiesis, without obvious disturbances in cell proliferation<sup>81</sup>. By contrast, *Cdk1*<sup>-/-</sup> embryos fail to develop beyond the two-cell stage. These observations led the authors to conclude that the mitotic CDK1 is the

only CDK required for normal cell proliferation<sup>81</sup> and that interphase CDKs cannot compensate for the activity of CDK1 (REF. 82). On the basis of these findings, it would seem that factors positively regulating cell cycle progression are not as crucial for normal cell proliferation in mammals as studies in yeast had originally implied. It has been suggested that oncogene-stimulated proliferation may result in the need for a specific CDK family member, as seems to occur with *CDK8* in colorectal cancer<sup>83</sup>. Given that the human CDK family consists of 13 members, it will be interesting to determine the extent of redundancy in development and cancer among members of this diverse family of kinases. Even though the combinations of CDKs involved in cell proliferation may change in different cell types, it is thought that E2Fs are the crucial downstream effectors of CDK activity that ultimately drive cell cycle progression. Although the loss of *E2f1–E2f3* leads to the complete arrest of mouse embryonic fibroblasts (mEFs) in cell culture and therefore supports this idea<sup>84</sup>, this hypothesis remains to be validated *in vivo*.

The idea that E2F function is indispensable for the control of cell proliferation has dominated several decades of experimentation. Incompatible with this view is the fact that mice deficient for individual or a combination of E2F genes did not have widespread defects in cell proliferation<sup>17,85–92</sup> (TABLE 1). Recent work has shown that the combined ablation of the entire subset of E2F activators (*E2f1–E2f3*) resulted in mid gestational lethality of mouse embryos with only a negligible effect on cell proliferation in most of the tissues examined<sup>214,215</sup>. These results seem to oppose the finding that mEFs lacking *E2f1–E2f3* are profoundly arrested at G1/S<sup>84</sup>. However, the inactivation of the p53 pathway was sufficient to stimulate quiescent triple-knockout MEFs to re-enter the cell cycle and be transformed by MYC and Ras<sup>93,94</sup>, indicating that conditions permissive for the proliferation of triple-knockout cells exist in a cell type- or oncogene-dependent manner. Collectively, these observations suggest that the redundant role of E2F activators in normal cell proliferation, as revealed by the recent E2F-knockin studies in mice, may be restricted to a fairly narrow period in the development of mammals and that compensatory factors exist to permit cell growth and division in the absence of E2F activators. Similar to CDKs, many studies have also suggested an acquired preference for specific E2F activators in oncogenic proliferation. For instance, E2F transgenic mice develop various tumours (TABLE 2), and overexpression and/or amplification of *E2F1* and *E2F3* has been observed in different human cancers (TABLE 3). In addition, E2F activators were shown to be necessary for the unscheduled proliferation of cells mutant for either *Rb1* or components of the Hippo tumour suppressor pathway<sup>42,43,48,95</sup>. Taken together, these results suggest a differential requirement for E2Fs in the control of cell proliferation in normal compared with oncogenic environments. Confirmation of this hypothesis will require a thorough evaluation of E2F function in both normal tissues and associated tumours.

**E2Fs linked to human cancer**—In the current RB–E2F paradigm (FIG. 3) it is thought that genetic alterations resulting in the loss of RB function cause cancer by unleashing E2F activity and deregulating cell proliferation. From this traditional view of how E2F factors control cell proliferation, clear predictions of their involvement in human cancer can be made. As a whole, E2F repressors may be expected to behave as tumour suppressors, and activators as oncogenes. Therefore, oncogenic alterations of repressor E2F genes would include loss-of-function mutations, chromosomal deletions and/or epigenetic silencing. Conversely, one would expect to observe gain-of-function mutations, amplification and/or overexpression of the activator E2F genes.

At least in humans, a clear theme regarding the oncogenic role of E2F1–E2F3 in human cancer has emerged. Several reports have described the amplification of the *E2F1* or *E2F3* gene locus as a frequent genetic event observed in hepatocellular carcinoma<sup>96–99</sup>, bladder cancer<sup>100–104</sup>, retinoblastoma<sup>105,106</sup>, liposarcoma<sup>107,108</sup> and many other malignancies.

Overexpression of *E2F1* or *E2F3* has also been detected in glioblastoma<sup>109</sup> and lung<sup>110–113</sup>, ovarian<sup>114,115</sup>, breast<sup>116–118</sup>, gastric<sup>119</sup> and colon cancer<sup>120,121</sup>. In some cases of neuroblastoma<sup>122,123</sup>, thyroid cancer<sup>122</sup> and pancreatic cancer<sup>124,125</sup>, large chromosomal deletions of regions including the *E2F1*, *E2F2* or *E2F3* genes have been detected. Generally, however, the levels of E2F activators are increased in most cancer types and are presumed to mediate the uncontrolled proliferation of cancer cells.

Inconsistent with the prediction of an involvement in tumour suppression, E2F repressors have not frequently been found to be mutated, deleted or silenced in human cancers. Instead, current data support a role for E2F repressors in tumour promotion. For example, the E2F4–p130 complex was shown to mediate the repression of the DNA repair genes *RAD51* and *BRCA1* in response to hypoxic stress *in vitro*<sup>126,127</sup>. Additionally, an increased gene copy number of *E2F5* was detected in two independent cohorts of patients with breast cancer<sup>128,129</sup>. In one of these studies, there was a positive association of *E2F5* amplification with a basal-like phenotype (an oestrogen receptor-, progesterone receptor- and ERBB2-negative phenotype) and worse clinical outcome<sup>129</sup>. Finally, the evaluation of E2F7 in human cancer has yielded conflicting results<sup>130–132</sup> and further investigation will be required to understand its function in cancer.

In summary, deregulated expression of E2F family members is common in human cancer, but whether this contributes to the genesis of these cancers has not been unequivocally established. Future studies will need to determine whether their potential contribution to the tumorigenic process involves deregulation of cell cycle progression or extends beyond the framework of RB and cell cycle control.

## E2Fs: beyond proliferation

Generally, cell transformation results from the accumulation of stochastic mutations or ‘hits’ that confer a selection advantage over neighbouring cells, and so mutations affecting multiple components in the same pathway that yield a similar outcome are rarely found in cancer. However, a close examination of the genetics of *RB1*-mutant human cancers reveals a pervasive association between RB and E2F expression that is inconsistent with the current view of how deregulation of the RB–E2F pathway leads to cancer.

*RB1* is mutated in essentially all patients with hereditary or sporadic retinoblastoma. Amplification of 6p22, which contains the *E2F3* locus, has been reported in more than 50% of cases of retinoblastoma and leads to increased expression of *E2F3* mRNA and protein<sup>105,106,133</sup>. This trend has also been observed in other types of cancers, including bladder cancer. Loss of heterozygosity (LOH) at the *RB1* locus is found in 29% of patients with bladder cancer and is associated with 6p22 amplification, *E2F3* over expression and advanced tumour stage<sup>100–104</sup>. In human SCLC, *RB1* is mutated in more than 90% of cases<sup>110,134</sup>, and most SCLCs also have *E2F1* or *E2F3* overexpression<sup>112–113</sup>. A similar but less pronounced relationship between *RB1* and *E2F1* was reported for non-small-cell lung cancer<sup>111,113,135</sup>. Finally, the Tlsty laboratory recently unmasked a strict inverse correlation between *RB1* and *E2F3* expression in the basal-like subtype of human breast cancer<sup>118</sup>. In their study, global gene expression profiling revealed low expression of either *CCND1* or *RB1*, consistent with their mutually exclusive association in many cancers, but high levels of *E2F3*. This overlap between *RB1* inactivation and *E2F3* hyperactivity in retinoblastoma and bladder, lung and breast cancer is not readily explained by current paradigms of RB–E2F action.

Why do *RB1* inactivation and E2F amplification and/or overexpression coexist in tumour cells? In several knockout-mouse models the inactivation of *Rb1* resulted in unrestrained E2F3 activity and cell proliferation, and the concomitant loss of *Rb1* and *E2f3* suppressed



most of this ectopic cell proliferation<sup>43,48</sup>. This demonstrates that the levels of E2F3 activity achieved by RB inactivation are sufficient to promote uninhibited cell proliferation. We propose, therefore, that in cancers such as retinoblastoma and bladder cancer the deregulation of E2F3 activity (as might occur through the loss of RB function) and the further increase in *E2F3* expression (as might occur through the amplification of 6p22) may represent separate molecular events with different physiological outcomes. In this context, *RB1* mutation would provide a proliferative advantage to tumour cells, whereas E2F3 hyperactivity would provide functions that extend beyond the traditional control of the cell cycle. A preliminary example of such a function is evident in mice in which the targeted ablation of *E2f3* from tumour cells overexpressing the oncogene *ErbB2* is associated with an infiltration of innate and adaptive immune cells in the tumour microenvironment, leading to tumour regression (G.L., unpublished observations). Several developmental studies in mice, flies and worms also support a role for E2Fs that is independent of cell cycle control. For instance, loss-of-function studies have identified roles for *lin-35* (an *RB1* orthologue) and *efl-1* (an E2F orthologue) in epidermal growth factor-mediated cell fate determination in *C. elegans*<sup>70,71</sup> and for mammalian E2Fs in angiogenesis<sup>136</sup>, adipogenesis<sup>31,137</sup> and cell migration<sup>21</sup>. On the basis of these examples, it can be argued that the role of E2Fs in cell cycle control might be a recent evolutionary adaptation and that the role of E2Fs in cancer might also include more evolutionarily ancient functions.

The observations outlined above suggest that the tight control of E2F1, and E2F3 in particular, is crucial for tissue homeostasis and that disruption of their expression may have a causative relationship with cancer development. A thorough molecular analysis of mice that either lack or have misregulated E2F1 and E2F3 should begin to clarify the mechanisms underlying their link with cancer. Genetic and gene expression profiling studies of human tumour samples, together with the analysis of mice deficient for multiple E2Fs, have begun to reveal functions of E2Fs beyond the control of the cell cycle and apoptosis. The extent to which these poorly defined functions contribute to the cancer phenotype will need to be evaluated in the coming years, with the promise that they might represent events that could be targeted by therapeutic regimens.

## Conclusions and future perspectives

The prevailing view is that E2F repressors and activators cooperate to orchestrate proper cell cycle progression and that disruption of this carefully coordinated network contributes to cancer. However, recent clinical and mouse studies have begun to challenge this view. We suggest that the simple paradigm of proliferation control by E2F repressors and activators does not correlate with the complexity of E2F function observed during development and tumorigenesis.

As factors that control the cell cycle, apoptosis, differentiation and stress responses, the E2F family of transcription factors has earned the status of master regulators of cell proliferation. We have learned that many signals can regulate the activity of E2F family members and that E2Fs can in turn regulate many different targets that affect a wide range of biological processes that are intertwined with the control of cell proliferation. Given that E2Fs have the capacity to determine whether cells proliferate or not, what more could be learnt about E2Fs other than the finer details of their work ethic in specific contexts and cell types? Quite a bit we think, and we could begin by asking whether E2Fs are necessary for the proliferation of all cell types in mammals and other organisms. If not, then identifying the crucial accomplices that function in concert with E2Fs, and how their activities are coordinated to commit cells to proliferation, would be important. It might be that E2F functions are not required in some cell types. Second, are the biological roles of E2Fs strictly dependent on their activation or repression functions and when are such activities relevant during the

developmental life of a cell? We should further address why different levels of E2F-dependent activation and repression induce such complex outcomes in mammalian cells. The combined use of new genetic mouse models and molecular techniques such as ChIP–sequencing, gene expression arrays and proteomics could help us map and elucidate the mechanism of E2F action in intact tissues. From a pessimistic standpoint, the complexity of E2F activities might simply represent an imperfect evolutionary adaptation to perform the vital task of cell proliferation in multicellular organisms. Once these normal functions of E2Fs have been fully understood, we could then ask whether the classic cell proliferation functions long ascribed to E2Fs are relevant to the development of cancer. If so, for which cancers and at what point in their malignant evolution might they be especially crucial? If not, then what additional pro-oncogenic or anti-oncogenic roles might they have?

So, despite  $\approx 3,500$  scientific publications focusing on E2Fs since the first family member was discovered two decades ago, we know embarrassingly little about what E2Fs do *in vivo* or how they do it. We have certainly garnered volumes of data relating to how E2F activities can be controlled and which targets and biological processes E2Fs can in turn potentially regulate. But from this bulk of information, we have yet to identify a single physiological circumstance in which a defined signal elicits the E2F programme to exert its powers on a specific biological outcome. Perhaps by studying these factors in whole animals, we may finally have the opportunity to discover the extent to which E2Fs function in specific capacities or as versatile modulators poised at biological crossroads that can be commandeered to execute diverse processes in health and disease.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Glossary

<b>Endocycle</b>	A specialized type of cell cycle, consisting of alternating S and G phases, that is widely used by both plants and animals. For example, mammalian cells using the endocycle include trophoblast giant cells, hepatocytes and megakaryocytes. Cells achieve greater than 2N genomes by uncoupling DNA replication from mitosis.
<b>Chimera</b>	An animal produced by mixing wild-type cells with mutant cells at the pre-implantation stages of embryonic development to overcome embryonic lethal mutations and facilitate the identification of the primary site of action or the function of a gene in later lineages.

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represent just two in a series of elegant studies demonstrating a role for RB–E2F in the regulation of cell fate specification in *C. elegans*.

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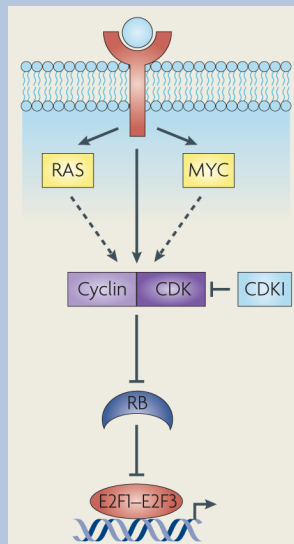
### At a glance

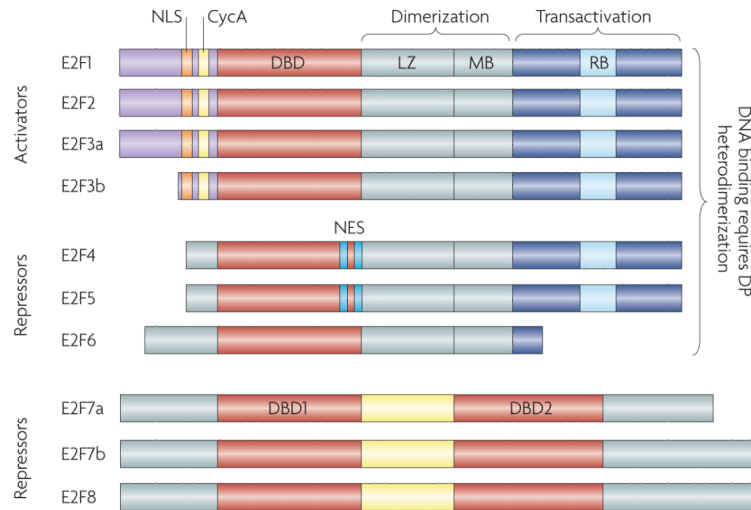
- A long-standing paradigm has been that E2F activity is tightly regulated by the RB tumour suppressor and that the disruption of this regulation leads to unscheduled progression through the cell cycle.
- Based on structure–function studies *in vitro*, the mammalian E2F family of transcription factors has been artificially subdivided into activators (E2F1–E2F3) and repressors (E2F4–E2F8).
- E2F1–E2F3 activators are highly redundant during development.
- Tumour models using RB–E2F compound-mutant mice and E2F-transgenic mice show dual roles for E2Fs in tumour promotion and suppression. These results suggest tissue-specific functions and argue against a uniform role for E2Fs in cancer.
- Mice lacking E2F1, E2F2 or E2F3 survive to mid gestation without global defects in the cell cycle, suggesting that the activators are not essential for normal mammalian cell proliferation. We propose that under normal conditions E2Fs do not substantially contribute to the proliferative potential of a cell.
- Deregulated expression or activity of most members of the E2F family has been detected in many human cancers. We propose that the requirement for certain E2F family members in proliferation under oncogenic conditions represents a recent evolutionary adaptation.
- RB inactivation and E2F amplification coexist in cancer. RB inactivation leads to inappropriate cell cycle progression through the deregulation of E2F function. We propose that the additional increase in E2F activity caused by amplification has cell proliferation-independent functions in cancer.

**Box 1****Alterations of the CDK–RB–E2F pathway in human cancer**

Uncontrolled cell growth is an invariable characteristic of human cancer<sup>138</sup>. The proliferation of cancer cells is sustained in the absence of growth factors and is insensitive to growth-inhibitory signals<sup>138</sup>. Members of the human epidermal growth factor receptor family of receptor tyrosine kinases are deregulated in many types of human cancer<sup>139</sup>. Overexpression or mutation of these receptors leads to the constitutive activation of downstream signalling pathways<sup>140,141</sup>.

On mitogenic stimulation, intracellular levels of D-type cyclins (D1, D2 and D3) increase, resulting in the formation and nuclear localization of cyclin D–cyclin-dependent kinase 4 (CDK4) and cyclin D–CDK6 complexes that phosphorylate RB early in G1 phase<sup>142</sup>. Subsequently, levels of mitogen-independent E-type cyclins accumulate and these cyclins associate with CDK2 to further phosphorylate RB in preparation for progression into S phase<sup>142</sup>. Genetic alterations of G1/S regulators have been extensively documented in human cancers (see the figure)<sup>29,59,143</sup>. The G1 cyclins, in particular D1 and E1, and their catalytic partners CDK4 and CDK6, are overexpressed, mutated or their genomic loci amplified in a wide variety of tumours<sup>29,59,143</sup>. The activity of CDKs is regulated by two families of CDK inhibitors (CKIs), with the first family comprised of the INK4 proteins (INK4A, INK4B, INK4C and INK4D). The second family consists of p21, p27 and p57. Decreased expression of these negative G1/S regulators in human cancer has been attributed to genetic and epigenetic changes<sup>3–5,29,59,143</sup>. In short, deregulation of factors that control the G1/S transition, which ultimately relies on the engagement of E2F activity in the current view of cell cycle regulation, seems to be a universal theme in the process of neoplastic transformation. LOH, loss of heterozygosity.

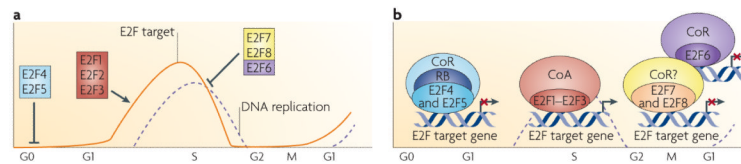




### Figure 1. The mammalian E2F family of transcription factors

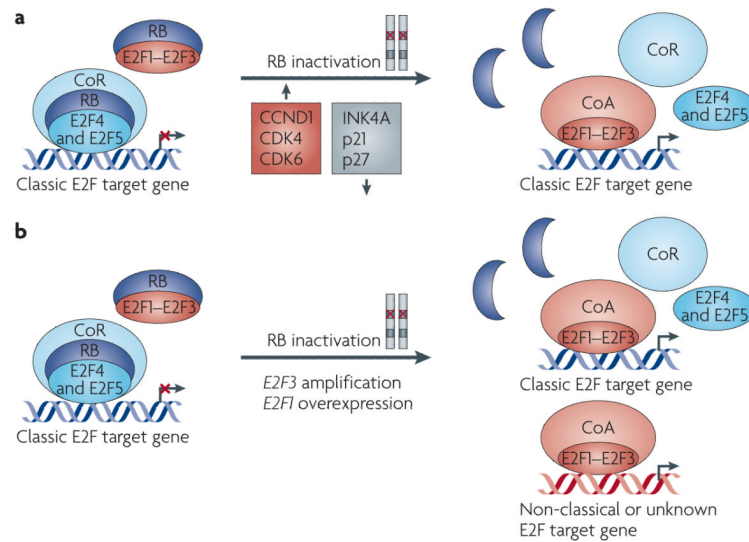
This family is defined by their signature winged-helix DNA binding domain (DBD), and all members are expressed from eight chromosomal loci that encode nine distinct gene products. Based predominantly on results from *in vitro* studies, the E2F family has been traditionally divided into activator (E2F1–E2F3) and repressor (E2F4–E2F8) subclasses. Most E2F family members (E2F1–E2F6) bind DNA as heterodimers with one of three dimerization partner (DP) proteins, TFDP1, TFDP2 and TFDP3, and heterodimerization is mediated by the leucine zipper (LZ) and marked box (MB) domains. RB binds within the transactivation domain (RB) of E2F1–E2F3. Alternative promoters at the *E2F3* locus drive the expression of two highly related isoforms, *E2F3a* and *E2F3b*<sup>51</sup>. Unlike the activating E2F1–E2F3, E2F4 associates with all three pocket protein family members and E2F5 associates predominantly with p130 (REFS 1,2,8). Ectopically expressed E2F activators are localized to the nucleus owing to their amino-terminal nuclear localization signal (NLS) sequence<sup>143,144</sup>, which is adjacent to the cyclin A-binding site (CycA). E2F4 and E2F5 have bipartite nuclear export signals (NES) that mediate their export to the cytoplasm<sup>145,146</sup>. Repressors E2F6–E2F8 do not possess the canonical carboxy-terminal features of E2F4 and E2F5 and so are presumed to repress E2F-responsive genes independently of RB and related pocket proteins<sup>40,41,147,148</sup>. Indeed, E2F6 can repress E2F target expression when overexpressed in cell culture<sup>147–149</sup>. The structurally unique and most recently identified E2Fs, E2F7 and E2F8, comprise a separate and highly evolutionarily conserved repressor arm in the E2F family. Unlike the *E2F3* isoforms that are transcribed from distinct promoters, *E2F7a* and *E2F7b* isoforms are produced by alternative splicing of the primary transcript<sup>41</sup>.





**Figure 2. The expression and activity of mammalian E2F family members during the cell cycle**

**a** In quiescent (G0) cells, the ubiquitously expressed E2F4 and E2F5 associate with pocket proteins and other co-repressors to maintain repression of E2F-responsive genes (line labelled E2F target) that promote entry into the G1 phase of the cell cycle. On mitogenic stimulation, the sequential phosphorylation of RB by activated cyclin-dependent kinases results in the loss of RB function, release of E2F repressors<sup>142</sup> and the accumulation of newly synthesized free E2F1, E2F2 and E2F3 late in G1 phase. Together, these events initiate a transcriptional programme driving cells into S phase<sup>150</sup> (dashed line labelled DNA replication). This G1/S-specific transcriptome is then attenuated on completion of S phase in G2 by the action of the repressors E2F6, E2F7 and E2F8, which are thought to function independently of RB and RB-related proteins<sup>40,41,151</sup>. E2F7 and E2F8 can also specifically mediate the repression of E2F activators such as *E2f1* (REFS 17,41). Heterodimeric or homodimeric E2F7 and E2F8 complexes directly bind the *E2f1* promoter and repress its expression throughout S phase to restrain activator E2F function. Without this brake, E2F1 proteins would persist inappropriately and continue the activation of genes encoding components of the transcriptional machinery, resulting in ectopic DNA replication. **b** In quiescent or differentiated cells, pocket protein-bound E2F4 and E2F5 have been found to associate with various co-repressors (CoRs), such as histone deacetylases, the DNA methyltransferase DNMT1 and C-terminal binding protein (CtBP), leading to chromatin compaction and transcription inhibition<sup>152–155</sup>. Conversely, during cell proliferation, when RB is hyperphosphorylated, E2F activators recruit the basal transcription factor TFIID and other co-activators (CoA), such as histone acetyltransferases, p300 and CBP, GCN5 and TIP60, to specific gene promoters<sup>156–158</sup>, leading to an open chromatin configuration and transcription initiation. The more recently identified repressors E2F6–E2F8 mediate repression of E2F-responsive genes independent of pocket protein binding. Although E2F6, through interaction with the polycomb complex<sup>149</sup>, still requires dimerization with a dimerization partner protein to function in transcription repression, E2F7 and E2F8 are unique in that they form homodimers (E2F7–E2F7 and E2F8–E2F8) or heterodimers (E2F7–E2F8) to repress transcription of cell cycle-related genes<sup>17,40,41</sup>. The co-repressors that associate with E2F7 and E2F8 are currently unknown.



**Figure 3. The paradigm of RB–E2F function in human cancer**

**a** | In the nuclei of non-proliferating cells, RB remains hypophosphorylated and forms RB–E2F repressor complexes to inhibit the expression of genes that promote S phase entry. Hypophosphorylated RB also directly binds to and inhibits the activity of E2F activators (E2F1–E2F3). RB is inactivated in most types of human cancer and this occurs through the direct mutation of *RB1*, located on the long arm of chromosome 13 (13q14.3), or through a disruption in the regulatory components of the RB–E2F pathway. For instance, *CCND1* is overexpressed or amplified in cancer<sup>143</sup>, an event that leads to deregulated E2F function by activating cyclin-dependent kinase 4 (CDK4) and CDK6 and by stimulating RB hyperphosphorylation. *CDK4* and *CDK6* are also overexpressed, amplified and mutated in cancers<sup>29,59,143</sup>, leading to the loss of RB function. Many types of tumours exhibit decreased expression or mutations of CDK inhibitors, such as *INK4A*, *p21* and *p27*, which normally antagonize RB phosphorylation<sup>29,59,143</sup>. In human cancer, it is the prevailing view that the inactivation of RB leads to the disassembly of RB–E2F co-repressor complexes, lifting the repression of genes that are necessary for progression through the cell cycle. Furthermore, the dissociation of hyperphosphorylated RB from E2F activators leads to the inappropriate accumulation of free E2F1, E2F2 and E2F3 with unmasked transactivation domains, resulting in additional transactivation of these genes. **b** | Amplification of the genomic locus 6p22 harbouring *E2F3* is detected in more than 50% of cases of retinoblastoma<sup>105,106,133</sup>, as well as in bladder tumours that have complete loss of RB function<sup>100–104</sup>. *RB1* is mutated in more than 90% of small-cell lung cancer cases, many of which also exhibit overexpression of *E2F1* or *E2F3* (REF. 110). The inactivation of RB in these cancers leads to increased proliferation of tumour cells as a result of deregulated E2F function and increased expression of classic E2F target genes. The additional increase in the level of E2F activators might contribute to the activation of genes important for tumour initiation or progression that function independently of cell cycle control. CoA, co-activator; CoR, co-repressor.

Table 1

## Developmental defects in E2F-knockout mouse models

Genotype	Phenotype	Refs
<i>E2f1</i> <sup>-/-</sup>	Defective thymic-negative selection, testicular atrophy and increased susceptibility to tumorigenesis in different tissues	85,86
<i>E2f2</i> <sup>-/-</sup>	Development of autoimmune disease owing to combined hematopoietic defect	88
<i>E2f1</i> <sup>-/-</sup> ; <i>E2f2</i> <sup>-/-</sup>	Development of exocrine pancreatic insufficiency and diabetes	159,160
<i>E2f3</i> <sup>-/-</sup>	Embryonic lethal in pure FVB (E12.5) or 129Sv genetic backgrounds, reduced viability in mixed (C57BL/6 × 129Sv) genetic background	31,87,161
<i>E2f3a</i> <sup>-/-</sup>	Reduced WAT deposits with age in pure genetic background; no specific defects observed in mixed genetic background	31,32
<i>E2f3b</i> <sup>-/-</sup>	None observed	31,32
<i>E2f1</i> <sup>-/-</sup> ; <i>E2f3a</i> <sup>-/-</sup>	Death by ~1 month of age, runted growth with under-developed sexual organs, reduced WAT deposits, reduced number of pancreatic exocrine cells and multiple other tissue-specific developmental defects	31,32
<i>E2f1</i> <sup>-/-</sup> ; <i>E2f3b</i> <sup>-/-</sup>	Viable and fertile	31,32
<i>E2f1</i> <sup>-/-</sup> ; <i>E2f2</i> <sup>-/-</sup> ; <i>E2f3a</i> <sup>-/-</sup>	Neonatal lethality (~E19.5) in mixed genetic background	31
<i>E2f1</i> <sup>-/-</sup> ; <i>E2f2</i> <sup>-/-</sup> ; <i>E2f3b</i> <sup>-/-</sup>	Runted growth with under-developed sexual organs in mixed genetic background	31
<i>E2f1</i> <sup>-/-</sup> ; <i>E2f2</i> <sup>-/-</sup> ; <i>E2f3</i> <sup>-/-</sup>	Embryonic lethal (E10.5) with no obvious disturbances in cell proliferation	214,215
<i>E2f4</i> <sup>-/-</sup>	Neonatal lethality owing to increased susceptibility to opportunistic infections resulting from craniofacial defects. Maturation defects in multiple hematopoietic lineages and the gut epithelium, macrocytic anaemia owing to impaired erythroid proliferation	89,90,162
<i>E2f5</i> <sup>-/-</sup>	Shortened lifespan owing to development of hydrocephalus resulting from overproduction of cerebrospinal fluid by the choroid plexus	91
<i>E2f6</i> <sup>-/-</sup>	Normal lifespan with homeotic transformations of the axial skeleton	92
<i>E2f7</i> <sup>-/-</sup>	None observed	17
<i>E2f8</i> <sup>-/-</sup>	None observed	17
<i>E2f7</i> <sup>-/-</sup> ; <i>E2f8</i> <sup>-/-</sup>	Embryonic lethal (E12.5) with widespread apoptosis, vascular dilation and haemorrhage	17

E, embryonic day; WAT, white adipose tissue.

Table 2

## E2F-transgenic mouse models

Promoter	Tissue with E2F expression	Phenotype	Refs
<i>E2f1</i>			
<i>Glaf</i>	Brain	Development of medulloblastoma, choroid plexus carcinoma and primary neuroectodermal tumour in young mice; development of malignant gliomas in aged mice	163
<i>Alb</i>	Liver	Altered lipid metabolism and early development of preneoplastic lesions, with 100% of these progressing to adenomas	164
$\alpha$ A-crystallin	Retina (post-mitotic lens fibre cell)	Cell cycle re-entry and subsequent apoptosis of lens fibre cells	165
<i>Pf4</i>	Megakaryocyte	Impaired terminal differentiation and apoptosis of megakaryocytes resulting in thrombocytopenia	166
<i>HmgA</i>	Testis	Testicular atrophy and sterility	167
<i>K5</i>	Basal epidermal layer and squamous epithelial tissues	Hyperplasia and p53-dependent apoptosis of epithelial tissue, development of spontaneous skin tumours by ~1 year of age and reduced response to two-step carcinogenesis protocol	168, 169
<i>E2f2</i>			
<i>E4</i> and <i>Pim1</i>	Thymic epithelium	Development of cortical thymomas	170
$\alpha$ A-crystallin	Retina (post-mitotic lens fibre cell)	Cell cycle re-entry and subsequent apoptosis of lens fibre cells	165
<i>E2f3a</i>			
<i>K5</i>	Basal epidermal layer and squamous epithelial tissues	Development of spontaneous skin tumours and increased response to two-step skin carcinogenesis protocol	171
$\alpha$ A-crystallin	Retina (post-mitotic lens fibre cell)	Cell cycle re-entry and subsequent apoptosis resulting in microphthalmia	172
<i>E2f4</i>			
<i>K5</i>	Basal epidermal layer and squamous epithelial tissues	Increased response to two-step skin carcinogenesis protocol	173
$\alpha$ A-crystallin	Retina (post-mitotic lens fibre cell)	Moderate level of cell cycle re-entry with normal-size lens	172
<i>E2f5</i>			
$\alpha$ A-crystallin	Retina (post-mitotic lens fibre cell)	Inhibition of E2F1- or E2F3a-induced ectopic cell cycle entry together with p130 overexpression	174

*Alb*, albumin; *E4*, immunoglobulin heavy chain gene enhancer; *Glaf*, glial fibrillary acidic protein; *K5*, keratin 5; *HmgA*, hydroxy-methyl-glutaryl-Coenzyme A reductase; *Pf4*, platelet factor 4.

Table 3

## Genetic alterations of E2F family members in human cancers

Gene (chromosome)	Genetic alteration	Human cancer	Notes
<i>E2F1</i> (20q11.2)	Amplification	HCC <sup>96,98</sup> , oesophageal SCC <sup>175</sup> , NSCLC <sup>176</sup> , and cancer <sup>177-179</sup>	None
	Increased expression	NSCLC <sup>111,135,179,180</sup> , SCLC <sup>112,181</sup> , glioblastoma <sup>109</sup> , oesophageal SCC <sup>181,182</sup> , HCC <sup>183,184</sup> , pancreatic ductal carcinoma <sup>185</sup> , and GI stromal <sup>186</sup> , breast <sup>187</sup> and ovarian cancer <sup>130,131,188</sup>	None
	Decreased expression	Gastric adenocarcinoma <sup>189</sup> , oral SCC <sup>190</sup> , and colon <sup>191</sup> and bladder cancer <sup>192</sup>	None
<i>E2F2</i> (1p36)	Amplification	SCLC <sup>193</sup> , alveolar rhabdomyosarcoma <sup>194</sup> and osteosarcoma <sup>195</sup>	Detection of 1p32-1p36 amplicon in SCLC
	Deletion	Neuroblastoma <sup>196</sup> , pheochromocytoma <sup>197</sup> and breast cancer <sup>198</sup>	None
	Increased expression	Ovarian cancer <sup>130,131</sup>	None
<i>E2F3</i> (6p22)	Amplification	Retinoblastoma <sup>105,106,133</sup> , uveal melanoma <sup>199</sup> , and breast <sup>200,201</sup> and bladder cancer <sup>103,104</sup>	Detection of 6p21.2 amplicon in breast cancer; complete <i>RB1</i> inactivation through LOH in retinoblastoma
	Increased expression	NSCLC <sup>111</sup> , SCLC <sup>111</sup> , and bladder <sup>105,104</sup> , breast <sup>118</sup> , ovarian <sup>188</sup> and prostate cancer <sup>202</sup>	Decreased <i>RB1</i> expression in breast cancer
<i>E2F4</i> (16q21-q22)	Amplification	Bladder cancer <sup>101</sup>	CGH analysis carried out on 12 transitional cell carcinoma lines
	Deletion	HCC <sup>203</sup> and breast cancer <sup>17,204,205</sup>	None
	Increased expression	Breast <sup>117</sup> and colon cancer <sup>206</sup>	None
	Mutation (AGC repeat)	GI cancer <sup>207-209</sup>	Expanded or reduced polyserine stretch and LOH frequently observed
<i>E2F5</i> (8q21.2)	Amplification	Osteosarcoma <sup>195</sup> and bladder <sup>102</sup> , colon <sup>210</sup> and breast cancer <sup>128,200</sup>	Minimal common region of 8q21.3-8q23 in osteosarcoma; <i>MOS</i> and/or <i>MYC</i> amplification in breast cancer
	Increased expression	Ovarian <sup>115,188</sup> and breast cancer <sup>200</sup>	None
<i>E2F6</i> (2p25.1)	Amplification	Neuroblastoma <sup>211</sup> and ganglioneuroblastoma <sup>212</sup>	Detection of 2p25 amplicon in ganglioneuroblastoma; <i>MYCN</i> amplification in neuroblastoma
<i>E2F7</i> (12q21.2)	Increased expression	Cutaneous SCC <sup>132</sup>	None
	Decreased expression	Ovarian cancer <sup>115</sup>	None
<i>E2F8</i> (11p15.1)	Deletion	Medulloblastoma <sup>213</sup>	LOH of subchromosomal region 11p13-11p15.1
	Increased expression	Ovarian cancer <sup>114</sup>	None



CGH, comparative genomic hybridization; GI, gastrointestinal; HCC, hepatocellular carcinoma; LOH, loss of heterozygosity; NSCLC, non-small-cell lung cancer; SCC, squamous cell carcinoma. SCLC, small-cell lung cancer.