The transcription factor E2F: a crucial switch in the control of homeostasis and tumorigenesis

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Summary. The transcription factor E2F plays a crucial role in governing cell proliferation through manipulation of the expression of many genes required for cell cycle progression. As studies are exploring in depth, E2F has grown into a multimember family and has been required for the regulation of a large number of genes involved in various cellular processes. The expanding E2F membership and biological function provide us some new insights relating to the evolution of E2F. One of them is to understand the exact mechanisms by which E2F executes in these different cellular processes during ontogenesis. This review summarizes recent advances in this field, with an emphasis on a notion that E2F acts as a molecular switch in the control of both normal cell and tumor development.

Key words: E2F, Proliferation, Differentiation, Apoptosis, Development, Tumorigenesis

Introduction

E2F was originally defined as a cellular activity able to mediate the transcriptional activation of the adenovirus E2 promoter (Kovesdi et al., 1986, 1987; Reichel et al., 1987; Yee et al., 1987). Subsequent work corroborated that the transcription factor E2F is a downstream effector of both positive and negative growth-regulatory signals (Sherr and Roberts, 1999), which determine whether or not a cell will divide, and is required for the timely regulation of numerous genes essential for DNA replication and cell cycle progression (Dyson, 1998). As studies are exploring in depth, several additional homologs have been cloned as E2F family members. Meanwhile, using genome-wide approaches to discover novel target genes of E2F has led to the identification of a large magnitude of genes that are involved not only in cell cycle progression, but also in apoptosis, DNA damage repair, differentiation and development. Moreover, disruption of E2F activity invariably accompanies the development of tumor. Undoubtedly, E2F is crucial to control or determine a cell’s fate. For this reason, it is highly important to understand how E2F fulfills its transcription-regulating function and coordinates these different cellular processes. In this review, we focus on recent advances understanding these two issues and its link with tumorigenesis.

Molecular bases for E2F’s transcriptional regulation

E2F has now been identified as a family consisting of one protein subunit encoded by the E2F family of genes and the other by the DP gene family, and to date, more than ten distinct polypeptides have been cloned from mammalian cells (Fig. 1). These proteins are characteristic of sharing a conserved DNA-binding domain. Based on structural and functional considerations, E2F family members can be divided into two subclasses: E2F1, E2F2 and E2F3a as transcription activators, E2F3b, E2F4, E2F5, E2F6, E2F7 and E2F8 as transcription repressors. Among the latter subclass, E2F4 shares a similar structure with E2F5, and E2F7 with E2F8. The DP proteins are essential, but not responsible, for E2F activity (Dyson, 1998; Trimarchi and Lees, 2002). The function of E2F as transcriptional regulator is intimately linked to its association with pocket proteins, represented by the pRB and its relatives p107 and p130 (Dyson, 1998; Liu et al., 2004a). Recent identification of E2F direct, physiological target genes by large-scale new approaches has revealed that many additional genes unrelated to cell cycle progression are also induced by E2F (Bracken et al., 2004). The expanding membership and targets of the E2F family have significantly enriched our view of E2F and call for a better understanding of the mechanisms by which E2F exerts its regulation to target genes.
Mediation of both transcription activation and repression

It is known that E2F1, E2F2, and E2F3a are potent transcriptional activators. This view is supported by overexpression of any of these proteins or microinjection of anti-E2F antibodies that activates transcription of genes and drives cell cycle progression or represses gene expression and causes cell cycle arrest, respectively, and by analysis of mouse embryonic fibroblasts (MEFs) deficient E2Fs that underscore their essentiality for cellular proliferation (Dyson, 1998; Trimarchi and Lees, 2002). Unexpectedly, a considerable amount of information has accumulated that this subclass may yet act as transcriptional repressors (Ishida et al., 2001; Muller et al., 2001; Vernell et al., 2003; Young et al., 2003). The mechanisms by which E2F1-3a negatively regulates the expression of genes are unknown. Bracken and colleagues presume two possibilities in their review (Bracken et al., 2004). One is that E2F1-3a recruits corepressors to block transcription in a manner similar to the capacity of MYC to repress the expression of some target genes directly (Wanzel et al., 2003). The other is that because of the emerging of antisense regulation in the human genome (Yelin et al., 2003), E2F1-3a binds to the promoters of antisense transcripts and activates their transcription. These antisense mRNA sequences subsequently bind to the corresponding target mRNA and downregulate its expression in the absence of protein synthesis.

The potential mechanisms for E2F4 and E2F5 to act as transcription repressors can be demonstrated from two aspects. First, unlike E2F1, E2F2 and E2F3, which share a canonical basic nuclear localization signal, E2F4 and E2F5 have nuclear export signals. Consistent with this observation, E2F1-3 is constitutively nuclear, whereas E2F4 and E2F5 are predominantly cytoplasmic, which indicates that E2F4 and E2F5 can not activate E2F-responsive genes by themselves (Muller et al., 1997; Verona et al., 1997). On the other hand, association with pocket protein is sufficient to induce nuclear localization of E2F4 and E2F5 (Verona et al., 1997) and the binding of these complexes to promoters diminishes acetylation of histones and represses gene transcription in quiescent cells (Takahashi et al., 2000). Despite all that, it is possible that E2F4 and E2F5 could activate transcription under some circumstances, because E2F4 has been detected on certain mouse promoters in late G1/S phase and ectopically expressed E2F4 is able to induce proliferation (Wang et al., 2000; Wells et al., 2000).

Relatively little is known about E2F3b, E2F6, E2F7 and E2F8, which have been recently identified as E2F-family members and also act as repressors of transcription. E2F3b is an alternative form lacking the N-terminal region present in E2F3, now designated as E2F3a, and is transcribed from a previously unrecognized promoter in the first intron of E2F3a (He et al., 2000; Leone et al., 2000). A recent report provides evidence that E2F3b acts as a repressor by shutting off the expression of p19Arf (Aslanian et al., 2004), coincident with the high level of p19Arf in E2F3-/mouse embryonic fibroblasts (MEFs) (Humbert et al., 2000b). Notably, E2F6, E2F7 and E2F8 diverge considerably from the other members since they lack sequences that mediate transcriptional activation and pocket protein-binding domain (Morkel et al., 1997; Cartwright et al., 1998; Trimarchi et al., 1998; de Bruin et al., 2003; Di Stefano et al., 2003; Logan et al., 2004; Maiti et al., 2005). Additionally, E2F7 and E2F8 are the
most extraordinary because of possessing two distinct DNA-binding domains and lacking a dimerization domain, but little is known about the specific property related to their unusual structure. These observations suggest that the mechanisms for them to mediate gene silencing may differ from the conventional mode of E2F regulation. E2F6 is thought to repress its target genes either through its direct binding to polycomb group (PcG)-related proteins or through the formation of a large multimeric complex containing Mga and Max proteins (Trimarchi et al., 2001; Ogawa et al., 2002). Interestingly, a latest study (Attwooll et al., 2005) has identified a novel E2F6-PcG complex which may regulate genes required for cell cycle progression through interaction with EZH2. Instead, E2F7/8-mediated repression does not depend on association with pocket protein or dimerization with DP proteins. They share a number of characteristics that could reflect their unique function. Both are expressed in a cell cycle-dependent manner, with peak level found during S phase, and are expressed in the same adult tissues of mice. Biochemical evidence reveals that both bind DNA as homodimer. Importantly, their overexpression significantly reduces the expression of E2F target genes and leads to a pronounced decrease in the proliferative capacity of cells. These findings imply that they may have overlapping and perhaps synergistic effects on cell proliferation (de Bruin et al., 2003; Di Stefano et al., 2003; Logan et al., 2004; Maiti et al., 2005). Whether other co-factors participate in the process of transcription repression mediated by E2F7 and E2F8 remains to be determined.

\[\text{Regulation of E2F by the pRB family}\]

pRB and its relatives, p107 and p130, which belong to a family of proteins called pocket proteins, have been proved to be key negative regulators that bind to E2Fs through a conserved carboxyl-terminal domain. This binding not only directly attenuates the action of E2F in stimulating transcription via masking its transactivation domain but also serves to recruit a repressor module to E2F-responsive promoters (Harbour and Dean, 2000; Trimarchi and Lees, 2002). This co-repression function of the pRB family seems to involve a number of distinct components, including histone deacetylases, components of the mammalian SWI/SNF chromatin-remodeling complex, histone methyltransferases, heterochromatin proteins, DNA methyltransferases, and Polycomb group proteins (Robertson et al., 2000; Strobeck et al., 2000; Zhang et al., 2000; Dahiyat et al., 2001; Nielsen et al., 2001). These factors can alter the chromatin structure and lead to gene silencing. Based on biochemical characterization of individual pRB interacting proteins, it has been hypothesized that pRb family members must assemble relatively stable complexes involving multiple co-repressors to mediate transcriptional repression of E2F-responsive genes. Furthermore, these co-repressors are critical for the stability of pRB residence on chromatin (Angus et al., 2003).

The pocket-protein-binding domain is embedded in the transactivation domain of most E2F family members, so most of them are regulated by suppressive association with pocket proteins in the aforementioned manner. Specifically, different E2F members show a strong preference for different pRb family proteins: E2F1, E2F2 and E2F3 bind almost exclusively to pRb; E2F4 binds with high affinity to p107 and p130 but also associates with pRb in some cell types; E2F5 binds to p130 (Dyson, 1998; Stevaux and Dyson, 2002). While E2F6, E2F7 and E2F8 are not regulated by the pocket proteins because they lack sequences homologous to pocket-protein-binding domain of other E2Fs. At the same time, these proteins are expressed and combine together during different stages of the cell cycle: p130/E2F complexes are found primarily in quiescent or differentiated cells and p107/E2F complexes are most prevalent in S phase cells but can also be found in G1. pRB/E2F complexes can be found in quiescent or differentiated cells, but are more evident as cells progress from G1 into S phase (Dyson, 1998).

To better understand the regulation of E2F by pRB family, many studies focus on the crystal structure of E2F/pRB complex. pRB contains two pocket domains that are critical for the biological activity of pRB and the formation of complex with E2F (Lee et al., 1998, 2002a). Recently, Xiao and colleagues (Xiao et al., 2003) have identified an additional interaction of pRB with the marked box of E2F1, that contributes to stronger affinity of these two proteins. This specific interaction, also identified by Dick and Dyson, seems to prevent binding of E2F1 to DNA and antagonize the ability of E2F1 to induce apoptosis (Dick and Dyson, 2003).

\[\text{Regulation of E2F target genes}\]

As the E2F responsive genes are involved in diverse events during cell evolution, the activation of E2F target genes have been investigated in detail. Bracken et al. (2004) have summarized that the models of transactivation of E2F target genes may vary between different genes. In that literature, these genes and their regulating models have been divided into three subgroups based on their activation at the stage of cell cycle. The first subgroup is activated at the G1/S transition such as CCNE1 and CDC6. During G0 and early G1 this group of genes is repressed by E2F4 or E2F5 with recruitment of a pocket protein and co-repressors. As cells progress through G1 to S phase, the pocket protein is phosphorylated by cyclin/cyclin-dependent kinase complexes, resulting in a striking diminution of these repressive complexes binding to promoters which are replaced subsequently by E2F1, E2F2, and E2F3 that activate the expression of these genes. The second subgroup accumulates subsequent to G1/S such as CCNA2 and CDC2. These genes are presumably regulated by additional repressors and/or...
activators because accumulation of their mRNA is delayed with respect to the first group genes. The third subgroup is activated in early G1 such as MYC and CCND1. These genes are also repressed in the same way as the first group genes in G0, but are activated before the accumulation of cyclin/cyclin-dependent kinase activity. The mechanisms to stimulate these genes are still elusive. As such, there would exist a more complex network in regulating the E2F target genes and in ensuring the periodical expression of genes activated at different stage of cell cycle. To support that, a serial studies (Schlisio et al., 2002; Giangrande et al., 2003, 2004a; Sim et al., 2004; Zhu et al., 2004) show that the activity of an activator or a repressor E2F is often dictated by the presence of the partner protein, pointing to the role for cooperative interactions between E2F and other transcription factors as a mechanism to achieve the temporal control of gene expression as cells move through the cell cycle. Another study (Giangrande et al., 2004b) demonstrates that E2F6 can act as a specific repressor of E2F-regulated G1/S genes and functions to distinguish G1/S and G2/M transcription during the cell cycle.

And besides, there may be some additional groups of genes, for example, apoptotic target genes and genes participating in differentiation, development, and tumorigenesis. The regulation of these genes may differ from that of cell cycle dependent genes, but little is known. A derepression model might be involved in these specific targets of E2F. An experimental evidence comes from analysis of embryonic motoneuron gene regulation which suggests that derepression of the activators (such as E2F) is necessary to permit motoneuron-specific gene Hb9 expression (Lee et al., 2004). In conclusion, the exact mechanisms involved in the transactivation of E2F responsive genes need to be further elucidated.

How does E2F coordinate different cellular processes?

The biological function of E2F is just beginning to emerge. More recently, new approaches that harness the power of ChIP (Chromatin Immunoprecipitation) and combine this technique with DNA microarray and bioinformatics have identified a large number of E2F target genes involved not only in DNA replication and cell cycle progression, but also in DNA damage repair, G2/M checkpoint, apoptosis, differentiation and development (Kel et al., 2001; Muller et al., 2001; Weinmann et al., 2001, 2002). This provides more compelling evidence that E2F may act as a pivotal regulator of several cellular processes far beyond the originally described cell cycle progression and proliferation. However, different cellular processes must be coordinated during ontogenesis, thereout a question arises: how does E2F control these processes precisely. The understanding in this field has been explored in some experiments and establishes a notion that the activity of E2F may act as a determinative switch in

several cellular processes that are essential for individual development (Fig. 2).

A molecular switch modulates the progression of proliferation into terminal differentiation

In order for differentiation to occur, proliferating cell must be stimulated to undergo growth arrest. Once growth is arrested, cells are able to respond to differentiation-inducing stimuli, resulting in both morphological and biochemical alterations. The inhibition of E2F has been demonstrated to be a prerequisite for initiation of squamous differentiation by two independent manners, the promotion of growth arrest and relief of the differentiation-suppressive properties of E2F. First, in proliferating keratinocytes, the pro-proliferative properties of E2F actively repress the ability of keratinocytes to respond to differentiation stimuli and the expression of differentiation-specific genes (Dicker et al., 2000). Because of this, the activating E2F inhibition is a key event allowing keratinocytes to undergo differentiation. This has been demonstrated by the observation that inhibition of E2F sensitizes proliferating keratinocytes and differentiation-incompetent squamous cell carcinoma cell lines to respond to differentiation stimuli (Wong et al., 2003). To further understand the function of E2F genes in epidermal morphogenesis, the expression patterns of E2F in primary keratinocytes or in cells induced to...
differentiate has been examined, which present a switch in expression from E2F1-3 in undifferentiated, proliferating cells to E2F5 in terminally differentiated keratinocytes (D’Souza et al., 2001). This is consistent with the previous reports that the activating E2F (E2F1, E2F2 and E2F3a) is able to promote cell cycle progression and proliferation, and the repressive E2F (E2F4 and E2F5) to induce cell cycle exit and terminal differentiation (Trimarchi and Lees, 2002). On the contrary, E2F6 is not involved in the modulation of squamous differentiation (Wong et al., 2004). The model of squamous differentiation may be a primary paradigm for how E2Fs modulate the progression of proliferation into terminal differentiation. Other evidence underlying E2F inhibition of cell differentiation is provided by observation of the effect of constitutive E2F1 overexpression on chondrocyte differentiation. The overexpression of E2F1 disturbs chondrocyte maturation, leading to a delayed endochondral ossification (Scheijen et al., 2003).

However, the activation of E2F might assume an equally important role in differentiation because the activating E2F members upregulate the expression of several differentiation-associating genes (Muller et al., 2001; Bracken et al., 2004). It would conflict with the concept that proliferating cells must be stimulated to exit from the cell cycle to trigger differentiation, since the activating E2F is known to promote cell cycle progression. For that reason, it is difficult to understand how activation of E2F mediates differentiation compared to inhibition of E2F. A possible explanation is that the cells stimulated to differentiation have to undergo several additional cell cycles before terminal differentiation (Brown et al., 2003), which is likely to allow E2F to activate differentiation-associating genes before the E2F inhibition occurs. Coincident with this supposition, cell cycle machinery and tissue-specific factors are concomitantly upregulated in differentiating cells (Fajas et al., 2002). It is therefore presumed that there is an unknown mechanism to coordinate the function of activation and inhibition of E2F in regulating the progression of proliferation into terminal differentiation.

A biological switch balances proliferation and apoptosis

It is worth noticing that E2F1 is capable of promoting both cell cycle progression and apoptosis (Trimarchi and Lees, 2002). Several key apoptotic genes, including ARF, ATM, p73, Apaf-1 and some genes of the BH3-only proteins family are E2F targets and involved in E2F1-mediated apoptosis (Bates et al., 1998; Irwin et al., 2000; Lissy et al., 2000; Stiewe and Putzer, 2000; Muller et al., 2001; Furukawa et al., 2002; Berkovich and Ginsberg, 2003; Hershko and Ginsberg, 2004). Furthermore, E2F1-mediated induction of apoptosis seems to be an activity responding to DNA damage, in which the regulation of E2F1 apoptotic target genes is associated with ATM-mediated phosphorylation of E2F1 (Lin et al., 2001; Pediconi et al., 2003) and 14-3-3 that mediated further stabilization of E2F1 (Wang et al., 2004). Of note, p53 is a key effector molecule that induces apoptosis in response to DNA damage. A serial studies (Hsieh et al., 2002; Fortin et al., 2004; Powers et al., 2004; Rogoff et al., 2004; Chen et al., 2005; Fogal et al., 2005; Hershko et al., 2005) show that E2F can cooperate with p53 to induce apoptosis through a number of parallel and perhaps synergistic mechanisms. First, E2F1 can directly activate p53 in a protein-protein interaction fashion. Second, E2F1 contributes to stabilization and general activation of p53 by transcriptionally activating ARF, ATM, Nbs1, Chk2 and PIN1. Thirdly, E2F1 induces expression of the proapoptotic cofactors of p53, ASPP1, ASPP2, JMY and TP53INP1, thereby directing p53 to its proapoptotic targets. Finally, E2F1 directly activates some of the p53 proapoptotic target genes including Apaf1, PUMA, Noxa, SIVA and probably also p53AIP1, thereby augmenting the upregulation of the corresponding transcriptions by p53. Another facet of the response of E2F1 to DNA damage is also supported by the presence of many checkpoint and DNA damage repair genes among E2F1 targets (Ren et al., 2002). Many of them have been proposed to function physiologically to prevent the refiring of replication origins and to suppress genomic rearrangements resulting from DNA replication or chromosome segregation errors during the normal cell cycle (Bracken et al., 2004). Collectively, these data demonstrate that E2F1 directly links cell cycle progression with the coordinate regulation of genes essential for both the synthesis of DNA as well as its surveillance. Although the molecular mechanism underlying this surveillance activity of E2F1 has not been well understood, the ultimate consequence of this surveillant system is survival or apoptosis.

In this way, E2F1-mediated induction of apoptosis is an activity acting as a switch which is shut off under normal physiological conditions and will be turned on once cells suffer from serious stress such as DNA damage. Therefore, there must be some specific mechanisms to switch off physiologically the activity of E2F1-induced pro-apoptosis. Recent studies (Dick and Dyson, 2003; Xiao et al., 2003) have identified an additional E2F1-binding site in the C-terminal portion of pRB, that allows it to bind specifically to E2F1 on a domain distinct from the previously characterized pocket protein-binding domain. This interaction seems to switch off E2F1-induced apoptosis in physiological conditions. Most interestingly, this specific interaction is disrupted upon DNA damage that may subsequently modify E2F1 and allow it to activate cell apoptosis. Other studies have provided differential mechanisms by which E2F1 can inhibit its own pro-apoptotic activity in normal, cycling cells through the induction of Gab2 and the activation of the PI3K/AKT pathway (Chausseped and Ginsberg, 2004) or through the induction of TopBP1 that inhibits E2F1-dependent apoptosis in a feedback manner (Liu et al., 2004b).
Besides ensuring genomic stability, the regulation of E2F1-induced apoptosis might also act extensively to maintain homeostasis during individual development. Previous work has shown that loss of the apoptotic activity of E2F1 reduces the apoptosis seen in lens of Rb-deficient embryos (Yamasaki et al., 1998; Hyde and Griep, 2002) and also severely impair the thymocyte negative selection (Field et al., 1996; Zhu et al., 1999; Garcia et al., 2000), in which E2f1-/- mice have developed an overgrowth of T cells resulting from incapability of driving apoptosis. These observations reveal that one significant role of E2F is to keep homeostasis by balancing proliferation and apoptosis. And future analysis will need to focus this activity on more tissues during individual’s growth and development.

A delicate equilibrium in tissue or organ’s development

As we know, the development of multicellular organisms relies on the temporal and spatial control of cell proliferation and differentiation, and E2F family members might be implicated in this process because it can regulate genes involved in proliferation, differentiation and development. Analysis of E2F mutant mouse strains has shown that the individual E2Fs play overlapping and unique role in controlling tissue and organ development. For example, E2F1-/- mice have various tissue-specific abnormalities including T cell heteroplasia and testicular atrophy (Field et al., 1996; Yamasaki et al., 1996; Zhu et al., 1999; Garcia et al., 2000) as well as a high predisposition to the development of Insulin-dependent diabetes mellitus (IDDM) and Sjogren’s syndrome (SS) (Iglesias et al., 2004; Salam et al., 2004). Studies using the E2F1/E2F2 compound mutant mouse strains indicate that E2F1 and E2F2 exert essential influences in expansion and maturation of hematopoietic progenitor cells during hematopoiesis (Li et al., 2003a), and in postnatal pancreas development and maintenance of differentiated pancreatic phenotypes in the adult as well (Li et al., 2003b). E2F4 deficient mice generate defects in the development of hematopoietic lineage and the gut epithelium, in conjunction with an increased susceptibility to opportunistic infections that seems to result from craniofacial defects (Humbert et al., 2000a; Rempel et al., 2000). In contrast, a high frequency of neonatal lethality caused by the loss of E2F3 and simultaneous inactivation of E2F4 and E2F5 in mice (Gaubatz et al., 2000; Humbert et al., 2000b; Cloud et al., 2002), hints that they perform overlapping functions during embryonic development. In spite of serious disturbances found in E2F mutant mouse development, how the E2Fs act in each normal tissue or organ is poorly understood. But anyhow, a precise balance of proliferation versus apoptosis and/or differentiation must exist in many normal tissues. This appears to be controlled by E2F switch that might mediate a delicate equilibrium throughout embryonic development and adult life, and await further confirmation.

E2F and tumorigenesis: promotes or suppresses tumor formation?

Cancer is a multifaceted disease where cell proliferation is no longer under normal growth control. Eventually, this unrestrained growth and division of the cancer cells is accountable for deregulated cell cycle progression or incapacity of apoptosis (Hanahan and Weinberg, 2000; Garrett, 2001). As discussed above, the individual E2Fs have both positive and/or negative functions that are integrated to regulate cell proliferation and to maintain cellular homeostasis. In this manner, deregulation of E2F activity is very prone to facilitate tumor formation.

Among the E2F transcription family members, E2F-1 is unique in its ability to regulate a number of key genes that participate in both cell cycle progression and apoptosis, raising a potential link with its role in tumorigenesis. The investigations on cells or animals present us with a wonderful paradox that E2F1 behaves as both an oncogene and a tumor suppressor gene. On one hand, enhancement of E2F1 activity in tissue culture cells can stimulate cell proliferation and be oncogenic. On the other hand, E2F-1 has been demonstrated as a tumor suppressor by spontaneous development of multiple tumors in mice lacking E2F1 (Dyson, 1998; Bell and Ryan, 2004). Therefore, the effect of E2F1 on tumorigenesis can be mediated by either upregulation or downregulation of E2F1 activity in specific cells. Such different roles of E2F1 in tumorigenesis can now be explained by its uniquely dual functions in both cell proliferation and apoptosis (Bell and Ryan, 2004).

It seems more likely that changes of other E2Fs could either promote or suppress tumor formation. The work to demonstrate the tumorigenic properties of other E2F family members is subsequently carried out on various E2F mutant mice. E2F2 mutant mice are tumor prone and the combined mutation of E2F1 and E2F2 increases both the incidence and onset of tumorigenesis (Zhu et al., 2001). Conversely, the mutation of E2F3, either alone or in combination with mutation of E2F1 or E2F4 has no detectable effect on tumorigenesis (Humbert et al., 2000a,b; Rempel et al., 2000; Cloud et al., 2002), suggesting that loss of E2F3 can rescue the tumor-suppressive ability of E2F1, although its mechanism is unknown. Significantly, the tumorigenic properties of the individual E2F genes appear quite different when analyzed in the context of the RB defective background. In this setting, E2F3 clearly displays opposing effects on pituitary tumors (suppressing) and thyroid tumors (promoting) (Ziebold et al., 2003), indicating that it behaves as both an oncogene and a tumor suppressor as E2F1. The oncogenic activity of these E2Fs is widely believed, resulting from their roles in transcription activation and the induction of cell proliferation (Wu et al., 2001). Nevertheless, there is still considerable debate about the
underlying basis for the E2Fs’ tumor-suppressive activity. One of the most popular models which account for the E2Fs’ tumor-suppressive activity is due to its capacity to activate apoptosis (Dyson, 1998; Trimarchi and Lees, 2002; Bell and Ryan, 2004). In agreement with this possibility, the studies of compound RB/E2F mutation show that the absence of either E2F1 or E2F3 greatly suppresses the p53-dependent and -independent apoptosis arising in Rb-deficient embryos (Tsai et al., 1998; Ziebold et al., 2001). The phenotype of E2F4/RB compound mutant mice adds to this complexity (Lee et al., 2002b). In this study, the loss of E2F4 suppresses tumorigenesis in Rb mutant mice via a novel mechanism: E2F4 loss allows p107 and p130 to bind and inhibit E2F1 and E2F3. Taken together, these findings present us an intricate but undoubted fact that deregulation of the individual E2Fs can induce the formation of tumor via similar or different mechanisms. Obviously, additional experiments will be required to address all potential mechanisms.

Recent studies have also shown that E2F can contribute to tumor proliferation through stimulating the expression of certain oncoproteins or suppressing the expression of some tumor suppressor gene. For instance, E2F-1 upregulates the expression of ICBP90 by binding to the intron of ICBP90, which contains two E2F-1-binding motifs (Mousli et al., 2003; Unoki et al., 2004). The accumulation of ICBP90 was found in breast-cancer cells, where it might suppress expression of tumor suppressor genes via recruitment of HDAC1 which causes deacetylation of histones (Unoki et al., 2004). E2F1 is also implicated in hepatocarcinogenesis by regulating the transcription of dbpA which can accelerate the step of inflammation-induced hepatocarcinogenesis (Arakawa et al., 2004). Simultaneously, recent clinical researches also support E2F to be a crucial regulator at the process of tumorigenesis. The increasing expression of E2F1 is regarded as a highly informative biomarker in many human tumors association with their malignancy and prognosis (Yasui et al., 1999; Brake et al., 2003; Fujita et al., 2003; Han et al., 2003; Niu et al., et al., 2003; Ebihara et al., 2004; Onda et al., 2004). In addition, it has been reported that deregulation of E2F1 activity contributes to enhanced proliferation and resistance to cytotoxic drugs in human cancer cells (Halaban et al., 2000). E2F3 amplification was strongly associated with invasive tumor phenotype and high tumor grade in a subset of bladder tumors and prostate cancer (Foster et al., 2004; Oeggerli et al., 2004). However, E2F5 was reported under-expression in serous ovarian carcinomas (Collins et al., 2004). These findings facilitate better understanding of the effects of E2Fs on tumorigenesis.

**Conclusion and perspective**

The E2F transcription factors are key regulators required for controlling the expression of a large magnitude of genes involved in cell cycle progression, apoptosis, DNA damage repair pathway, differentiation, and development. Biochemical and functional studies of the family of E2F transcription factors exhibit a miraculous paradox that E2F has the ability to mediate transcriptional activation or repression, to promote cell proliferation or apoptosis, and to promote or suppress tumor formation in a tissue specific manner. Therefore, to well establish the fundamental roles of E2F in such a multitude of cellular processes, a comprehensive understanding of the mechanisms by which the individual E2Fs regulate their specific targets in these cellular events will be required. Clearly, there are other dubious or unknown issues which need to be resolved, such as how the E2F switch balancing proliferation and apoptosis works during development and whether the newly identified members including E2F6, E2F7 and E2F8 act as tumor repressors.

Finally, the story of E2F’s evolution implies that E2F might act as a molecular switch in maintaining cellular homeostasis. Once the E2F switch has been disrupted during development, the individual may suffer from a series of disorders and cancer as well. Meanwhile, the recognition of the important role of E2F in tumorigenesis may raise expectations for E2F as a fascinating target for anticancer intervention.

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