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ROLE OF E2F IN CELL CYCLE CONTROL AND CANCER

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TABLE OF CONTENTS

1. ABSTRACT

E2F transcription factors regulate the expression of a number of genes important in cell proliferation, particularly those involved in progression through G1 and into the S-phase of the cell cycle. The activity of E2F factors is regulated through association with the retinoblastoma tumor suppressor protein (Rb) and the other pocket proteins, p107 and p130. Binding of Rb, p107 or p130 converts E2F factors from transcriptional activators to transcriptional repressors. The interplay among G1 cyclins (D-type cyclins and cyclin E), cyclin-dependent kinases (cdk4, 6, and 2), cdk inhibitors, and protein phosphatases determines the phosphorylation state of the pocket proteins which in turn regulates the ability of the pocket proteins to complex with E2F. E2F activity is further regulated through direct interactions with other factors, such cyclin A, Sp1, p53 and the ubiquitin-proteasome pathway. Deregulated expression of E2F family member genes has been shown to induce both inappropriate S phase entry and apoptosis. An important role for E2F in the development of cancer is suggested by the finding that in most human neoplasias, genetic or epigenetic alterations occur that ultimately result in the deregulation of E2F-dependent transcription. This review will highlight recent findings on the specific roles of the individual E2F species in regulating transcription, proliferation and apoptosis, and discuss the growing link between E2F and cancer.

2. INTRODUCTION

E2F transcription factors play a key role in cell proliferation control by linking the activities of the cell cycle machinery with the transcriptional regulation of genes whose products are required for S-phase entry and DNA synthesis. E2F DNA-binding sites (TTTC/GG/CCGC/G) are found in the promoters of genes encoding enzymes directly involved in DNA synthesis such as DHFR, thymidine kinase, HsOrc1, and DNA polymerase alpha (1-6). Several regulators of the cell cycle, such as cdc2, cyclin E, cyclin A, and the E2F1 gene itself also contain E2F binding sites in their promoters (5,7-12). In addition, some proto-oncogenes involved in cell proliferation, including c-myb, B-myb, c-myc, and N-myc, have been shown to be regulated by E2F (1,2,13-15). In many cases, the E2F binding sites identified in these gene promoters have been demonstrated to be critical for their cell cycle-regulated expression.

E2F factors appear to be key components in a cell cycle checkpoint that determines whether a cell will arrest in G1 or enter into S-phase. Multiple mitogenic signaling pathways, as well as growth inhibitory signals, ultimately converge upon E2F at this G1 phase checkpoint. In addition to regulating S-phase entry, E2F factors have also been implicated in regulating growth inhibition, differentiation, apoptosis and oncogenic transformation. The E2F family appears to accomplish these diverse activities through the regulated expression of distinct E2F factors and through their associations with a variety of cell cycle-regulatory proteins. Recent studies are beginning to shed light on the specific roles of the different E2F species and of the various multiprotein complexes containing E2F factors. In addition, direct links are now being made between the loss of normal E2F-dependent transcriptional control and the development of cancer.

3. DISCUSSION

3.1. The E2F family

The E2F transcription factor family consists of at least seven distinct genes divided into two groups. E2F1, E2F2, E2F3, E2F4, and E2F5 constitute one group, while the related DP1 and DP2 genes constitute the other group.

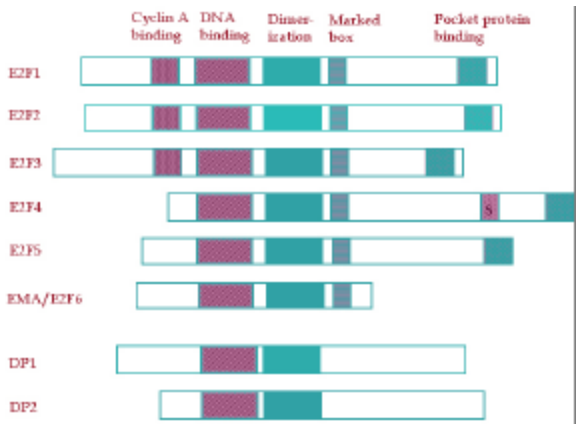

Figure 1. Schematic Representation of the E2F Family.

Several forms of the DP2 (also referred to as DP3) protein can be produced as the result of alternative splicing, thus providing additional complexity to the E2F family (16). A functional E2F transcription factor consists of a heterodimer containing an E2F polypeptide and a DP polypeptide (17-21). Each of the five E2F polypeptides can heterodimerize with either DP1 or DP2 (DP3) (16-22). Furthermore, each of these E2F/DP heterodimers (referred to as E2F factors hereafter) can bind consensus E2F sites *in vitro* and stimulate transcription when overexpressed (20,23-26).

All of the E2F subgroup proteins have a similar structure, although E2F1, E2F2, and E2F3 are more closely related to each other than to E2F4 and E2F5 (figure 1). The DNA-binding domain found in the amino terminus represents the area of greatest homology between the five E2F species (22,25-27). Adjacent to the DNA-binding domain is the DP dimerization domain, which contains within it a leucine heptad repeat. The carboxy termini of the five E2F polypeptides contain the defined transcriptional activation domains, which are characterized by an abundance of acidic residues. Embedded within the transactivation domain of each E2F is a region of homology involved in binding to the pocket proteins (Rb, p107, and p130) (28). An additional region of homology, termed the Marked box, lies between the DP dimerization and transcriptional activation domains. Although this Marked box motif is highly conserved between the different E2Fs, its function is unknown. The amino termini of E2F1, E2F2, and E2F3 contain an additional region of homology not found in E2F4 or E2F5. This region has been demonstrated to have several functions, including binding to the cyclin A protein (29,30). The E2F4 protein contains a stretch of consecutive serine residues between the Marked box and the pocket protein binding domain not found in other E2F family members (22-24). DP1 and DP2 polypeptides contain DNA-binding and dimerization domains related to the E2F proteins but do not contain transcriptional activation domains or regions homologous to the pocket protein binding or Marked box domains (20, 21, 27,31).

An additional E2F family member has recently been isolated and termed EMA (E2F-binding site modulating activity) or E2F6 (32-34). EMA/E2F6 shares homology with the E2F polypeptides in the DNA-binding domain, the DP dimerization domain and the Marked box, but lacks the pocket protein binding domain and acidic transcriptional activation domain found in the carboxy terminus of the other E2F species (figure 1). Like the other E2F polypeptides, EMA/E2F6 dimerizes with DP1 or DP2 and, in conjunction with a DP partner, binds E2F DNA-binding sites with preference for a subset of sites with the core sequence TCCCGCC. EMA/E2F6 appears to function as a repressor of E2F site-dependent transcription independent of pocket protein binding. The mechanism of repression is either through competitive inhibition with other E2F species or through an active transcriptional repression domain located in the amino terminus of EMA/E2F6 (32-34).

The expression of the various E2F family members differs during the cell proliferation response. Following stimulation of serum-starved fibroblasts or resting T cells to enter the cell cycle, the level of E2F1 mRNA dramatically increases at the G1/S-phase boundary (3,7-9,27,35). E2F2 is expressed with similar kinetics although at an overall lower level in fibroblasts (27,36). E2F3 and E2F5 expression levels rise in early-to-mid G1 and do not significantly increase thereafter as cells enter S-phase (22,27). E2F4 is expressed constitutively, even in growth-arrested cells, although a slight increase in expression does occur in mid G1 (22,24). E2F4 is the most abundant E2F gene message, and this is consistent with the finding that in most cells the E2F4 protein is a constituent of the majority of E2F DNA-binding activity (24,37,38). There is also some evidence for tissue-specific expression of E2F family genes. For example, E2F1, E2F2, and E2F3 are all expressed in placenta; E2F1 and E2F3, but not E2F2, are expressed in the heart; and only E2F1 is expressed in the brain (27,39-41).

Like E2F4, DP1 is expressed constitutively with a peak in mid G1 (27). In many cell types, DP1 is a component of the vast majority of E2F DNA-binding complexes. The cell cycle-regulated expression of DP2 has not been determined, but its expression has been found to be tissue specific. High levels of DP2 are found in the heart, where DP1 levels are relatively low, but DP2 is not expressed in the lung or brain (20).

Recent studies are finding that different E2F factors may be responsible for regulating different E2F target genes. Tao and coworkers have found that specific E2F/DP heterodimers select for different E2F DNA-binding site sequences *in vitro* (42). Both the E2F and DP subunits appear to determine E2F site selection. Association of Rb with E2F1/DP1 heterodimers also alters E2F site selection. Several groups have also identified variant E2F sites that preferentially bind some E2F species but not others. In the hamster DHFR promoter, E2F2 was found to preferentially bind to only one of two overlapping E2F sites (43). In the human cyclin A gene promoter, a

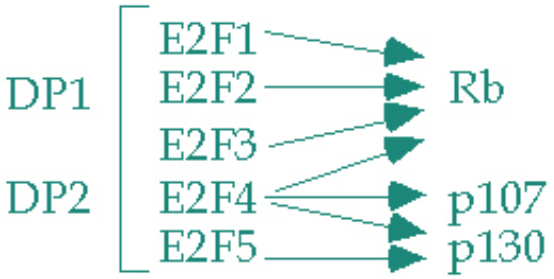

Figure 2. Interactions among E2F family members and the pocket proteins.

variant E2F site is found to bind some E2F species but not others (10).

Experiments analyzing endogenous gene expression following infection of REF52 cells with recombinant adenoviruses have found that overexpression of different E2F species stimulate distinct sets of E2F target genes (44). For example, E2F1, but not E2F3, could efficiently stimulate expression from the cyclin A gene, while E2F3, but not E2F1, could efficiently transactivate the cdk2 gene. These studies also demonstrated that E2F5, and to some extent E2F4, are overall less potent activators of transcription than the other E2Fs. The same was true when the transcriptional activation capacities of E2F1, E2F4, and E2F5 were compared in transient transfection experiments using a variety of E2F-responsive promoters (45). This finding may be related to the subcellular localization of the different E2Fs or to the relative strengths of their respective transcriptional activation domains (45-48). In either case, these findings suggest that the various E2F factors have distinct roles in regulating E2F-dependent transcription.

3.2. Regulation of E2F factors by the Rb family of pocket proteins

The activity of E2F factors is regulated through association with members of the Rb family. This family includes Rb, p107, and p130 proteins, which are collectively known as the pocket proteins. All of the pocket proteins can regulate cell proliferation, in part, through their ability to associate with E2F factors (28,49-53). Pocket protein binding inhibits the transcriptional activation capacity of E2F factors, likely through masking of the transcriptional activation domain and, in at least some cases, converts E2F factors to repressors of transcription (see below). The importance of the Rb-E2F interaction in cell growth control is illustrated by the finding that all naturally occurring Rb mutants isolated from human tumors lack the ability to bind and negatively regulate E2F. Further studies have demonstrated a direct correlation between the ability of Rb to bind E2F and to arrest cells in the G1 phase of the cell cycle (54-56).

The various E2F family members differ in their associations with the pocket proteins (figure 2). E2F1, E2F2, and E2F3 associate exclusively with Rb *in vivo* and not with p107 or p130 (28,37,38). E2F4 associates with all three of the pocket proteins in the cell cycle-regulated manner described below (23,37,38). E2F5 associates with only the p130 protein *in vivo* (26). The DP heterodimerization partner does not appear to be involved in determining the binding specificity of E2F factors for the pocket proteins. However, DP factors do function in stabilizing the interaction between E2F factors and the pocket proteins (17,18).

D-type cyclins modulate the function of Rb during the G1 phase of the cell cycle by activating associated cyclin-dependent kinases (cdk4 and cdk6), which in turn phosphorylate Rb. In fact, D-type cyclins not only function as regulatory subunits for their associated cdks, but also act to target Rb for phosphorylation through direct protein-protein binding (57-59). Cyclin E, in association with cdk2, also likely plays a role in the phosphorylation of Rb during G1 progression (60). A consequence of Rb phosphorylation by G1 cyclin kinases is the release of "free", transcriptionally active E2F factors since only the underphosphorylated form of Rb is functional in E2F binding (59,61). The p107 and p130 proteins have also been shown to be targets for G1 cyclin kinases, and like Rb, phosphorylation regulates their ability to associate with E2F factors (62-64). There is also evidence demonstrating that phosphorylation of E2F factors by cyclin-dependent kinases regulates E2F binding to Rb (65,66). Thus, through the regulation of E2F-pocket protein association, G1 cyclin kinases can regulate the expression of numerous genes required for S-phase entry.

The presence of E2F-pocket protein complexes varies as cells enter, traverse, and exit the cell cycle. In quiescent cells, such as serum-starved fibroblasts or resting T cells, the major E2F DNA-binding activity contains the p130 protein (28,37,38,67). Following stimulation of quiescent cells to enter the cell cycle, the p130 complex is lost in mid-to-late G1, and free E2F heterodimers begin to accumulate. E2F-p130 complexes have also been shown to accumulate as some cell types, including myoblasts and melanocytes, undergo terminal differentiation (28,68-70). At the G1/S-phase transition, complexes containing p107 in association with E2F4 can be detected (37,38). These E2F4-p107 complexes also contain cyclins E or A and cdk2 (71-73). The cyclins and cdk2 in these complexes interact indirectly with E2F4 through stable binding to the spacer domain of p107. The p130 spacer domain can also directly bind cyclins E and A, but stable E2F-p130-cyclin-cdk2 complexes are not usually detected in electrophoretic mobility shift assays (EMSA) (74,75). The physiological relevance of E2F4-p107 complexes containing cyclins and cdk2 is at present unclear. It has been shown that these complexes have both E2F-site specific DNA-binding activity as well as cdk activity (76). However, recent studies have demonstrated that E2F4-p107-cyclin A-cdk2 complexes are found primarily in the cytoplasm where they would be unable to directly regulate transcription (47,48).

The regulation of E2F-Rb complex formation during cell cycle progression is somewhat paradoxical. Rb is hypophosphorylated in quiescent cells and becomes hyperphosphorylated in late G1 through the action of G1

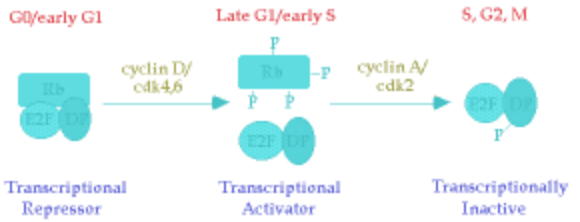

Figure 3. Transcriptional regulation by E2F during cell cycle progression.

cyclin kinases. However, E2F-Rb complexes are not usually detected by EMSA in quiescent cells when Rb is hypophosphorylated and active for E2F binding. Instead, EMSA experiments demonstrate that E2F-Rb DNA-binding complexes accumulate in late G1 and persist throughout S-phase when the vast majority of Rb is hyperphosphorylated and unable to associate with E2F (37,38,77). The reason for this discrepancy is unclear. There is some evidence to suggest that E2F-Rb complexes are present in G0 cells but that these complexes are inactive in binding DNA, at least *in vitro*. (78,79). It has also been shown that E2F1 and E2F3 make unequal contributions to the E2F-Rb complex found in activated T cells. Although both E2F species are present, the majority of E2F1 is in the free form while the majority of E2F3 is in complex with Rb (38).

3.3. Transcriptional repression by E2F

It is now clear that association of pocket proteins with E2F factors does not simply result in an inactive or inert complex. Rather, E2F-Rb, E2F-p107, and E2F-p130 complexes can function as active transcriptional repressors. In several gene promoters, such as B-myb, RB1, and E2F1, E2F DNA-binding sites function as negative regulatory elements (8,14,80). Mutation of the E2F sites in these promoters results in a significant upregulation of promoter activity, particularly in quiescent and early G1 phase cells. This is in sharp contrast to what is observed when the E2F sites of the adenovirus E2 gene promoter are mutated (49). In this case, the overall activity of the E2 promoter decreases, and it becomes unresponsive to activators of E2F such as E1A. In G0/early G1 cells, when E2F sites function as repressor elements, E2F is found primarily in complex with p130 and Rb (37,38,67,81). There is also evidence to suggest that E2F-p107 complexes may function to transcriptionally repress some gene promoters (82).

The idea that E2F complexes containing pocket proteins function as transcriptional repressors is consistent with the finding that Rb, p107, and p130 can each actively repress transcription when brought directly to a promoter through fusion with the yeast Gal4 DNA-binding domain (45,83-87). Repression by pocket proteins requires both the conserved A and B domains of the pocket protein and appears to involve the association of other proteins in addition to E2F factors. Furthermore, phosphorylation of pocket proteins by G1 cyclin kinases inhibits their ability to repress transcription independent of E2F interaction. Several possibilities exist for the molecular mechanism by which pocket proteins actively repress transcription. It is possible that pocket proteins interact directly with other promoter-bound transcription factors, such as AP-2 and PU.1, and block their ability to interact with the basal transcription complex (86). It is also possible that pocket proteins repress transcription by directly binding basal factors such as $TAF_{II}250$ (88). Finally, it may be that pocket proteins repress transcription by remodeling chromatin structure through interaction with proteins such as hBRM, BRG1 and HDAC1 (88-93). hBRM and BRG1 are mammalian homologs of components of the yeast chromatin remodeling complex SNF2/SWI2 (90). HDAC1 is a histone deactylase that has recently been shown to be recruited to E2F complexes by Rb and to function in repressing cyclin E gene expression (92,93). Whatever the molecular mechanism, the role of the E2F factor appears to be to tether the pocket protein to specific target promoters containing E2F sites.

What determines whether a given E2F site will function as a positive or negative regulatory element is at present unclear. Recent studies are finding that the chromatin structure found *in vivo* and the specific sequence of the E2F site may determine if a given E2F-pocket protein complex binds to a given E2F site. *In vivo* footprinting experiments have demonstrated that the E2F site in the B-myb promoter is occupied only in quiescent and early G1 cells when the B-myb promoter is repressed by E2F (94). Thus, although free, transcriptionally active E2F factors can bind the B-myb promoter *in vitro*, this does not appear to occur *in vivo*. A variant E2F site in the human cdc2 gene promoter is also only occupied in G0/early G1 cells *in vivo* (11). *In vitro*, this variant E2F site from the cdc2 promoter binds E2F-p130 complexes but not E2F-p107 complexes found during S-phase. In the p107 gene promoter, only one of two tandemly repeated E2F sites were shown to mediate transcriptional repression by Rb (53). As more studies carefully examine this issue, it is likely that many E2F sites in cellular gene promoters will be found to function, at least in part, as negative regulatory elements.

3.4. Regulation of E2F activity by other factors

E2F1, E2F2, and E2F3 each contain a domain involved in direct binding to the cyclin A protein (29,30,95). This allows cyclin A-associated kinases to downregulate these three E2F factors by targeting the DP1 heterodimerization partner for phosphorylation. The result is an inhibition of DNA-binding activity likely as a consequence of destabilizing the E2F/DP1 heterodimer (96)(figure 3). The amino termini of E2F1, E2F2, and E2F3 also contain a domain involved in interacting with Sp1 that is not found in E2F4 or E2F5 (97-99). Interaction of these three E2F species with Sp1 appears to be important for the regulation of several E2F target genes including DHFR and thymidine kinase.

The amino termini of E2F1, E2F2, and E2F3, but not E2F4 or E2F5, also contain nuclear localization signals (48,100). Thus, E2F1, E2F2, and E2F3 are found almost exclusively in the nucleus, while the subcellular location of E2F4 and E2F5 is regulated through association with other factors (47,48). Some splice variants of DP2 (DP3) also

contain a nuclear localization signal, while DP1 does not (46,47,101). Coexpression of DP2, but not DP1, efficiently promotes the nuclear localization of E2F4 and E2F5. Coexpression of p107 and p130 can also promote nuclear localization of E2F4 and E2F5 (46,47). In quiescent cells, E2F4, in association with pocket proteins, is found predominantly in the nucleus, where these complexes likely function as transcriptional repressors (47). In cycling cells, however, the majority of endogenous E2F-p107, E2F-p130, and free E2F are found in the cytoplasm, while E2F-Rb complexes are found almost exclusively in the nucleus (47,48).

It has been reported that the p53 tumor suppressor protein makes functional interactions with both E2F1 and DP1 (102,103). Expression of p53 can inhibit transcription by E2F1/DP1, while overexpression of E2F1 or DP1 can inhibit p53-dependent transcription. Inhibition of E2F-dependent transcription by p53 may involve competitive interference with E2F1/DP1 heterodimer formation. A regulator of p53, the MDM2 oncogene, may also directly interact with and regulate E2F1. MDM2 binds to the E2F1 transactivation domain *in vitro*, and coexpression of MDM2 augments E2F1 transcriptional activity in transient transfection assays (104). Members of this same group have also demonstrated *in vitro* binding and augmentation of E2F1 activity by the coactivator CBP (105). Other factors that have been demonstrated to bind E2F proteins include the TATA-binding protein, cdk3, the interferon-inducible p202 protein, DDB, NF-κB p50, and BRCA1 (106-111). Whether the apparent interactions of all of these molecules with E2F factors are physiologically relevant is still unclear.

Finally, the levels of some E2F family proteins are regulated through degradation by the ubiquitin-proteasome pathway. The carboxy termini of both E2F1 and E2F4 contain sequences that target them for degradation (112,113). Binding of pocket proteins to the E2F factors blocks them from being ubiquitinated, thus causing E2F-pocket protein complexes to be relatively stable. Interestingly, E1A, which releases pocket proteins from E2F factors, also protects E2F1 and E2F4 from degradation (112), suggesting that other cellular factors may also regulate E2F protein stability.

3.5. Control of S phase entry and apoptosis by E2F

The first direct demonstration that E2F factors regulate cell cycle progression came from studies demonstrating that microinjection of E2F1 expression vectors can induce serum starved REF52 cells to enter S-phase as measured by BrdU incorporation (114). The only other cellular transcription factor that is known to have this S-phase-promoting activity is the c-myc proto-oncogene product (15,115). E2F1 expression also has the ability to overcome G1 phase cell cycle blocks induced by several other factors. E2F1 can overcome a G1 phase block induced by Rb (116), TGF-β (117) and γ irradiation (118). In addition, the cell cycle inhibitory activities of p16/INK4A, p21/CIP1, and p27, can each be overcome by E2F1 overexpression (118,119). Surprisingly, E2F1 can overcome these blocks in cell cycle progression in the absence of G1 cyclin kinase activity (118).

More recently it has been shown that E2F2 and E2F3 can also induce S-phase in serum-starved or p16/INK4A-overexpressing cells (44,119). E2F4 and E2F5 can not induce S-phase on their own under these conditions, but can when coexpressed with DP1. At least in the case of E2F4, this lower S-phase-promoting activity appears to be related to its lack of a nuclear localization signal. When a nuclear localization signal from E2F1 or SV40 T antigen is fused to E2F4, it can now induce S-phase in the absence of DP1 coexpression (100). Further evidence that E2F plays a critical role in the G1 to S-phase transition comes from the finding that a dominant-negative mutant of DP1, which forms E2F heterodimers inactive in DNA-binding, causes cells to arrest in G1 (120).

Related to the ability of E2F to bypass growth arrest induced by a number factors, ectopic E2F1 expression has also been found to inhibit terminal differentiation in both *in vitro* and *in vivo* model systems. In one study, deregulated E2F1 expression in C2C12 myoctes blocked myogenic differentiation and prevented cells from exiting the cell cycle under differentiation conditions (121). In a transgenic model, targeted expression of E2F1 in megakaryocytes blocked differentiation during development, resulting in severe thrombocytopenia and massive megakaryocyte accumulation (122).

Several groups have also found that overexpression of E2F1 can induce cells to undergo programmed cell death or apoptosis (123-125). In cultured fibroblasts, induction of apoptosis is largely dependent upon wild-type p53 activity. In p53-null mouse embryo fibroblasts, E2F1 is much less efficient at inducing apoptosis. In addition, mutant p53 can inhibit apoptosis by E2F1 in cells containing wild-type p53. Thus, like some other oncogenes that induce proliferation such as c-myc, overexpression of E2F1 can lead to p53-dependent apoptosis. Others have found that E2F1 can induce some cell types to undergo apoptosis in a p53-independent manner. Adenovirus-mediated overexpression of E2F1 in human breast and ovarian carcinoma cell lines induces apoptosis without the requirement for functional p53 (126). In SAOS-2 osteosarcoma cells, E2F1 can induce apoptosis even though these cells lack a p53 gene (127,128). Interestingly, these same groups found that induction of apoptosis by E2F1 in SAOS-2 cells requires the DNA-binding and heterodimerization domains but does not require a functional transcriptional activation domain (127,128).

Induction of apoptosis by E2F1 does not appear to be a simple consequence of abnormal S-phase entry, but rather a specific function of this particular E2F family member. Other E2F family members do not efficiently induce apoptosis in serum-starved rat embryo fibroblasts although E2F2 and E2F3 induce S-phase entry just as efficiently as E2F1 (44,129). The ability of E2F1 to induce apoptosis may be related to its unique ability to induce the

accumulation of p53 (129,130). This in turn may be related to the ability of E2F1 to bind MDM2 (104). The MDM2 protein has been shown not only to block the transcriptional activation capacity of p53 but also to induce p53 degradation (131,132). E2F1 may compete for MDM2 binding, thus relieving MDM2-mediated degradation of p53. This model is consistent with the finding that overexpression of MDM2 in rat fibroblast cells can inhibit apoptosis induced by E2F1 (129). The ability of the other E2F species to induce apoptosis in the p53-independent systems described above has not been reported.

3.6. Role of E2F in cancer

There is much indirect evidence to suggest that the activation of E2F transcription factors, via alterations in the p16-cyclin D-Rb pathway, is a key event in the development of most human cancers. The Rb gene is deleted or mutated in a variety of human tumors including retinoblastomas, osteosarcomas, and small cell lung carcinomas (133). Rearrangement and overexpression of cyclin D1 (also known as the PRAD1/Bcl1 oncogene) also contributes to the development of several types of human tumors including parathyroid adenomas, B-cell lymphomas, and squamous cell carcinomas (134). Deregulated expression of other G1 cyclins (D2, D3, and E) has also been reported to be associated with tumorigenesis (134,135). The finding that the cyclin D/cdk inhibitor, p16/INK4A, is lost in several different tumor types provides further evidence that increased or inappropriately timed G1 cyclin kinase activity can be oncogenic (134). It is believed that deregulated G1 cyclin kinase activity leads to the functional inactivation of Rb and in turn the constitutive activation of E2F. This is supported by the finding that transient overexpression of G1 cyclins can transcriptionally stimulate at least some E2F target genes in the absence of any other growth signals (8,81,88).

Consistent with a role for E2F activation in cancer development, several E2F family member genes have been shown to function as oncogenes in culture. Deregulated expression of E2F1, in cooperation with an activated *ras* gene, can lead to oncogenic transformation of primary rat embryo cells (136). Removal of sequences involved in regulation by Rb, or inclusion of DP1, increases the oncogenic potential of E2F1. Cells transformed by E2F1 and *ras* form foci, grow in soft agar, and form tumors when injected into nude mice. On its own, E2F1 has been shown to induce anchorage-independent growth in immortalized 3T3 or REF cells (137,138). E2F2, E2F3, E2F4, and DP1 have each been shown to behave as an oncogene when tested in similar transformation assays (23,24,138,139).

A role for the E2F1 gene as a tumor suppressor has recently been established through the generation of mice lacking a functional E2F1 allele (140,141). Mice lacking E2F1 are viable, fertile, and demonstrate little evidence of the hypoproliferative defects one might expect from the absence of a positive regulator of cell cycle progression. Instead, as E2F1 knockout mice age some of their tissues exhibit hyperplasia and even neoplasia. A range of tumors are seen, including sarcomas of the reproductive tract, lung tumors, and lymphomas. The finding that the E2F1 gene functions as a tumor suppressor in the context of a whole mouse is quite surprising since all of the data from studies on human cancers point to an oncogenic role for E2F in tumorigenesis. This finding also means that either the studies demonstrating an oncogenic potential for E2F1 were artifacts of *in vitro* systems or that the E2F1 gene is truly unique in that it functions as both an oncogene and a tumor suppressor gene.

To examine the effect of increased E2F1 activity in an animal model system, our laboratory has recently generated transgenic mice expressing E2F1 under the control of a keratin 5 (K5) promoter (142). Overexpression of E2F1 in the skin results in epidermal hyperplasia and, in a high-expressing line, a hair growth defect as the result of aberrant, p53-dependent apoptosis in developing hair follicles. Increased E2F1 activity has been found to contribute to tumorigenesis in at least two ways. First, the K5 E2F1 transgene cooperates with a v-Ha-ras transgene to induce benign skin papillomas (142). Second, K5 E2F1 transgenic mice that are also heterozygous or nullizygous for a functional p53 allele develop spontaneous skin carcinomas (143). These findings are the first to make a direct link between increased E2F activity and tumorigenesis.

The ability of E2F1 to function as both an oncogene and a tumor suppressor gene can be rationalized by several conceptual models. One is related to the ability of E2F to transcriptionally repress, as well as activate, some gene promoters. It is possible that in some tissues, complexes between E2F1 and Rb function to repress transcription of genes involved in proliferation. In E2F1 knockout mice, loss of this Rb-E2F1 complex might then result in the activation of these genes, leading to deregulated cell growth. If an important role of Rb in some tissues is to repress certain genes targeted by E2F1, then loss of E2F1 might be expected to result in a phenotype similar to loss of Rb. One potential problem with this model is that the E2F1 gene itself is a target of E2F-mediated transcriptional repression (8). Moreover, when E2F1 is expressed in late G1/ early S phase, it is predominately found in the free form and not in complex with Rb (38).

Another, not necessarily mutually exclusive, explanation for the dual activity of E2F1 in tumorigenesis is its ability to induce both proliferation and apoptosis. A physiological role for E2F1 in regulating apoptosis comes from the finding that E2F1-deficient mice have an excess of mature T cells, perhaps due to a maturation-stage specific defect in thymocyte apoptosis. In culture, thymocytes from E2F1 knockout mice had a reduced ability to undergo apoptosis as compared with normal thymocytes (141). There is also accumulating evidence to suggest that E2F1 participates in a protective, apoptotic pathway that functions to eliminate cells that have lost normal cell cycle control. In many model systems, loss of Rb function leads to apoptosis rather than proliferation (144-147). Apoptosis as a consequence of Rb inactivation is largely dependent

upon wild-type p53 function. This finding is consistent with the observation that in many human cancers with Rb mutations, p53 is also inactivated. It also likely explains why DNA tumor viruses such as SV40, adenovirus, and human papilloma virus (HPV) encode proteins that target both Rb and p53. Given that ectopic expression of E2F1 can induce p53-dependent apoptosis (123,125), it is likely that deregulation of E2F1 participates in this protective apoptotic response as a consequence of Rb inactivation. The loss of this Rb-E2F1 apoptotic pathway may well explain why mice lacking E2F1 are predisposed to cancer (140). In the case of the K5 E2F1 transgenic mice, factors such as p53 loss or ras activation may alter the balance between E2F1-mediated apoptosis and E2F1-mediated proliferation leading to deregulated cell growth and tumorigenesis (142,143).

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