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Opposing Roles of E2Fs in Cell Proliferation and Death

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Abstract

Progression through the cell cycle is dependent upon the temporal and spatial regulation of the various members of the E2F family of transcription factors. Two of these members, E2F1 and E2F4, have opposing roles in cell cycle progression, which were defined over a decade ago. While E2F1 is an activator of cell cycle progression, E2F4 functions as a transcriptional repressor. Recent data indicate that these transcription factors also play a role in the cellular response to DNA damage. In the case of E2F1, its overexpression leads to apoptosis. In contrast, the decreased expression of E2F4, in response to siRNA-mediated knockdown or to certain therapeutic agents, induces apoptosis. Conversely, increased levels of E2F4 may confer resistance to apoptosis-inducing therapies used in the clinic. The balance between the activities of these two proteins in tumor cells is of great interest. Directed control of E2F1 and E2F4 action may lead to better diagnosis of disease and improved therapeutic modalities.

Keywords

E2F1; E2F4; apoptosis; cell cycle; chromatin immunoprecipitation

Introduction

The E2F family of transcription factors is comprised of eight different members (E2F1–8), which coordinately regulate the cell cycle.[1,2] Based on their function, these eight members have been divided into two subgroups: activating (E2F 1–3a) and repressing (E2F 3b–5; 6–8) transcription factors. E2F activity is dependent upon its dimerization with a DP protein, as well as its interaction with the pocket proteins, pRb, p107 or p130.[3] Together, these factors elicit signaling that either promotes cellular growth, cell cycle exit, or terminal differentiation.[2] Because the various E2F family members share a great deal of homology, some functionality is redundant, yet specificity is still maintained.

E2F1 and E2F4 are spatially and temporally regulated in development, with E2F1 and E2F4 opposing each other in their function as a transcription activator or repressor, respectively. The tight regulation of E2Fs is responsible for the timely expression of genes promoting DNA synthesis, cell cycle progression, and mitosis.[4] Many new target genes have been recently discovered through gene expression profiling arrays combined with chromatin immunoprecipitation (ChIP) assays. Specificity for the regulation of these genes occurs

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through the E2Fs' subcellular localization and respective interactions with different pocket proteins and chromatin modifiers, such as Suv93H, HDAC, BRG1 and BRG2, throughout the cell cycle.[5-7]

The classical model for E2F regulation indicates that E2F4/p130 and HDAC complexes are predominant during the G_0/G_1 phase of the cell cycle and serve to repress E2F target genes that are important for cell cycle progression.[8] As cells enter the cell cycle and transit into the G_1/S phase, E2F1/pRb becomes the predominant complex. After being phosphorylated by cyclin-dependent kinases (CDKs), pRb leaves the complex, allowing E2F1 to function as a transcriptional activator and promote cell cycle progression. pRb is rarely found bound to the chromatin, or interacts with chromatin in a transient manner.[3] In contrast, E2F4/p130 and E2F4/p107 complexes may persist on the chromatin until complexes containing E2F1 and other activator E2Fs may bind to promoters during S phase.[1] However, earlier work stresses that pRb is extremely important for the temporal expression of target genes and that without its presence, mouse embryonic fibroblasts (MEFs) exhibit deregulated cell cycle kinetics, E2F1 target gene expression (e.g., DHFR, thymidylate synthase), and became sensitized to DNA damaging agents.[9] Recent data also suggest that E2Fs may bind to multiple promoter elements, thereby invoking the ability to dynamically regulate promoter elements in either positive or negative manners.[10] In addition to the genes that are regulated in the cell cycle, ChIP analyses have also revealed that dynamic gene regulation by the E2Fs encompasses genes that are important for the DNA damage checkpoint and repair pathways, chromatin assembly/condensation, chromosome segregation, and the mitotic spindle checkpoint.[11-13]

Although a great deal of attention has been placed on understanding the pattern of in vivo E2F activity in development, questions entailing the pattern of gene regulation and specificity have yet to be fully answered. This is especially true in the context of characterizing molecular mechanisms implicated in cancer progression and treatment. Thus, we hope to provide an overview of the current literature, with a particular focus on the dynamics of two opposing E2F family members, E2F1 and E2F4.

E2F1: A Multitasking Mediator

A member of the activator E2Fs, E2F1 was the founding member of the E2F family and is essential for cellular proliferation.[14] Although the activities of E2F1 have been defined in development, the downstream molecular complexities related to its deregulation are currently under investigation. Recent work has shown that E2F1 may function as an oncogene[15] or as a tumor suppressor.[16] The nature of this duality is likely to be based on the degree to which E2F1 is expressed in the context of the cell cycle and/or following DNA damage and the transactivation of its target genes.

As an oncogene, the overexpression or untimely expression of E2F1 can advance cells from the G_0 phase to the S phase.[17,18] E2F1 overexpression has been identified in HEL erythroleukemia cells, as a result of the amplification of the *E2F1* gene.[19] E2F1 overexpression is also known to cause neoplastic transformation in astrocytes in vitro.[20] In colon cancer patients, the levels of E2F1 and a target gene, thymidylate synthase were elevated, effectively promoting cell cycle misregulation and oncogenesis.[21]

However, in other circumstances, the overexpression of E2F1 can lead to apoptosis through p53-dependent[17,22,23] and -independent pathways.[24] Early studies indicated that increased levels of E2F1 resulted in increased stability of p53 and apoptosis, which could be blocked by mdm2 induction.[25] Following treatment with the DNA damaging agent, etoposide, Chk2 induction increases E2F1 levels through protein stabilization, leading to transcriptional induction of target genes, such as *p73*, *INK4/arf*, *apaf-1* and *caspase-7*, which may lead to apoptosis.[26,27] A positive feedback loop involving the induction of *chk2* by E2F1 and

increased Chk2 stability by Atm and Nbs1 enhances p53 stability through its phosphorylation on Ser15[28] and promotes apoptosis.[29] Alternatively, TNFα increases the levels of E2F1, leading to degraded and decreased TRAF2 levels; this results in a loss of JNK/SAPK activity and antiapoptotic signaling.[16]

Reasons for this heterogeneity may result from different threshold levels of E2F1 required for differential gene transactivation of its target gene promoters, which may favor either apoptosis or survival. Indeed, the E2F1 promoter contains sites for both activation and repression, and E2F1 levels are dynamically regulated during the cell cycle.[30] The cellular response to DNA damage adds another level of complexity to E2F1's transcriptional regulation and downstream target effectors. However, although both E2F1 and E2F2 are able to cause quiescent cells to enter S phase, only E2F1 has been shown to promote apoptosis, which delineates its function from other activating E2Fs that could otherwise cause aberrant cell cycle regulation.

Increases in levels of E2F1 may result in deregulated gene expression that commits cells to undergo apoptosis.[31] Certainly post-translational modifications, as in the case of E2F1 acetylation, which promote the induction of p73 have already been identified.[32] Additionally, an E2F transactivation-independent mechanism was proposed in which increased levels of E2F1 protein could complex with increased levels of either p53 or Cyclin A, resulting in apoptosis or survival, respectively.[22]

E2F4: A Remarkable Repressor

E2F4 serves as a member of the repressor E2Fs and is known to function in growth suppression and differentiation.[33] Although less is known about the activities of E2F4, it is clear that it represses genes during quiescence,[2] heterodimerizes with p130 after cells undergo cell cycle exit and thereby induces differentiation in neurons.[34] E2F4 is unique compared to E2F1 in that it is primarily cytoplasmic, contains a nuclear export signal, and is dependent on CRM1 for its cytoplasmic localization.[35] Its heterodimerization with the pocket proteins pRb, p107, or p130[36] is responsible for nuclear import.

Aside from functioning during quiescence and differentiation, E2F4, like E2F1, appears to act outside of these conventional roles. E2F-4 functions as an oncogene when it is introduced into untransformed cells in vitro[37] In tumors, E2F4 loss, in combination with $pRb^{-/-}$, blocks inappropriate gene expression and cellular proliferation that would otherwise occur in pRb-deficient cells and potentially functions as a tumor suppressor.[38] Indeed, E2F-4 mutations have been identified in gastric adenocarcinomas, ulcerative colitis-associated neoplasms, colorectal carcinomas, endometrial cancers and prostatic carcinomas, indicating that E2F4 plays a key role in tumorigenesis.[37] Mutations of coding repeats within the *e2f4*, are critical targets of microsatellite instability in many kinds of cancers, including childhood and adult leukemias.[39]

E2F4 does not appear to be necessary for cell cycle progression, but it is important for the pocket protein-mediated G_0/G_1 arrest of cycling cells, as $E2F4^{-/-}$ MEFs fail to arrest in response to p16INK4a.[40] In addition, E2F4 contributes to the DNA damage response and the ensuing cell cycle arrest following exposure to ionizing radiation (IR) during the G_2/M phase of the cell cycle in the C4-2 prostate carcinoma cells (Crosby ME, Almasan A, unpublished data). In the C4-2 cells, the levels of E2F4 increase in response to IR and seem to confer cell survival.[41] However, in contrast to the increased levels of E2F1 following DNA damage and activation of apoptosis, E2F4 levels in H1299 lung adenocarcinoma cells decrease after treatment with cyclin-dependent kinase inhibitors and with DNA damaging agents, but not with microtubule inhibitors, and are associated with apoptosis.[42] Similarly, C4-2 cells treated with siRNA E2F4 and IR undergo apoptosis, as evidenced by the cleavage of pro-caspase 3 and of poly(ADP-

ribose) polymerase.[41] Thus, as in the case of E2F1, aberrant changes in E2F4 expression can result in a cellular response that may promote cell death or cell survival.

Although the mechanism underlying E2F1-induced apoptosis has been well characterized, the role of E2F4 in apoptosis has not. The changes in the levels of E2F4 are thought to promote increased sensitivity, as siRNA against E2F4 promoted apoptosis,[41,42] but the underlying mechanism has not been well studied. Because of the observation that E2F4 levels are higher in $E2F1^{-/-}$ cells and that levels of E2F4 decrease after levels of E2F1 increase, it is thought that E2F4 may be transcriptionally repressed by E2F1.[42] Because the overexpression of E2F4 does not protect cells from drug- or E2F1-induced apoptosis, it has been proposed that E2F4 does not function to block E2F1.[42]

Decreased levels of E2F4, however, may promote apoptosis by vacating the E2F1 binding sites present in the promoters of proapoptotic genes, which would otherwise block the E2F1 transactivation and apoptosis.[42] Multiple binding sites for E2Fs have been characterized in a number of promoters for target genes, which may also add to the complexity of gene transactivation.[10] Thus, the integration of the relative promoter capacities or competition for discrete E2F binding sites reflects the ability of E2F1 or E2F4 to enhance or resist the induction of apoptosis. E2F4 deficiency sensitizes cells and E2F1 deficiency decreases sensitivity to apoptotic stimuli. Thus, it is proposed that E2F1 and E2F4 oppose each other not just in their control of the cell cycle function, but also in the context of the response to DNA damaging agents, which indicates that they provide a critical balance for gene regulation.

Conclusions

Our current knowledge indicate that coordinate E2F regulation is associated with the control of cell cycle. Moreover, E2F1 and E2F4 may be regulated aberrantly, as in the case of DNA damage response. In an effort to reconcile these activities, we look to cancer cells, which have cell cycle pathways containing E2F/Rb mutations. These cells have aberrant growth profiles that result from deregulated E2F expression, where E2Fs are either induced or repressed. In the case of $Rb^{-/-}$, premature S-phase entry could lead to deregulated gene expression and apoptosis, indicating that the role of the pocket proteins is vital to cell cycle maintenance.[9] An integral part of this system of checks and balances, the levels of E2F1 and E2F4 have opposing roles within normal cell cycle regulation, as well as in their response to certain therapeutics, such as DNA damaging agents.

Increased levels of E2F1, E2F2 and E2F3a lead to apoptosis, where E2F1 is the most potent inducer, but E2F4 expression does not promote apoptosis. However, decreased levels of E2F4 sensitizes cells to chemotherapeutic or radiation induced apoptosis.[41,42] In contrast, increased levels of E2F4 serve to increase the survival of cells. In $E2F1^{-/-}$ cells, levels of E2F4 are higher in MEFSs treated with cisplatin, flavopiridol, VP-16, paclitaxel or roscovotine.[42] Therefore, agents that decrease levels of E2F4 and block its interaction with p130 can potentially enhance the effect of cancer therapeutics.

As many studies have focused on the overexpression of the E2Fs and examining its activities based on reporter constructs, the next step is to determine biologically relevant circumstances where levels of the E2Fs are deregulated. Thus, future studies should focus on mutating E2F binding sites and address the overall contribution of these defined changes. Studies must also be conducted to unravel the complexity of having promoters that contain multiple E2F binding sites.

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Abbreviations

CDK
cyclin-dependent kinase

IR
ionizing radiation

siRNA
small interference RNA

ChIP
chromatin immunoprecipitation

MEF
mouse embryonic fibroblast