Extra Views

Life, Death and E2F

Linking Proliferation Control and DNA Damage Signaling via E2F1

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ABSTRACT

Proper regulation of cellular proliferation is critical for normal development and cancer prevention. Most, if not all, cancer cells contain mutations in the Rb/E2F pathway, which controls cellular proliferation. Inactivation of the retinoblastoma (Rb) family of proteins can occur through Rb loss, mutation, or inactivation by cellular or viral oncoproteins leading to unrestrained proliferation and, often times, results in apoptosis. The loss of growth control occurs primarily by derepression and activation of the E2F transcription factors. E2F1 in particular, serves as the primary link between loss of Rb function and activation of p53-dependent apoptosis. E2F1 function is crucial for responding to loss of proper Rb-mediated growth control to activate p53 and the apoptotic program. Recently, we described the requirement for the DNA damage response proteins Atm, Nbs1, and Chk2 in the E2F1 apoptosis pathway. These findings suggest that there may be a more intimate relationship between the apoptosis pathways resulting from loss of proper Rb-mediated growth control and apoptosis resulting from the accumulation of DNA damage.

The role of the E2F transcription factor family in promoting cell cycle entry has been well documented. Most cellular genes associated with the entry into S phase are regulated by the E2F family, including genes involved in controlling cell cycle regulation, DNA synthesis, and DNA replication.[1] The importance of the activator E2F family members in promoting entry into the cell cycle is demonstrated by the severe proliferative defects in mice and cells lacking *e2f1, e2f2*, and *e2f3*.[2] This ability of activator E2F family members to promote transition into S phase is normally kept in check by the retinoblastoma protein (Rb). The importance of Rb regulating the G_1 to S phase transition is apparent in the observation that loss of Rb-mediated growth control occurs in the vast majority of human tumors.

Deregulation of Rb/E2F mediated growth control can result in activation of an apoptotic pathway that eliminates cells from the body that harbor defects in the proliferation pathway. Inactivation of Rb by cellular or viral oncoproteins, or loss of Rb, will stimulate cells to bypass G_0/G_1 checkpoints and enter into S phase, and this unscheduled entry into S phase can trigger apoptosis. Ectopic expression of viral oncogenes, such as the high risk HPV-16 E7 protein, can also lead to the induction of apoptosis. The studies with high risk HPV and other small DNA tumor viruses suggested a link between Rb/E2F cell cycle control and p53-mediated apoptosis.[3] Our group and others have demonstrated that E2F1 serves as a link between perturbations in Rb-mediated growth control and p53-dependent apoptosis.[4-6] We have recently shown that inactivation of Rb by the HPV-16 E7 protein results in apoptosis that depends specifically on activation of an E2F1-induced apoptosis pathway.[5] These studies suggest that E2F1 is responsible for generating the apoptosis signal associated with Rb inactivation.

It had been hypothesized that the link between loss of proper Rb/E2F cell growth control and p53-dependent apoptosis was through activation of the p19[ARF]/Mdm2 pathway. However, after testing this hypothesis, our group and others have demonstrated that inactivation of Rb or overexpression of E2F1 results in apoptosis that is independent of *p19[ARF]*.[7-11] Recently, we described a role for the DNA damage response proteins Atm, Nbs1, and Chk2 as critical signaling molecules in the E2F1 apoptosis pathway.[5] Similar results have been obtained by others.[12] Surprisingly, we found that activation of Atm was not unique to E2F1, since expression of the related E2F family member, E2F2, also resulted in activation of Atm. However, we determined that the induction of *Chk2* expression was unique to E2F1, and correlated with an increase in the phospho-serine 20 form of p53 and with the induction of apoptosis. These observations demonstrate that E2F1 is signaling through a specific subset of the DNA damage response factors to activate p53 and kill cells.
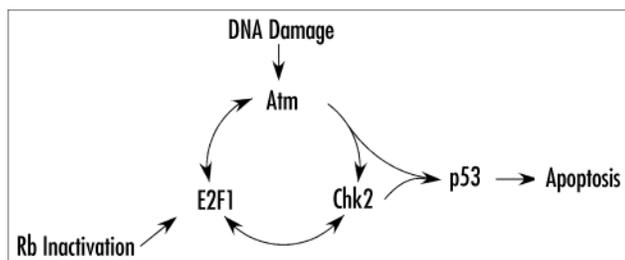
Figure 1. Proposed feedback loop linking loss of proliferation control, DNA damage signaling, and apoptosis. Inactivation of Rb leads to increased E2F1 activity, activation of Atm and Chk2, and subsequent phosphorylation of p53. Similarly, DNA damaging agents can lead to activation of Atm and Chk2, and subsequent phosphorylation of p53. E2F1 can also act downstream of Atm and Chk2 in the DNA damage response, leading to amplification of the apoptotic signals.

The observation that aberrant E2F1 activity results in activation of Atm and induction of *Chk2* expression suggests that E2F1 may function to sensitize cells to undergo apoptosis in response to other types of stimuli. Although we have demonstrated a central role for E2F1 in apoptosis signaling following Rb inactivation, these findings, when viewed together with the work of others, suggest that the contribution of E2F1 to the DNA damage response may be underappreciated. It has been known for some time that E2F1 protein levels increase following DNA damage.[13] This protein accumulation has been shown to be mediated, in large part, by an Atm-dependent phosphorylation of E2F1.[14] These observations suggest that activation of E2F1 and the E2F1-associated apoptosis pathway can be a component of the DNA damage response. In support of this hypothesis, there is a defect in apoptosis in response to DNA damaging agents in thymocytes lacking *E2F1*.[14] E2F1 is further linked to the DNA damage response through Chk2. We have shown that expression of E2F1 leads to an increase in *Chk2* mRNA and an accumulation of Chk2 protein. Others have found that Chk2 phosphorylates E2F1, altering the DNA binding specificity of E2F1 from S phase genes to the pro-apoptotic, *p73* gene.[15]

These observations suggest that there are common features in the apoptosis signaling pathways that respond to both loss of cellular proliferation control (inactivation of Rb) and generation of DNA damage. In response to Rb inactivation, E2F1 signals to p53 through activation of Atm and induction of *Chk2* expression, leading to phosphorylation of p53 and apoptosis. In response to DNA damage, activation of Atm leads to phosphorylation and activation of Chk2, leading to p53 modification and apoptosis. Additionally, certain forms of DNA damage lead to the stabilization and activation of E2F1 by covalent modifications mediated by Atm and Chk2. These results suggest a feedback loop involving integration of the DNA damage signaling molecules Atm and Chk2 with a cell cycle regulator, E2F1 (Fig. 1).

Determining both the mechanism of E2F1-induced apoptosis and the contribution of E2F1 to DNA damage-induced apoptosis is essential to our understanding of basic cell cycle biology and the molecular events that often precede tumorigenesis. The roles of E2F1 in responding to both loss of cellular proliferation control and DNA damage suggests that E2F1 may be an attractive target for either loss or amplification in tumors. However, while mutations in both the Rb and p53 regulatory pathways are common in human tumors, E2F1 mutations are rarely found. It is unclear why E2F1 is not often mutated in human tumors. Perhaps loss or amplification of E2F1 would be detrimental to tumor success due to the dual

functions of E2F1 in promoting cell cycle progression and apoptosis. Given the role of E2F1 in apoptosis signaling that results from Rb inactivation and DNA damage, it is possible that compounds that enhance endogenous E2F1 activity in conjunction with genotoxic drugs, may be useful as anti-tumor agents.

References

1. Cam H, Dynlacht BD. Emerging roles for E2F: Beyond the G_1/S transition and DNA replication. Cancer Cell 2003; 3:311-6.
2. Wu L, Timmers C, Maiti B, Saavedra HI, Sang L, Chong GT, et al. The E2F1-3 transcription factors are essential for cellular proliferation. Nature 2001; 414:457-62.
3. Helt AM, Galloway DA. Mechanisms by which DNA tumor virus oncoproteins target the Rb family of pocket proteins. Carcinogenesis 2003; 24:159-69.
4. Pan H, Yin C, Dyson NJ, Harlow E, Yamasaki L, Van Dyke T. Key roles for E2F1 in signaling p53-dependent apoptosis and in cell division within developing tumors. Mol Cell 1998; 2:283-92.
5. Rogoff HA, Pickering MT, Frame FM, Debatis ME, Sanchez Y, Jones S, et al. Apoptosis associated with deregulated E2F activity is dependent on E2F1 and Atm/Nbs1/Chk2. Mol Cell Biol 2004; 24:2968-77.
6. Tsai KY, Hu Y, Macleod KF, Crowley D, Yamasaki L, Jacks T. Mutation of E2f-1 suppresses apoptosis and inappropriate S phase entry and extends survival of Rb-deficient mouse embryos. Mol Cell 1998; 2:293-304.
7. Tolbert D, Lu X, Yin C, Tantama M, Van Dyke T. p19^{ARF} is dispensable for oncogenic stress-induced p53-mediated apoptosis and tumor suppression in vivo. Mol Cell Biol 2002; 22:370-7.
8. Tsai KY, MacPherson D, Rubinson DA, Crowley D, Jacks T. ARF is not required for apoptosis in Rb mutant mouse embryos. Curr Biol 2002; 12:159-63.
9. Russell JL, Powers JT, Rounbehler RJ, Rogers PM, Conti CJ, Johnson DG. ARF differentially modulates apoptosis induced by E2F1 and Myc. Mol Cell Biol 2002; 22:1360-8.
10. Rogoff HA, Pickering MT, Debatis ME, Jones S, Kowalik TF. E2F1 induces phosphorylation of p53 that is coincident with p53 accumulation and apoptosis. Mol Cell Biol 2002; 22:5308-18.
11. Lindstrom MS, Wiman KG. Myc and E2F1 induce p53 through p14ARF-independent mechanisms in human fibroblasts. Oncogene 2003; 22:4993-5005.
12. Powers JT, Hong S, Mayhew CN, Rogers PM, Knudsen ES, Johnson DG. E2F1 uses the ATM signaling pathway to induce p53 and chk2 phosphorylation and apoptosis. Mol Cancer Res 2004; 2:203-14.
13. Huang Y, Ishiko T, Nakada S, Utsugisawa T, Kato T, Yuan ZM. Role for E2F in DNA damage-induced entry of cells into S phase. Cancer Res 1997; 57:3640-3.
14. Lin WC, Lin FT, Nevins JR. Selective induction of E2F1 in response to DNA damage, mediated by ATM-dependent phosphorylation. Genes Dev 2001; 15:1833-44.
15. Stevens C, Smith L, La Thangue NB. Chk2 activates E2F-1 in response to DNA damage. Nat Cell Biol 2003; 5:401-9.