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The Transcription Factor E2F: Balancing Proliferation and Apoptosis

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Introduction

The E2F family of transcription factors plays a crucial role in the control of cell proliferation. E2F regulates the expression of many genes required for entry into and progression through the S phase of the cell cycle, and overexpression of E2F induces S phase entry of quiescent cells. E2F activity is negatively regulated by the product of the retinoblastoma tumor suppressor, RB, and the growth suppression activity of RB is dependent on its ability to interact with E2F. The RB/E2F pathway is often mutated in human cancer resulting in deregulated E2F activity. Deregulated E2F1 constitutes an oncogenic stress that induces apoptosis via both p53-dependent and p53-independent pathways. Our lab studies the molecular mechanisms underlying both the regulation and biological activities of E2F focusing on its involvement in the response to DNA damage and its ability to induce apoptosis.

Up-regulation of the protein kinase ATM and BH3-only proteins by E2F1 mediates apoptosis.

BH3-only proteins are pro-apoptotic members of the Bcl-2 protein family that trigger apoptosis in response to diverse stimuli. We identified four of the BH3-only proteins, PUMA, Noxa, Bim and Hrk, as direct transcriptional targets of E2F1. Our studies demonstrated that endogenous E2F1 binds to the promoters of these four genes and activates their expression leading to elevation in their mRNA and protein levels. Importantly, inhibition of the expression of either Noxa or PUMA by specific siRNA results in significant reduction in E2F1-induced apoptosis indicating that increased Noxa and PUMA levels mediate this E2F1-induced apoptosis.

We also show that E2F induces an increase in ATM levels and activity. The protein kinase ATM is a pivotal mediator of the response to genotoxic stress. In response to ionizing radiation ATM phosphorylates and activates numerous proteins including the tumor suppressor p53, a key player in the control of cell growth and viability. We demonstrated that over-expressed or deregulated E2F elevate ATM promoter activity and induce an increase in ATM mRNA and protein level that is accompanied by an increase in p53 phosphorylation. Furthermore, inhibition of ATM activity significantly reduces E2F1-induced apoptosis demonstrating that, similarly to the BH3-only proteins, ATM plays an important role in this E2F1-induced apoptosis.

E2F1 and Ras co-operate in inducing apoptosis.

Deregulation of E2F activity often occurs already in pre-malignant cells and it has been suggested that the apoptotic activity of E2F1 may serve to eliminate such pre-malignant cells before they become fully transformed. When cells that contain deregulated E2F sustain another oncogenic hit they make another step towards full transformation and, therefore, we hypothesized that this may increase their tendency to undergo apoptosis.

Indeed, we showed that oncogenic Ras and deregulated E2F1 co-operate in inducing apoptosis. We further demonstrated that this is due to differential regulation of E2F target genes: oncogenic Ras directs E2F1 preferentially to promoters of pro-apoptotic genes and, thus, in the presence of oncogenic Ras the E2F-dependent activation of its pro-apoptotic target p73 is enhanced while regulation of proliferation-related E2F-responsive genes is not altered. Moreover, inhibition of p73 activity abolished the cooperative induction of apoptosis by E2F1 and Ras demonstrating that p73 is a critical mediator of this cooperation.

E2F1 also has anti-apoptotic activities.

The PI3K/AKT survival pathway was shown to suppress E2F1 apoptotic activity. We found that the PI3K/AKT pathway is transcriptionally regulated by E2F1. We identified the adaptor protein Grb2-associated binder 2 (Gab2) as a direct E2F target gene and an essential effector of E2F-dependent AKT activation. Our results provide a mechanism 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.

E2F4 mediates sustained G2 arrest in response to genotoxic stress.

Exposure of cells to sub-lethal doses of genotoxic agents results in activation of checkpoint pathways leading to cell cycle arrest. These arrest pathways allow repair of damaged DNA before its replication and segregation, thus preventing accumulation of mutations. The tumor suppressor RB is required for both G1 and G2 checkpoint function. However, the molecular mechanism underlying the involvement of RB in the G2 checkpoint is not fully understood. We showed that sustained G2 arrest induced by genotoxic stress is E2F-dependent and involves a decrease in expression of mitotic regulators. Abrogation of E2F function leads to premature exit from G2 after genotoxic stress. Furthermore, genotoxic stress increases the levels of nuclear E2F4 as well as the in vivo binding of E2F4 and RB family members to promoters of mitotic genes. Thus, functional complexes containing E2F and RB family members appear to be essential for repressing expression of critical mitotic regulators and maintaining the G2 checkpoint.

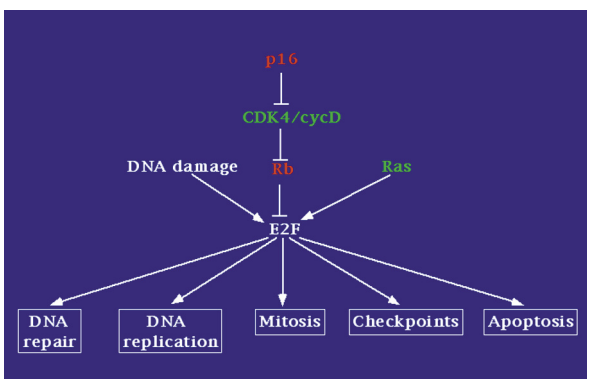

Fig. 1 *Regulations and activities of the transcription factor E2F. E2F is regulated by a number of mechanisms including negative regulation of its activity by pRB and up-regulation of its levels as well as changes in its DNA binding specificity by either DNA damage or activated Ras. E2F regulates the expression of genes involved in a number of biological processes including DNA replication, DNA repair, mitosis, checkpoints and apoptosis.*

Selected Publications

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Polager, S. and Ginsberg, D. (2003) E2F mediates sustained G2 arrest and down-regulation of Stathmin and AIM-1 expression in response to genotoxic stress. J Biol Chem, 278, 1443-1449.

Hershko, T. and Ginsberg, D. (2004) Up-regulation of Bcl-2 Homology 3 (BH3)-only Proteins by E2F1 Mediates Apoptosis. J Biol Chem, 279, 8627-8634.

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