Live or Let Die: E2F1 and PI3K Pathways Intersect to Make Life or Death Decisions

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In the current issue of *Cancer Cell*, Hallstrom et al. show that a subset of targets of the growth regulatory transcription factor E2F1 are repressed by a serum-induced PI3K activation, explaining how apoptosis can be suppressed while simultaneously engaging a proliferation program.

E2F1 can paradoxically function as both tumor suppressor and oncogene (Trimarchi and Lees, 2002). Induction of E2F1 could serve in the capacity of oncogene surveillance, whereby cells that have sustained a mutation in the RB pathway, known to be defective in many human cancers, could be eliminated. To this end, several components of the core apoptosis-inducing machinery (caspases, Apaf1) have been identified as E2F1 targets (Nahle et al., 2002). It is thought that cancer could arise through deregulation of oncogene-induced apoptosis.

The E2F family of transcription factors are known regulators of mammalian proliferation, held in check by the retinoblastoma tumor suppressor (pRB) family (Trimarchi and Lees, 2002; Blais and Dynlacht, 2004). The family can be subdivided into activators (E2F1-3) and repressors (E2F4-8). Mitogenic signaling promotes cyclin-dependent kinase (CDK)-mediated pRB phosphorylation, which releases an unrestrained, active transcription factor. Release of active E2F1 is a double-edged sword for cell survival: on the one hand, E2F1 can activate genes required for proliferation, but on the other, it could potentially activate apoptosis-related genes as well. E2F1 appears to be the strongest inducer of apoptosis among the activator E2Fs, and this specificity appears to stem from a protein domain ("marked box") restricted to this family member (Hallstrom and Nevins, 2003). Although at least one protein has been found to specifically interact with this domain (Hallstrom and Nevins, 2006), how the apoptotic decision is regulated at the cellular level and how the balance between disparate E2F1 functions—proliferation and apoptosis—is achieved are not completely understood.

Thus, one may ask the question: What is the molecular circuitry that tips the balance in favor of proliferation in some settings at the expense of apoptosis, and how does E2F mediate this process? Earlier work from the Nevins laboratory had indicated that growth factor-induced PI3 kinase (PI3K) and Akt pathways, but not activation of the mitogen-activated protein kinase kinase (MEK), could influence the outcome, promoting proliferation rather than apoptosis (Hallstrom and Nevins, 2003, 2006). Recent work from the Nevins laboratory now sheds considerable light on this subject (Hallstrom et al., 2008). Using genome-wide profiling of rat fibroblasts (REF52), Hallstrom and colleagues identified genes that are activated upon enforced expression of E2F1 yet repressed in a PI3K-dependent manner in the presence of serum growth factors. Interestingly, the subset of genes unaffected by serum represent canonical E2F target genes involved in cell-cycle progression and DNA replication (Ren et al., 2002), while the genes specifically repressed by serum appear to reflect a multitude of heterogeneous pathways without an obvious or intrinsic cell-cycle association. Although these studies were performed using a PI3K inhibitor, LY294002, complementary experiments in which PI3K was overexpressed confirm the observation.

Reasoning that these serum-repressed targets could influence the decision to commit suicide, eleven candidate

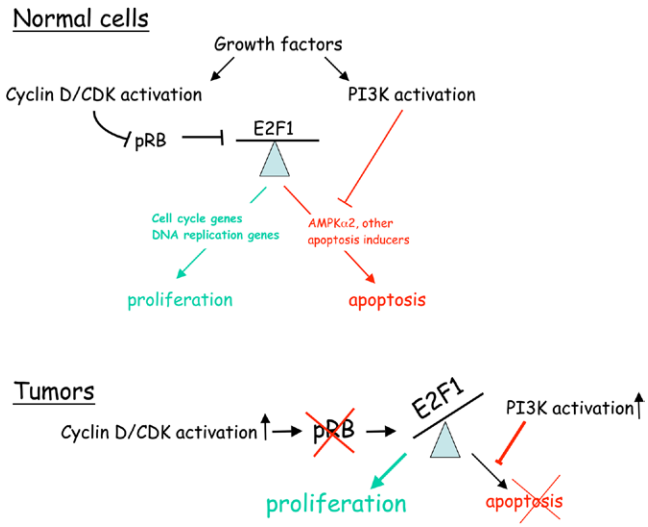

Figure 1. The Fateful Intersection of E2F1 and PI3K Pathways in Normal and Cancer Cells
Growth factors stimulate proliferation through two branches involving distinct CDK- and PI3K-Akt kinase pathways that simultaneously liberate E2F1 activity while suppressing apoptosis. Normal growth is characterized by an E2F1-mediated balancing act that promotes expression of cell-cycle genes and apoptosis to eliminate cells with deregulated E2F-1 activity. Hyperactivity or mutations in the PI3K pathway could abnormally suppress the apoptotic arm, promoting inappropriate proliferation and tumorigenesis.

genes were knocked down, and apoptosis was examined. The most impressive relief of E2F-mediated apoptosis was obtained by suppressing expression of AMP-activated protein kinase, AMPKα2, a central regulator of energy homeostasis in the face of nutrient and ATP depletion. Depletion of AMPKα2 renders both normal human fibroblasts and tumor cells resistant to E2F-mediated apoptosis. Conversely, ectopic expression of AMPKα2 synergistically enhances apoptosis in an E2F1-dependent manner. Given that E2F1 induces apoptosis in cells rendered quiescent through mitogen deprivation, it is likely that AMPKα2 is one critical sensor of this nutrient-poor state that pushes cells in the direction of death (Figure 1). If this hypothesis is correct, several mechanistic predictions can be made regarding a direct role for AMPKα2 activity in apoptosis. One prediction would be that inducers of AMPKα2 activity should actuate E2F1-mediated apoptosis. Indeed, as predicted, treatment of cells with an AMP analog, AICAR, which activates AMPK, induces apoptosis in an E2F1- and AMPKα2-dependent manner.

The importance of these findings would be greatly enhanced if they translated from a tissue culture model to the clinic. Moreover, given the well-established role of pRB and PI3K pathways in human cancer, prognostic and therapeutic possibilities might also be envisioned. To test this notion, Hallstrom and colleagues compared their genome-wide expression profiles (obtained from rat fibroblasts) with a compendium of microarray data obtained from several independent human breast and ovarian cancer data sets. The authors examined genes induced by E2F1 that fell into two categories: those that were not inhibited by PI3K and a second group that was repressed in response to serum-induced PI3K activation. Interestingly, using hierarchical clustering of microarray data, expression profiles could be used to reveal two groups of breast tumors with distinct signatures: one group displayed low levels of expression of the PI3K-repressed, proapoptotic class of genes at the expense of the PI3K-neutral ones, while the other group of tumors exhibited the inverse pattern. A cohort of ovarian tumors could be similarly segregated, allowing the authors to distinguish "high apoptotic" and "low apoptotic" cancer patients. When these tumor samples were analyzed using Kaplan-Meier survival curves, a remarkable trend was apparent: those patients with the lowest-level expression of PI3K-repressed genes exhibited a worse prognosis, including a shorter survival period and higher rate of recurrence. Advanced stage tumors had a greater association with reduced expression of these genes than did earlier stage tumors. Thus, it would appear that a PI3K activation signature could well determine the prognosis of at least a subset of human cancers.

This study further illustrates the use of metagene analyses in the delineation of critical pathways activated in tumors and provides fertile ground for additional prognostic and therapeutic predictions (Bild et al., 2006). Clearly, E2F1 occupies a nodal point that critically influences whether a cell proliferates or dies. Exactly how does PI3K modulate the activity of E2F1? It will be important to uncover the complete compendium of genes that convey the death signal emanating from E2F1, since *AMPKα2* and two other genes revealed in this study (*Cyp26b1* or *Nr4a3*, a regulator of retinoic acid metabolism and a nuclear receptor family member, respectively) are likely to represent the proverbial tip of the iceberg. Since secondary waves of expression occur upon enforced expression of E2F1 and drug treatment, it will be important to establish which of the noncanonical, presumptive E2F1 targets are indeed directly regulated by this factor in a PI3K-responsive manner and whether the poor prognosis associated with the cluster of tumors with relatively low expression of PI3K-repressed genes actually reflects diminished levels of apoptosis or altered proliferative properties of these tumors. As a final test of the power of this approach, it will be most exciting to test the therapeutic value of chemically inhibiting PI3K signaling in these tumors to assess whether the apoptotic arm emanating from the E2F1-associated fulcrum can be restored, thereby improving the prognosis (Figure 1). The results presented here reinforce an independent study that showed the importance of PI3K activation in breast cancer and that pinpointed the activation of this pathway as a biomarker for responsiveness to antitumor treatments in a cohort of breast cancer patients (Berns et al., 2007).

It is quite curious that, to date, few human tumors have been associated with amplification of the *E2F1* gene, while many tumors exhibit mutations or loss of the *RB* gene. This interesting study suggests that it may be worth taking a closer look at the occurrence of synergistic collaborations between amplified E2F1 and PI3K pathway defects in human cancer.

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