Molecular Pathways: Regulation of Metabolism by RB

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Abstract

The discovery of the retinoblastoma (RB-1) gene as a tumor suppressor that is disrupted in a majority of human cancers either via direct or indirect genetic alterations has resulted in increased interest in its functions and downstream effectors. Although the canonical pathway that links this tumor suppressor to human cancers details its interaction with the E2F transcription factors and cell-cycle progression, recent studies have shown an essential role for RB-1 in the suppression of glycolytic and glutaminolytic metabolism. Characterization of the precise metabolic transporters and enzymes suppressed by the RB-E2F axis should enable the identification of small molecule antagonists that have selective and potent antitumor properties. Clin Cancer Res; 18(22); 6096–100. ©2012 AACR.

Background

In the early 1970s, Alfred Knudson described a statistical study of 48 cases of a rare childhood malignancy, retinoblastoma, which indicated that only 2 mutational events were required for its development (1). Subsequent pedigree analyses of retinoblastoma patients identified a linked locus on chromosome 13 (2) and, within this locus, the retinoblastoma target gene RB-1 was sequenced by Lee and colleagues in the 1980s (3). Multiple RB-1 deletions and mutations that prevent the expression and function of the normal retinoblastoma protein product (RB-1) were then identified in retinoblastomas (4) and the two-hit hypothesis by Knudson was supported by the observations that one mutated allele and one wild-type allele were present in normal, somatic cells of hereditary retinoblastoma patients whereas their retinoblastomas contained 2 mutated alleles (3).

Apart from retinoblastoma, genetic alterations of RB-1 also have been found in osteosarcomas, renal cell carcinomas, soft-tissue sarcomas, and adenocarcinomas of breast, lung (small cell), prostate, cervix, and the bladder, suggesting an extensive role for RB dysfunction in the development of human cancers (4). In addition, mutations of gene products that lead to suppression of RB function have been found to be a ubiquitous feature of malignancies. For example, genetic loss of p16INK4A, which is common in non–small cell lung and pancreatic adenocarcinomas, melanomas, and hepatocellular carcinomas, causes the activation of cyclin-dependent kinases (CDKs) that phosphorylate and suppress the activity of RB-1 (5, 6). Given that introduction of wild-type RB-1 into RB-1 negative cancer cells suppresses their anchorage-independent growth in soft agar and as tumors in mice (7), RB-1 became known as the first gene to fulfill the equivalent of Koch’s postulates for a tumor suppressor. On the basis of these prior genetic and biologic studies, it is now anticipated that understanding the precise biochemical functions of RB-1 will identify proteins that become activated from the loss of RB-1 and that have utility as molecular targets for the development of novel chemotherapeutic approaches.

Canonical Function of RB in Cell-Cycle Regulation

In normal cells, activation of signaling pathways downstream of growth factor receptor tyrosine kinases causes increased expression of D-type cyclins that form the regulatory domains of CDK-4 and CDK-6 to enhance catalytic activity and nuclear translocation (8). Activated CDKs phosphorylate RB-1, which causes its dissociation from the E2F transcription factors that, depending on their RB-binding state, modulate the expression of genes such as CDC25A that are essential for the G1/S cell-cycle progression (9). Perhaps not surprisingly, overexpression of wild-type RB-1 protein in RB-1 negative cells in the early G1 phase of the cell-cycle causes a G1 arrest and cytostasis (10). The E2F family consists of nine distinct members, which are differentiated by their putative activator (E2F-1, 2, 3a) or repressor functions (E2F-3b, 4, 5, 6, 7, 8). RB-1 associates specifically with the E2F activator transcription factors and, when bound, recruits selective corepressor complexes, such as histone deacetylases (HDAC), to silence gene transcription (11–15). Although the precise mechanisms for RB-E2F-mediated modulation of gene expression remain controversial, several developmental mouse models in which E2F activators have been genetically deleted support this canonical pathway (9).
Control of Glucose Metabolism by RB

Until recently, the central dogma related to mutations that facilitate neoplastic transformation by disabling the RB-E2F suppressive activity supported a scenario whereby loss of RB function resulted in transcriptional upregulation of cell-cycle mediators which in turn caused a shift in the diffusion of nutrients and biosynthetic enzyme equilibria. However, an early indication that RB-E2F might directly regulate metabolic enzyme expression came from the observation that two enzymes required for nucleotide synthesis, thymidine kinase (TK1) and dihydrofolate reductase (DHFR), are direct transcriptional targets of E2F family members (16–23). Because RB suppresses the transcription of these nucleotide biosynthetic enzyme mRNAs via physical interaction with E2Fs, investigators postulated that RB might also suppress the transcription of mRNAs required to translate the multitude of regulators, transporters, and enzymes required for energetic and anabolic metabolism.

Glycolysis provides carbons required for the synthesis of ribose, amino acids, lipids, and ATP necessary for biomass expansion and cytokinesis. Early studies identified elevated glycolytic enzyme expression and activity levels in retinoblastomas. For example, the expression of the first enzyme of the glycolytic pathway, hexokinase (isozyme II), is significantly elevated in retinoblastomas relative to the normal retina (24) and the glycolytic hub enzyme, lactate dehydrogenase (LDH), has been found to be activated in the aqueous humor surrounding retinoblastomas compared with non-malignant ocular disorders (25). The precise mechanisms for increased expression and activity of glycolytic enzymes in retinoblastomas as well as other RB-1 deleted cancers have not been completely defined. However, derepressed E2F-1 activity leads to increased expression of c-Myc through direct transcriptional activation of the MYC promoter, and the role of c-Myc in stimulating glycolytic flux to lactate is well established via its control of glycolytic mRNAs including GLUT1, HK2, PKM2, and LDH-A (26–29). In addition, loss of RB function in 3T3 fibroblasts causes a 30-fold increase in modulation of glutamine transporters and glutaminase. Over the last 10 years, the activation of several oncogenes and deletion of tumor suppressors have been found to regulate glutaminolysis via direct modulation of glutamine transporters and glutaminase. Importantly, overexpression of c-Myc increases expression of glutamine transporters and glutaminase 1 (GLS1) along with concomitant and obligatory reliance on glutamine for survival (48–50). Given the symbiotic interaction between oncogenes and tumor suppressors that drives neoplastic transformation (51), it is not surprising that genetic deletion of the RB family in mouse embryonic fibroblasts also causes increased glutamine uptake and usage via elevated expression of the glutamine transporter ASCT2 and the activity of GLS1 (52). Additionally, exposure to a physiologic concentration of glutamine has been found to markedly increase oxygen consumption, ATP production, and glutathione formation in RB-null cells relative to RB wild-type cells, indicating high reliance on glutaminolysis as a result of an inactive RB pathway. Importantly, the effects of RB deletion are mediated in part by the RB-E2F cascade as ChIP analysis has revealed specific E2F family members associated with promoter regions of glutaminolytic enzymes (52). Together, these
studies extend the tumor suppressor role of the RB-E2F pathway to a metabolic pathway, glutaminolysis that is essential for the promotion of the growth and survival of neoplastic cells and identifies several metabolic enzymes that may be useful for the development of novel anti-neoplastic strategies.

Recent studies also have showed a direct role for the RB-E2F interaction in mitochondrial biogenesis and electron transport chain activity, both of which are involved in glutaminolysis. Adipocyte-specific deletion of RB causes increased expression of peroxisome proliferator-activated receptor γ co-activator 1-α (PGC-1α) and estrogen receptor related α (ERRα), which directly promote mitochondrial transcription, biogenesis, and oxygen consumption (53). This has been similarly observed in gastrocnemius myocytes from E2F-1−/− knockout animals (41). In addition, several genes involved in electron transport chain activity and oxidative phosphorylation have been found to be directly suppressed by RB-E2F-1, including subunits of ATP synthase, cytochrome c oxidase, ubiquinol-cytochrome c reductase, and the succinate dehydrogenase complex (41). Combined, these reports suggest that dysfunction of RB should facilitate the usage of glutamine for oxidative phosphorylation.

Clinical–Translational Advances

Targeting the loss of RB-E2F function in cancer

Several metabolic transporters and enzymes that are transcriptionally repressed by the RB-E2F pathway may prove useful as targets for the development of antineoplastic agents (Fig. 1). For example, gossypol and its derivatives are competitive inhibitors of the NADH binding site of the RB-
E2F-c-Myc target LDH that inhibits xenograft growth in mice (54) and another target, Glut1, confers sensitivity to the hexokinase inhibitor, 3-bromopyruvate (3BP; ref. 55). In addition, a competitive antagonist of the PKFB family of glycolytic regulators was recently discovered, 3-[(3-pyridyl)-1-(4-pyridyl)-2-propen-1-one (termed 3PO), that reduces the glucose uptake, intracellular F2,6BP, lactate secretion, steady-state concentration of ATP, and in vitro and in vivo growth of multiple transformed cells (56). Importantly, H-RasV12–transformed bronchial epithelial cells that have low RB function are markedly more sensitive to the cytotoxic effects of 3PO than are normal bronchial epithelial cells (56). Finally, the induction of glutaminolysis in RB-null cells indicates that this metabolic pathway may be selectively required for their survival and growth. Indeed, glutamine withdrawal is cytotoxic to RB-null cells and the cells can be rescued by the addition of the TCA cycle intermediate, α-ketoglutarate (52). These studies suggest that suppression of glutamine metabolism may be catastrophic to RB-deficient tumors. Unfortunately, glutamine antagonists have not been proven to be effective in clinical trials (for example, ref. 57) thereby highlighting the need for a more targeted therapeutic approach against enzymes whose activity may be altered in neoplastic cells lacking RB function. For example, several small molecular inhibitors of glutaminase, including BPTES and compound 968, have been found to have potent antitumor activities (58, 59). To validate these approaches and justify clinical trials, analyses of the relative sensitivities of RB-null human cancer cells versus RB wild-type normal cells to pharmacologic inhibition of the RB-E2F-regulated metabolic enzymes are necessary.

Summary

The discovery of mutations that disrupt the activities of the RB-E2F pathway in a majority of human cancers has highlighted the clinical importance of downstream effector enzymes that regulate cell-cycle progression. More recent studies have showed that this pathway also regulates the expression and activities of several metabolic transporters and enzymes that are required for the production of ATP and anabolic precursors. Given that these metabolic effector proteins are directly suppressed by the RB-E2F axis and are required for the immortalization and subsequent transformation of normal cells into neoplastic cells, small molecule antagonists that target their substrate-binding domains may have selective antitumor properties.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: B.F. Clem, J. Chesney
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