REVIEWS IN BASIC AND CLINICAL GASTROENTEROLOGY AND HEPATOLOGY

Robert F. Schwabe and John W. Wiley, Section Editors

Targeting PI3K Signaling as a Therapeutic Approach for Colorectal Cancer

JING ZHANG,[*,‡] THOMAS M. ROBERTS,[*,‡] and RAMESH A. SHIVDASANI[§,‖]

*Departments of *Cancer Biology and [§]Medical Oncology, Dana-Farber Cancer Institute, Harvard Medical School, Boston; [‡]Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston; and [‖]Departments of Medicine, Brigham & Women's Hospital and Harvard Medical School, Boston, Massachusetts*

Survival times of patients with colorectal cancer (CRC) have increased over the past decade, primarily as a result of treatment with combinations of conventional cytotoxic agents. Because CRC is commonly associated with mutations in genes that control growth factor signaling, therapies are being developed to target the products of these genes; individualized treatment might also be guided by specific mutations in tumors and by new biomarkers. Currently, targeted therapies confer limited clinical benefit; better drugs are therefore needed. Genomic studies indicate that phosphoinositide 3-kinase (PI3K) signaling is one of the most frequently deregulated pathways in several human cancers, including CRC. PI3K signaling has an important role in cancer cell proliferation, survival, motility, and metabolism and therefore could be an attractive therapeutic target. We review PI3K signaling in CRC and discuss current therapeutic approaches.

Keywords: Colon Cancer; Neoplasm; Signal Transduction; Genetics; Tumor.

Colorectal cancer (CRC) is one of the most common cancers worldwide and the second leading cause of cancer mortality in the United States. The lifetime risk of developing CRC in the United States is about 6%.[1] The disease develops gradually over many years, starting with the transformation of normal colonic epithelium to an adenomatous intermediate that can eventually progress into invasive adenocarcinoma.[2] Specific mutations, epigenetic changes, and defects in chromosomal stability or DNA repair promote disease progression and malignant behaviors. Although cytotoxic agents are the most commonly used therapies, both in the adjuvant setting and in metastatic disease, other strategies are being developed for management of metastatic CRC: inhibiting epidermal growth factor (EGF) receptor (EGFR) with monoclonal antibodies such as cetuximab and panitumumab and blocking angiogenesis with antibodies against vascular endothelial growth factor receptor such as bevacizumab.[3] Regrettably, the clinical benefits of these targeted therapies are short-lived and restricted to subgroups of patients, indicating a need for improvement in rational design of cancer therapy.

The phosphoinositide 3-kinase (PI3K) signaling pathway plays an essential role in cancer cell proliferation, survival, motility, and metabolism. It is frequently deregulated in CRC cells, making it an attractive therapeutic target.

Genetic Factors That Contribute to Formation and Progression of CRC

CRC is believed to arise and progress as a result of cumulative genetic and epigenetic changes in tumor cells; certain mutations appear at consistently high frequencies and at particular stages of disease progression.[4] Constitutive activation of Wnt/β-catenin signaling initiates growth of benign adenomas. Mutations in the oncogene *KRAS* and related pathways stimulate adenoma growth and, together with inactivation or loss of p53, contribute to invasive and other malignant behaviors.[3] Loss of genomic stability is an important element. One way this arises is through chromosomal instability—multiple structural or numerical chromosomal changes that are observed in more than 80% of tumors.[5] Most colorectal tumors that lack chromosomal instability show microsatellite instability (MSI), which results from deficiencies in DNA mismatch repair.[6] Cells of these tumors have a near-diploid genome, with hundreds of point mutations, small deletions, and insertions near nucleotide repeat tracts.[7] CRC

Abbreviations used in this paper: CRC, colorectal cancer; EGF, epidermal growth factor; EGFR, epidermal growth factor receptor; GPCR, G-protein–coupled receptor; IRS1, insulin receptor substrate 1; MEF, murine embryonic fibroblast; MSI, microsatellite instability; mTOR, mammalian target of rapamycin; mTORC, mammalian target of rapamycin complex; PDK, phosphoinositide-dependent kinase; PI3K, phosphoinositide 3-kinase; PIP_2, phosphatidylinositol-4,5-bisphosphate; PIP_3, phosphatidylinositol-3,4,5-trisphosphate; PTEN, phosphatase and tensin homologue; RTK, receptor tyrosine kinase.

0016-5085/$36.00
doi:10.1053/j.gastro.2011.05.010

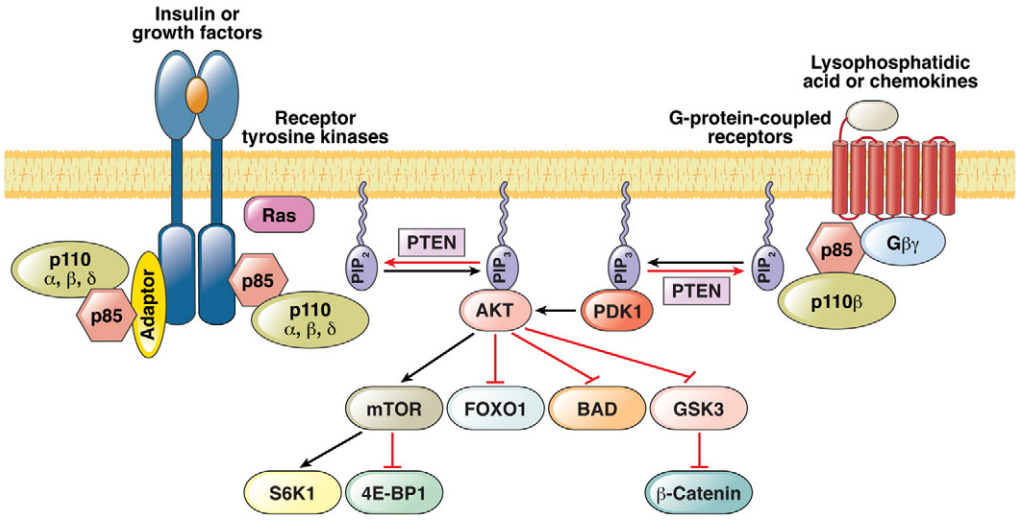

Figure 1. The class IA PI3K signaling pathway. Growth factor or insulin stimulation activates RTKs. Class IA PI3Ks, including p110α-p85, p110β-p85, and p110δ-p85, are recruited to the plasma membrane by direct interaction with the activated receptors (such as platelet-derived growth factor receptor) or by interaction with adaptor proteins associated with the receptors (such as IRS1). The p110β subunit can also be activated by GPCRs, which are activated by lysophosphatidic acid or chemokines. Activated PI3K converts PIP_2 to PIP_3, which provides docking sites for signaling proteins such as PDK1 and serine-threonine kinase AKT. Once activated, AKT phosphorylates many downstream effectors to regulate cell processes such as protein synthesis, cell survival, proliferation, and metabolism. PTEN functionally antagonizes PI3K activity by dephosphorylating PIP_3.

cells also have epigenetic instability—either overall DNA hypomethylation[8] or a high frequency of hypermethylation that is associated with promoter CpG islands (CIMP).[9] CIMP hypermethylation is detected in many tumors that have MSI[9] or the oncogenic *BRAF* V600E mutation.[10,11]

In colorectal tumors, genetic alterations are detected in growth pathways that sustain normal colonic epithelial cells, particularly the intracellular signaling cascades triggered by activation of receptor tyrosine kinases (RTKs) of the EGFR family. Mutations that activate products of the oncogenes *KRAS*, *PIK3CA*, and *BRAF* are frequently detected in colorectal tumors; these proteins are important intermediates of EGFR and other RTK-induced signals, and mutations can confer growth factor independence.[12] Whereas *KRAS* mutations do not usually occur in cancer cells that have *BRAF* mutations, *PIK3CA* mutations that activate PI3K signaling are sometimes detected in cancer cells that have mutations in these other oncogenes.[13,14] Recent large-scale sequencing and other genomic analyses of human colorectal tumors indicate that there are up to 80 nonsilent mutations in each tumor and that individual tumors have different mutations.[15] Although the mutations are many and varied, classification of common and rare mutations revealed that 38 pathways are disrupted with particular frequency; many of these pathways intersect with PI3K signaling.[15]

The PI3K Pathway

PI3Ks belong to a family of intracellular lipid kinases that phosphorylate the 3′-hydroxyl group of phosphatidylinositol and phosphoinositides. The lipid products of PI3K reactions create binding sites for specific, lipid-binding domains on many intracellular signaling proteins and thereby regulate a range of cellular processes, including cell survival, proliferation, and differentiation. There are 3 classes of PI3Ks (I, II, and III), based on their structural characteristics and substrate preferences.[16] The best characterized are the class I PI3Ks, which are generally coupled to extracellular stimuli. This class is further divided into class IA PI3Ks, which are activated by RTKs, G-protein–coupled receptors (GPCRs), and certain oncoproteins such as the small G-protein RAS (Figure 1), and class IB PI3K, which is regulated exclusively by GPCRs.

Class IA PI3Ks have been most frequently associated with human cancer. They are heterodimeric proteins that comprise a p85 regulatory subunit and a p110 catalytic subunit. The genes *PIK3R1*, *PIK3R2*, and *PIK3R3* encode p85α, p85β, p55γ, and their respective splicing variants, which are collectively called p85 (Figure 2). The mammalian catalytic isoforms (p110α, p110β, and p110δ) are encoded by *PIK3CA*, *PIK3CB*, and *PIK3CD*, respectively. p110α and p110β are expressed ubiquitously, whereas p110δ is largely confined to the immune system.[17] All p110 subunits in class IA have a p85-binding domain at the N-terminus, followed by a Ras-binding domain that interacts with small G-proteins of the RAS family, a C2 domain, a helical domain, and a C-terminal catalytic domain (Figure 2). Dimerization with p85 stabilizes p110 but maintains the enzyme in a low-activity state through inhibitory interactions.[18,19]

In response to stimulation of RTKs by growth factors, PI3K is recruited to the plasma membrane via the interaction of its p85 subunit with phosphotyrosine motifs on activated receptors, such as platelet-derived growth factor receptor, or on adaptor proteins associated with these