CHAPTER

6

PIK3CA Mutations in Colorectal and Breast Cancer: Impact on Oncogenesis and Response to Nonsteroidal Anti-Inflammatory Drugs

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Abstract

Genetic alterations, including somatic activating mutations of oncogenes and loss of tumor suppressor genes, are initiators of oncogenesis. The phosphatidylinositol-3-kinase (PI3K) signaling pathway regulates a broad spectrum of normal physiological processes, and when altered, contributes to tumor formation, progression, and resistance to therapy. Specifically, mutations in *PIK3CA*, the gene encoding the PI3K catalytic subunit, are found in a variety of human cancers and are associated with more aggressive disease and worse prognosis. Cumulative evidence has demonstrated that regular use of aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) is effective in the prevention and treatment of colorectal cancer, breast cancer, and other malignancies. However, the mechanism by which NSAIDs influence cancer risk and survival are poorly understood. Emerging data from observational studies and clinical trials suggest that *PIK3CA* mutational status in conjunction with other biomarkers might help to identify individuals who will benefit most from the NSAID therapy. This chapter reviews the PI3K signaling pathway with a focus on *PIK3CA* mutations and the role of aspirin and other NSAIDs as promising chemopreventive therapies in human cancer.

ABBREVIATIONS

AMPK	5′ Adenosine monophosphate-activated protein kinase
APC	Adenomatous polyposis coli
5 ASA	5-Aminosalicyclic acid
COX	Cyclooxygenase

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COXIBs	COX-2-selective inhibitors
CRC	Colorectal cancer
EGFR	Epidermal growth factor receptor
ER	Estrogen receptor
FAP	Familial adenomatous polyposis
GPCR	G protein-coupled receptor
HER	Human epidermal growth factor receptor
HNPCC	Hereditary nonpolyposis colorectal cancer
HNSCC	Head and neck squamous cell carcinoma
IBD	Inflammatory bowel disease
IGF-1	Insulin-like growth factor-1
MAPK	Mitogen-activated protein kinase
mCRC	Metastatic colorectal cancer
MDSC	Myeloid-derived suppressor cell
MEK	Mitogen-activated protein kinase kinase
mTOR	Mammalian target of rapamycin
NF-kB	Nuclear factor kappa B
NHGRI	National Human Genome Research Institute
NSAIDs	Nonsteroidal anti-inflammatory drugs
PDK1	Phosphoinositide dependent protein kinase-1
PGE_2	Prostaglandin E_2
PGH_2	Prostaglandin H2
PH	Pleckstrin homology
PI	Phosphatidylinositol
PIP	Phosphatidylinositol (3)-phosphate
PIP_2	Phosphatidylinositol (3,4)-biphosphate
PIP_3	Phosphatidylinositol (3,4,5)-triphosphate
PI3K	Phosphatidylinositol-3-kinase
PR	Progesterone receptor
PTEN	Phosphatase and tensin homolog deleted on chromosome ten
RTK	Receptor tyrosine kinase
TCGA	The Cancer Genome Atlas
VEGF	Vascular endothelial growth factor

6.1 INTRODUCTION

There is a growing body of literature supporting the regular use of nonsteroidal anti-inflammatory drugs (NSAIDs), particularly aspirin, in the prevention and treatment of colorectal cancer (CRC) and colorectal adenomas [1]. CRCs are thought to arise from a series of molecular alterations that transform normal colonic epithelial cells into premalignant adenomas and subsequently into carcinomas [2]. Pro-inflammatory prostaglandins, which are produced by the enzyme cyclooxygenase (COX), are believed to support CRC carcinogenesis [3]. Early studies with rodent CRC models showed that treatment with the COX inhibitor, indomethacin, decreased the incidence and size of carcinogen-induced colonic tumors [4–6]. A similar effect in humans has been suggested by small, randomized clinical trials evaluating the efficacy of the NSAIDs sulindac and celecoxib in protecting against colonic polyp formation and reducing the risk of CRC in patients with familial adenomatous polyposis (FAP) [7–9]. These encouraging findings in patients with FAP prompted evaluation of the anticancer properties of aspirin and other NSAIDs in the general population of CRC patients.

Multiple epidemiological studies have examined the chemopreventive properties of NSAIDs in reducing the incidence of CRC and colorectal adenomas [10–12]. In a case–control study of 1326 patients with CRC and 4891 control patients, Rosenberg et al. [10] showed that sustained use of NSAIDs reduced the risk of human colon cancer. A similar investigation from The Nurses' Health Study examined a cohort of 89,446 women and found that regular use of aspirin (two or more times per week), at doses similar to those recommended for prevention of cardiovascular disease, reduced the risk of CRC [12]. However, this clinical benefit was only observed in patients after a decade of aspirin use. Another population-based control study from Australia found a similar reduction in the risk of CRCs among individuals taking aspirin [13]. These findings have been further substantiated in two prospective cohort studies, the Cancer Prevention Study II [14] and the Health Professionals Follow-up Study [11], which demonstrated that regular use of aspirin reduced the risk of fatal CRC and colorectal adenoma recurrence.

Emerging from these cumulative preclinical and observational studies are two landmark, randomized, double-blind clinical trials led by Sandler et al. [15] and Baron et al. [16]. These studies demonstrated that aspirin use was associated with a substantial reduction in incidence of CRCs and colorectal adenomas among patients with a prior history of either CRC or adenomas [15,16]. However, the benefit of aspirin in reducing the risk of CRCs or adenomas was found to disappear when aspirin use was discontinued [16]. A subsequent observational follow-up study reported that patients who discontinued aspirin, and subsequently started using a different NSAID, exhibited significant protection against CRC and adenomas [17]. These findings have encouraged additional clinical investigations evaluating the effects of aspirin on CRC-specific and overall survival [18–20]. In fact, in April 2016, the U.S. Preventive Services Task Force found substantial evidence to recommend that people who are between 50 and 69 years of age, and are not at increased risk for bleeding, consider taking aspirin to prevent CRC and cardiovascular events [21,22].

Although generally well tolerated, use of aspirin daily is not without perils. The most commonly reported adverse side effects include dyspepsia, nausea, vomiting, and abdominal discomfort, while the more threatening complications include hepatotoxicity, gastrointestinal bleeding, and hemorrhagic stroke. Therefore, it is imperative to elucidate molecular biomarkers and mechanisms of aspirin response in the cancer microenvironment in order to better identify which subgroup of patients are most likely to benefit from aspirin's antitumor effect. With the growing population of CRC survivors, there is considerable interest in developing strategies for targeting signaling pathways in both the cancer and the immune system. This is reflected in several *in vitro and in vivo* studies that shed light on the association of specific *PIK3CA* mutations in CRCs with elevated expression of cyclooxygenase (COX-2), a critical enzyme in the synthesis of prostaglandin E_2 (PGE_2), a potent immunosuppressive molecule [23,24]. Notably, COX-2 is inhibited by aspirin (and other NSAIDs) [25]. Thus, it is perhaps not surprising that recent studies have shown that *PIK3CA* mutations are a promising predictive biomarker for successful therapy with aspirin in patients with CRCs. In this chapter, we review the genetic alterations in *PIK3CA* that are found in CRCs and their impact on tumor growth and response to aspirin. Further, we will discuss the potential of aspirin therapy as an emerging chemopreventive strategy in CRCs, breast cancer, and other malignancies.

6.2 ONCOGENIC SIGNALING IN CRCs

CRC is the third most common cancer and second leading cause of cancer-related mortality in the United States. Based on data from 2017, it is estimated that approximately 4.6% of men (1 in 22) and 4.2% of women (1 in 24) will be diagnosed with CRC in their lifetime [26]. The estimated 5-year survival rate varies with the pathological extent of disease (TNM stage), from 90% for localized stage I CRC to 50%–70% for advanced stage III CRC [26]. Major risk factors for CRC development include genetic and epigenetic abnormalities, diet, lifestyle, and chronic inflammation. Since the mid-1980s, enhanced CRC screening and treatment methods have contributed to the rise in CRC survival rates. The detection and removal of preneoplastic polyps is an effective preventive approach to mitigate CRC development. Unfortunately, not all individuals can be identified at the earliest stages of CRCs to reap maximal benefit from surgical resection alone. Thus, chemotherapy and radiation are often combined with surgery to treat patients with more advanced CRC that has spread to surrounding tissues and regional lymph nodes and/or metastasized to distant parts of the body. However, metastatic CRC is considered incurable with a 5-year survival rate of only 11%, and 90% of patients with metastatic disease acquire resistance to therapy [27]. The identification of molecules or pathways that can be targeted to overcome this resistance represents a major avenue of current research.

CRC develops from a cascade of mutations in key cell-cycle regulatory genes, tumor suppressor genes, and proto-oncogenes. The pathogenesis of CRC frequently involves mutations in components of the Wnt signaling pathway in intestinal crypt stem cells, with the *APC* gene being the most frequently altered [28]. This gene encodes the adenomatous polyposis coli (APC) protein, which normally prevents the accumulation of β-catenin, an adaptor protein present in the adherens junctional complex and a transcription regulator. When β-catenin aberrantly accumulates in the cytoplasm, as is the case in CRC with *APC* alterations, it undergoes translocation into the nucleus where it promotes transcription of Wnt target genes, including *c-Myc*, leading to enhanced cellular proliferation and growth [29]. However, it takes more than a "single" hit mutation for malignant transformation in CRC to occur [30]. This is because tumor suppressor genes present in the cell, including *PTEN* and *TP53*, survey dysregulated cellular activity and facilitate either rectification or trigger apoptosis. Eventually, cells acquire additional mutations in tumor suppressor genes leading to "double-hit" (or "multihit") oncogenesis. Abrogation of such tumor suppressor function allows for transformation of the intestinal stem cell into an invasive epithelial carcinoma cell [30].

Mutation or overexpression of proto-oncogenes also promotes CRC development. For example, the epidermal growth factor receptor (EGFR) plays an instrumental role in the transduction of proliferative and anti-apoptotic signals and is commonly overexpressed in metastatic colorectal cancer (mCRC). Accordingly, EGFR has been favorably targeted using two EGFR monoclonal antibodies: cetuximab and panitumumab. These molecular inhibitors selectively block the extracellular binding domain on EGFR and serve to augment conventional chemotherapy regimens in the treatment of mCRC [31,32]. However, dysregulation of signaling components downstream of EGFR, including components of the RAS/RAF, MEK/MAPK, and PI3K/AKT/mTOR pathways, promotes resistance to EGFR inhibitors. Moreover, in chemotherapy-refractory patients with mCRC, activating mutations in *K-Ras* mediate resistance to EGFR monoclonal antibody treatment [33].

6.3 THE PI3K SIGNALING PATHWAY

The phosphatidylinositol-3-kinases (PI3Ks) are intracellular signaling enzymes that translate information from exogenous stimuli including growth factors and cytokines into intracellular second messengers that induce multiple pathways involved in cell-cycle regulation and cellular metabolism, differentiation, migration, and survival [34]. PI3Ks are heterodimeric lipid kinases that phosphorylate 3'-hydroxyl groups on the phosphoinositol ring. By doing so, these kinases generate phospholipid molecules that are involved in critical cell signaling pathways and maintenance of cellular homeostasis. However, aberrant PI3K activity has been observed in numerous pathological conditions including diabetes, chronic inflammation, and cancer. Since their discovery, PI3Ks and the signaling pathways mediated by these kinases have been extensively studied for their promotion of oncogenesis in colorectal, breast, brain, and lung cancers via mechanisms involving increased genomic instability, enhanced cell proliferation, evasion of growth suppressors, resistance to cell death, and induction of angiogenesis and invasion [35–37]. Understanding PI3K signaling pathways will be integral to drug development strategies for these diseases.

Members of the PI3K family can be grouped into classes I, II, and III based on their primary structure, catalytic activity, and substrate specificity [38]. Class I PI3Ks primarily produce phosphatidylinositol (3,4,5)-trisphosphate (PIP_3) from phosphatidylinositol (3,4)-bisphosphate (PIP_2). Class I PI3Ks are further divided into subclasses IA and IB. Class IA PI3Ks consist of a p110 catalytic subunit that heterodimerizes with a p85 regulatory subunit [39]. The genes *PIK3CA*, *PIK3CB*, and *PIK3CD* encode three class IA catalytic isoforms (p110α, β, and δ, respectively) that associate with five different regulatory isoforms: p85α or its two splice variants p55α and p50α encoded by *PIK3R1*, p85β encoded by *PIK3R2*, and p55γ encoded by *PIK3R3* [40]. By contrast, class IB PI3Ks comprise a single catalytic subunit (p110γ) encoded by *PIK3CG* and a regulatory subunit (p101) encoded by *PIK3R5* [40].

Classes II and III PI3Ks are less well characterized. Class II PI3Ks consist of three catalytic isoforms C2α, C2β, and C2γ, but lack regulatory domains and catalyze the phosphorylation of phosphatidylinositol (PI) to produce phosphatidylinositol (3)-phosphate (PIP), with subsequent phosphorylation of PIP to produce PIP_2 [38]. Class II PI3Ks play a critical role in generating the PIP_2 substrate for class I PI3Ks. Class III PI3Ks resemble class I PI3Ks in heterodimeric structure, containing both catalytic (Vps34) and regulatory subunits (Vps15/p150), but differ in function, as class III PI3Ks predominantly produce PIP from PI and are thought to be involved in autophagy, endocytosis, and phagocytosis [41].

The binding of growth factors or cytokines to their cognate transmembrane receptor triggers class I PI3K signaling (Fig. 6.1). Upon activation of G protein-coupled receptors (GPCR) or receptor tyrosine kinases (RTKs), class I PI3Ks are recruited to the inner plasma membrane through interaction with GPCR-associated G protein subunits or tyrosine phosphorylated motifs present on RTKs. Recruitment to the activated receptors relieves the inhibition of p110 imposed by the p85 regulatory subunit and leads to the production of PIP_3 from PIP_2 [40]. PIP_3 is a lipid second messenger that induces AKT-dependent signaling pathways. Specifically, PIP_3 serves as a docking site for proteins containing a pleckstrin homology (PH) domain, including the serine/threonine kinases AKT1, AKT2, and AKT3, as well as phosphoinositide-dependent protein kinase-1 (PDK1). Once localized to the cellular inner membrane, PDK1

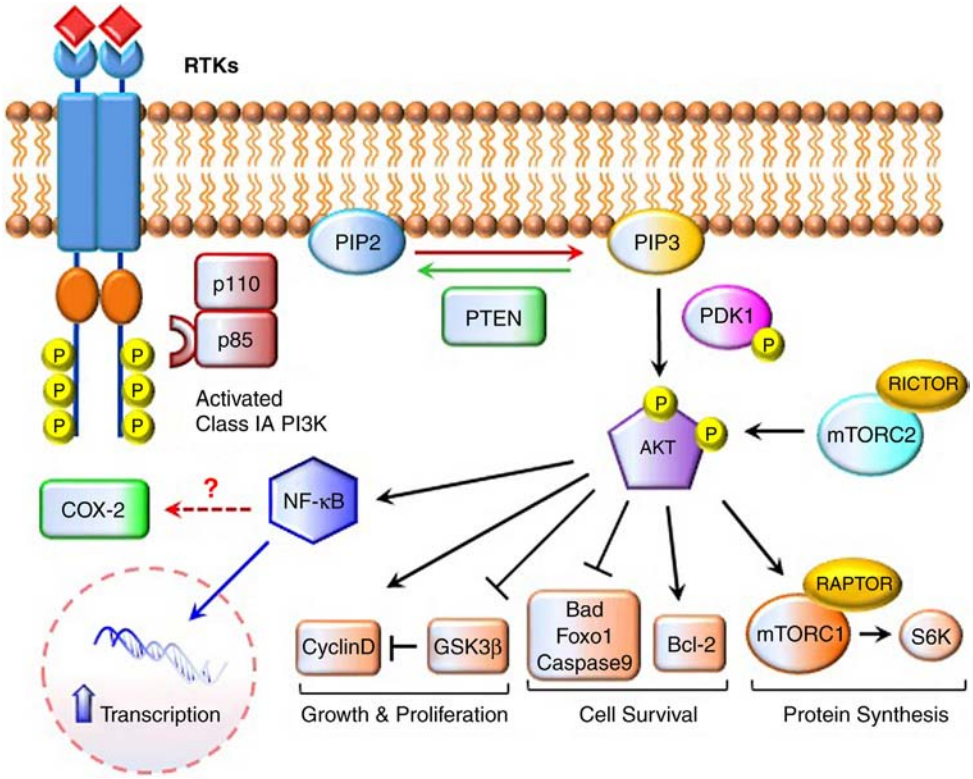

FIG. 6.1 **Class I phosphoinositide 3-kinase (PI3K) signaling pathway.** Upon ligand stimulation and subsequent activation of RTKs, class IA PI3K, consisting of p110/p85, is recruited to the cellular membrane via interaction of the regulatory p85 subunit with the tyrosine-phosphorylated RTK receptor, either directly or through an adaptor protein. The activated p110 catalytic subunit converts phosphatidylinositol-4,5-bisphosphate (PIP2) to phosphatidylinositol-3,4,5-triphosphate (PIP3), which provides docking sites for proteins containing pleckstrin-homology (PH) domains including phosphoinositide-dependent kinase 1 (PKD1). PDK1 phosphorylates and activates the seronine-threonine kinase, AKT, which regulates a broad spectrum of downstream activities that are involved in cell growth, proliferation, and survival. AKT can activate the nuclear transcription factor, NF-κB which is putatively linked to the production of pro-inflammatory prostaglandins by cyclooxygenase-2 (COX-2). Phosphatase and tensin homolog mutated on chromosome ten (PTEN) inhibits PI3K signaling by dephosphorylating PIP3 back to PIP2. Abbreviations: GSK3β, glycogen synthase kinase 3 β; mTORC, mammalian target of rapamycin complex; Bcl-2, B-cell lymphoma-2; S6K, ribosomal protein S6 kinase; Bad, Bcl-2-associated death promoter protein; Foxo1, Forkhead box protein O1; RTK, receptor tyrosine kinase.

phosphorylates AKT on threonine 308 with subsequent phosphorylation of serine 473 by cytoplasmic mTORC2 (complex of mTOR and RICTOR) [42]. Activation of AKT ultimately leads to activation of mTORC1 (complex of mTOR and RAPTOR), a downstream effector kinase that promotes cell growth, enhanced glucose metabolism, and protein and lipid synthesis [43]. Phosphatase and tensin homolog deleted on chromosome ten (PTEN) is a lipid phosphatase that removes the 3′ phosphate moiety from PIP_3, thereby inactivating class I PI3K signaling [40]. The equilibrium between phosphorylation of PIP_2 by kinase and dephosphorylation

of PIP_3 by phosphatase modulates the PI3K/AKT/mTOR signaling pathway to orchestrate a diverse array of cellular activities important for growth and proliferation. When aberrantly activated, this pathway is a potent inducer of oncogenesis.

Hyperactivation of the PI3K/AKT/mTOR signaling pathway is one of the most frequent events observed in human cancers. The *PTEN* gene, a critical negative regulator of PI3K signaling, is frequently mutated, lost, or downregulated in human brain, breast, and prostate cancers [44]. The PI3K signaling pathway can also be activated by mutations in the heterodimeric catalytic and regulatory subunits of PI3K. Class I PI3K mutations, particularly alterations in *PIK3CA* that encodes the p110α catalytic subunit, have been implicated as key drivers of CRCs and breast cancer and have emerged as potential drug targets [40]. The oncogenic potential of *PIK3CA* mutation was initially described by studies in the late 1990s, which demonstrated transformation of chicken cells by an avian retroviral oncogene homologous to the p110α catalytic subunit and transformation of human mammary epithelial cells by mutant forms of p110α and p110β [45,46].

Later studies on *PIK3CA* mutations in human cancers identified two hotspots for mutation in the p110α helical domain (E542K, E545K) and one hotspot for mutation in the p110α kinase domain (H1047R) [47]. The E542K, E545K, and H1047R mutations are commonly referred to as canonical PI3K mutations. These canonical mutations are gain-of-function mutations in p110α that promote hyperactivation of the PI3K pathway and have transforming capacity [46]. Mutant p110α proteins harboring the H1047R mutation exhibit increased catalytic activity, resulting in enhanced downstream signaling and oncogenic transforming ability [46]. Similarly, *PIK3CA* helical domain mutations diminish negative regulation by p85, leading to enhanced enzymatic activity [48,49]. Mutations in other genes (*PIK3CB*, *PIK3CD*, and *PIK3CG*) encoding class I PI3K catalytic subunits are more rare [40]. Further, while roles for class I PI3Ks in cancer are well described, the roles of class II and III PI3Ks in human malignancies remain unclear.

6.4 *PIK3CA* MUTATIONS IN CRCs

Advances in tumor tissue harvesting methods and high-throughput sequencing technologies have created new opportunities for identifying somatic PI3K mutations in human colorectal and breast cancers [50]. High-throughput sequencing of all PI3K genes in a panel of 234 CRCs by Samuels et al. [51] revealed that *PIK3CA* was mutated at a frequency of 32% in all CRC cases. All but three of the *PIK3CA* alterations identified in this screen were heterozygous missense mutations and no truncating or nonsense alterations were found. Remarkably, over 80% of the missense mutations in *PIK3CA* localized to the aforementioned hotspots in exon 9 (encoding helicase domain) and 20 (encoding kinase domain), a finding consistent with the presence of hotspot mutations in other CRC oncogenic drivers, including *K-Ras* and *B-Raf* [52,53]. Interestingly, *PIK3CA* mutations were found to be twice as common in tumors with microsatellite instability, indicating an inverse relationship between intact cellular renewal mechanisms and oncogenic *PIK3CA* mutations [51]. This study further examined 76 premalignant colorectal adenomatous polyps and found only two mutations in advanced-stage polyps, suggesting that, perhaps, *PIK3CA* mutations arise late in CRC progression to promote invasion and metastasis.

Other studies have correlated *PIK3CA* mutational status in CRCs to distinct pathologies. For instance, Miyaki et al. [54] found that *PIK3CA* mutations occurred at similar frequencies in sporadic CRCs and inherited CRCs [i.e., FAP and hereditary nonpolyposis colorectal cancer (HNPCC or Lynch syndrome)]. Mutations in patients with inherited predisposition to CRC occurred predominantly in the p110α kinase domain, whereas mutations in sporadic tumors arose primarily in the p110α helical domain [54]. Another study demonstrated a gender bias for *PIK3CA* mutations, with higher frequencies observed in women with CRC [55]. Lastly, several groups showed that *PI3KC*A mutation status was correlated with increased frequency of disease recurrence, resistance to conventional chemotherapy, and poor prognosis, even in patients with curatively resected CRC [56,57]. *PI3KCA* mutations in CRC were further described to confer clinical resistance to anti-EGFR monoclonal antibody treatment [58]. This comes as no particular surprise since inhibition of an upstream signal would not be expected to suppress pathway signaling due to mutation of a downstream signaling protein.

Additional progress in the characterization of *PI3KCA* alterations and other tumor-specific mutations in CRC has emanated from The Cancer Genome Atlas (TCGA) project, a collaborative venture between the National Cancer Institute (NCI) and the National Human Genome Research Institute (NHGRI). The TCGA applies high-throughput genome analysis technologies to explore the full spectrum of genomic changes in human cancer. To characterize genetic alterations in CRCs, the Colorectal Cancer Project conducted genome-wide analysis of 276 tumor samples, of which 224 were sequenced tumors, and found that the 10 most frequently mutated genes in CRCs are *APC*, *TP53*, *K-Ras*, *SYNE1*, *PIK3CA*, *LRP1B*, *FAT4*, *FBXW7*, *DNAH5*, and *LRP2* (Table 6.1) [59,60]. According to TCGA data, over 75% of CRC tumors harbor a mutation in one or more members of the Wnt signaling pathway, predominantly in APC [59].

Additional findings from TCGA have revealed that *PIK3CA* somatic mutation occurs at a frequency of 20.1% (45 of 224 sequenced cases) in CRC tumors [59,60]. Of these *PIK3CA* mutations, the most common are missense mutations that cluster in the p110α helical and kinase domains, a finding consistent with earlier studies. On the other hand, *PIK3CA* amplification, which is observed in 16.5% (83 of 504 sequenced cases) of head and neck squamous cell carcinoma tumors, does not appear to be a frequent event in CRC [61]. Mutations in *PTEN* resulting in loss of PTEN function occur at a frequency of 5.8% (13 of 224 sequenced cases) and are primarily deletion mutations, with truncation and missense mutations occurring at a lower frequency [59,60]. Additional evidence indicates that epigenetic changes may lead to loss of PTEN in a substantial number of cancers. Collectively, these studies suggest that while activation of the Wnt signaling pathway is nearly ubiquitous in CRCs, genetic alterations in the PI3K signaling pathway have also emerged as frequent triggers for oncogenesis and represent promising targets for anticancer strategies.

6.5 *PIK3CA* MUTATIONS IN BREAST CANCER

Breast cancer is the leading cancer diagnosed in women and the second leading cause of cancer deaths in women (United States), with an estimated 12% lifetime risk (1 in 8 US women). In 2017, an estimated 252,710 new cases of invasive breast cancer are expected to be diagnosed with an average 5-year survival rate of 89% [62]. *PIK3CA* is among the most

TABLE 6.1 Ten Most frequently Mutated Genes in CRCs

Gene	Class	Protein	Pathway affected	Number of mutations	Mutation frequency (cases)
APC	Tumor suppressor	APC	Wnt signaling	247	75% (168)
TP53	Tumor suppressor	Cellular tumor antigen p53	DNA repair, cell cycle, apoptosis, senescence	123	54% (121)
KRAS	Oncogene	GTP3se K-Ras	Signal transduction	94	42% (94)
SYNE1	N/A	Nesprin-1 or nuclear envelope spectrin repeat protein 1	Cerebellar neuronal signaling	101	21% (47)
PIK3CA	Oncogene	p110α catalytic subunit of PI3K	Signal transduction	53	20.1% (45)
LRP1B	Tumor suppressor	Low-density lipoprotein receptor-related protein 1B	Negative regulator of cell migration	80	17.9% (40)
FAT4	Tumor suppressor	Protocadherin Fat 4	Cell polarity, Hippo signaling	73	17.4% (39)
FBXW7	Tumor suppressor	F-box/WD repeat-containing protein 7	Ubiquitination	44	16.5% (37)
DNAH5	N/A	Dynein heavy chain 5, axonemal	Microtubule motility	70	15.2% (34)
LRP2	N/A	Low-density lipoprotein receptor-related protein 2	Cell signaling, ligand endocytosis	50	15.2% (34)

Results obtained from TCGA Colorectal Cancer Project [59]. Two hundred and seventy six CRC tumor samples from 276 patients were analyzed and mutation frequency data obtained from 224 sequenced tumors. The *PIK3CA* gene was among the top five most commonly mutated genes in CRC, at a frequency of 20.1% (45 out of 224 cases). Abbreviations: APC, adenomatous polyposis coli; CRC, colorectal cancer.

commonly mutated genes in breast cancer (Table 6.2). Two TCGA studies of primary breast cancers found that somatic *PIK3CA* alterations occur at a frequency between 32.5% (319 out of 982 sequenced cases) in all breast invasive carcinoma and 38% (308 out of 817 sequenced cases) in all invasive lobular breast cancers [63,64]. Specifically, *PIK3CA* mutation occurs in about 28%–47% of estrogen receptor (ER)-positive and progesterone receptor (PR)-positive breast cancers, in approximately 25% of human epidermal growth factor receptor 2 (HER2)-overexpressing breast cancers, and in 8% of triple-negative breast cancers [65]. Similar to the pattern observed in CRCs, the majority of *PIK3CA* mutations in breast cancer are missense mutations and are located in exon 9 (E542K and E545K) and exon 20 (H1047R).

PIK3CA amplification and *PTEN* loss can also augment PI3K activity in invasive breast cancer. These two events occur at higher frequencies in breast cancer compared to CRCs (Table 6.3). The TCGA provisional study on breast invasive carcinoma has observed *PIK3CA* amplification in 3.1% (30 of 982 sequenced cases) and *PTEN* loss in 5.1% (50 of 982

TABLE 6.2 Ten Most Frequently Mutated Genes in Breast Cancer

Gene	Class	Protein	Pathway affected	Number of mutations	Mutation frequency (cases)
PIK3CA	Oncogene	p110α catalytic subunit of PI3K	Signal transduction	355	32.5% (319)
TP53	Tumor suppressor	Cellular tumor antigen p53	DNA repair, cell cycle, apoptosis, senescence	304	30.7% (301)
CDH1	Tumor suppressor	Cadhem-1	Cell–cell interaction	114	11.4% (112)
GATA3	N/A	GATA Binding Protein 3	Mammary epithelial differentiation. Th2 differentiation	101	9.9% (97)
MAP3K1	Oncogene	Mitogen-activated protein kinase kinase kinase 1	Signal transduction	98	7.2% (71)
KMT2C	N/A	Lysine *N*-methyltransferase	Epigenetic transcriptional activation	83	7.1% (70)
MUC12	N/A	Mucin-12	Mucosal barrier, epithelial renewal	80	5.5% (54)
MUC4	N/A	Mucin-4	Mucosal barrier, epithelial renewal	62	5.4% (53)
FLG	N/A	Filaggrin	Epithelial differentiation	51	4.5% (45)
SYNE1	N/A	Nesprin-1 or nuclear envelope spectrin repeat protein 1	Cerebellar neuronal slanalinc	55	4.4% (43)

Results obtained from TCGA Breast Cancer Project, Provisional. One thousand hundred and five breast cancer tumor samples from 1098 patients were analyzed and mutation frequency data obtained from 982 sequenced tumors. The *PIK3CA* gene was the most frequently mutated gene in breast cancer, at a frequency of 32.5% (319 out of 982 sequenced cases).

sequenced cases) of tumors [63]. While loss-of-function mutations in the *PTEN* gene occur in fewer than 5% of breast cancer, reduced expression of PTEN protein due to promoter methylation or regulation at the RNA or protein level have been reported to occur in 30% of breast cancer cases [66]. *PIK3CA* amplification and/or *PTEN* loss were found to increase AKT phosphorylation and PI3K downstream signaling in 40% of breast invasive lobular carcinoma cases [64]. Inhibitors targeting p110α, mTOR, or AKT as well as dual PI3K/mTOR inhibitors have shown promise in early-phase clinical trials for hormone receptor-positive breast cancer, as well as other cancers, including CRCs and ovarian carcinoma [67,68]. However, as with other targeting agents, clinical response to PI3K pathway inhibitors in breast cancer may be short-lived due to the development of acquired resistance to

TABLE 6.3 *PIK3CA* and *PTEN* Alteration Frequencies in CRC and Breast Cancer

Alteration	Frequency (cases) in CRCs	Frequency (cases) in breast cancer
PIK3CA overall	20.1% (45)	35.6% (350)
PIK3CA mutation	20.1% (45)	32.5% (319)
PIK3CA amplification	N/A	3.1% (30)
PIK3CA deletion	N/A	0.1% (1)
PTEN overall	5.8% (13)	9.0% (88)
PTEN mutation	3.6% (8)	3.6% (35)
PTEN amplification	N/A	0.5% (5)
PTEN deletion	2.2% (5)	5.1% (50)

From TCGA, 276 CRC tumor samples were analyzed that generated 224 sequenced tumors. One thousand hundred and five breast cancer tumor samples were analyzed that generated 982 sequenced tumors. While CRC tumors were characterized by *PIK3CA* mutations, breast cancer tumors also exhibited *PIK3CA* amplification and deletion. *PTEN* deletion occurred at a frequency of 2.2% in CRC (5 of 224 sequenced cases) and 5.1% in breast cancer (50 of 982 sequenced cases). Abbreviation: CRC, colorectal cancer.

therapy. For instance, in the KPL-4PR breast cancer cell line, amplification of an activating mutant *PIK3CA* allele was found to confer resistance to a variety of PI3K inhibitors and PI3K/mTOR dual inhibitors [69]. Likewise, in a phase I clinical trial, loss of *PTEN* was determined to be a frequent mechanism of resistance to treatment with the PI3K inhibitor BYL719 in women with metastatic breast cancer [70]. These preclinical findings suggest that determination of the status of *PIK3CA* amplification and PTEN loss may provide important predictive value in identifying which patients will benefit from treatment with PI3K/AKT/mTOR pathway inhibitors.

Other mechanisms that lead to enhanced PI3K signaling in breast cancer include *HER2* amplification, at a frequency of 13% (107 out of 825 sequenced cases), and *AKT1* mutation, at a frequency of 2.4% (20 out of 825 sequenced cases) [63]. The *HER2* (*ERBB2*) gene encodes a cell-surface receptor tyrosine kinase, HER2, whose expression has been associated with worse prognosis in breast cancer. HER2 lacks a known ligand but heterodimerizes with other members of the HER family of receptors to induce signaling via the PI3K pathway. HER2 preferentially dimerizes with HER3, which contains six docking sites for the p85 subunit of PI3K, thereby allowing the HER2/HER3 heterodimer to trigger PI3K signaling [71]. In fact, HER2/PI3K activation has been shown to play an important role in conferring broad-spectrum chemotherapy resistance on human breast adenocarcinoma cells, suggesting that PI3K activation may prove to be a valuable predictive biomarker and molecular target for therapies aimed at improving outcomes in patients with chemotherapy-resistant breast cancer [72]. *AKT1* gene mutations in breast cancer (<8%) are exclusively restricted to tumors expressing both ER and PR [66].

Several studies on *PIK3CA* mutations in breast cancer have focused on the association of these alterations with clinical and pathological parameters. Saal *et al*. [73] reported that *PIK3CA* mutations correlate with expression of wild-type *PTEN*, positive hormone receptor expression, and node metastasis. Other studies have shown that *PIK3CA* mutations in breast

cancer correlate with worse survival and that mutations are more commonly found in larger tumors with ER and PR expression [74]. Moreover, higher frequencies of *PIK3CA* mutations are seen in lobular compared to ductal breast cancers [75]. *PIK3CA* mutational status has also been correlated with resistance to trastuzumab, a monoclonal antibody against HER2 receptor in *HER2*-amplified breast tumors [76].

6.6 *PIK3CA* AND COX-2 EXPRESSION

The mammalian intestinal epithelium undergoes rapid turnover, with cells turning over every 3–5 days [77]. This process is characterized by proliferation of intestinal crypt stem cells, progression of the daughter cells up the crypt-villi axis concurrent with differentiation into mature enterocytes, followed by apoptosis of the differentiated cells [77]. Dysregulation of intestinal epithelial cell proliferation, differentiation, and apoptosis is thought to play an important role in the carcinogenesis of CRC [78]. Prostaglandins are key mediators of coordinated intestinal epithelial cell turnover and homeostasis and are generated from arachidonic acid [79]. COX is the rate-limiting enzyme that catalyzes the conversion of arachidonic acid into prostaglandin-H2 (PGH_2), the precursor to mature prostaglandins. There are two COX isoforms, COX-1 and COX-2, which differ primarily in their pattern of expression and cellular activity. COX-1 is ubiquitously expressed and produces a variety of prostaglandins to coordinate normal metabolic functions. By contrast, COX-2 is primarily an inducible enzyme. Exposure to physiological stresses leads to COX-2 expression and production of prostaglandin-E2 (PGE_2) [80] (Fig. 6.2).

Mounting evidence suggests that COX-2 plays a critical role in CRC progression by enhancing cell survival and metastatic potential [81,82]. In fact, elevated COX-2 expression has been observed in approximately 85% of colorectal adenocarcinomas and 50% of premalignant adenomas and is associated with worse survival among CRC patients [83–85]. Preclinical studies indicate that enhanced COX-2 signaling and PGE_2 synthesis may facilitate CRC progression by augmenting the proliferation and survival of intestinal epithelial cells, increasing the production of pro-angiogenic factors, increasing expression of metalloproteases to promote tumor invasion and metastasis, and suppression of antitumor immunity [86,87]. On the other hand, inactivation of the *COX-2* gene in mice is associated with decreased colorectal tumorigenesis [88]. A similar study in Caco-2 colon carcinoma cells revealed that CRC cell growth may depend on COX-2 expression levels and activity and that COX-2 blockade results in induction of apoptosis [89].

PIK3CA mutations result in the constitutive activation of p110α kinase catalytic activity and appear to stimulate COX-2 expression through downstream AKT signaling. Other alterations in the PI3K signaling pathway, such as *PIK3CA* amplification or *PTEN* loss, may also lead to overexpression of COX-2 and high levels of PGE_2 production [89]. High levels of PGE_2 have been implicated in inflammatory bowel disease (IBD) and colorectal tumorigenesis, where PGE_2 acts to stimulate immunosuppressive myeloid-derived suppressor cells and promote an inflammatory environment [90–92]. Several studies have suggested a link between PI3K signaling and COX-2 expression and activity. For example, PI3K signaling can mediate the proliferative effects of insulin-like growth factor-1 receptor in CRC cells by increasing COX-2 expression and PGE_2 synthesis [89]. Another study on the chemopreventive activity of curcumin in CRC has shown that PI3K/AKT can activate nuclear factor kappa B (NF-κB),

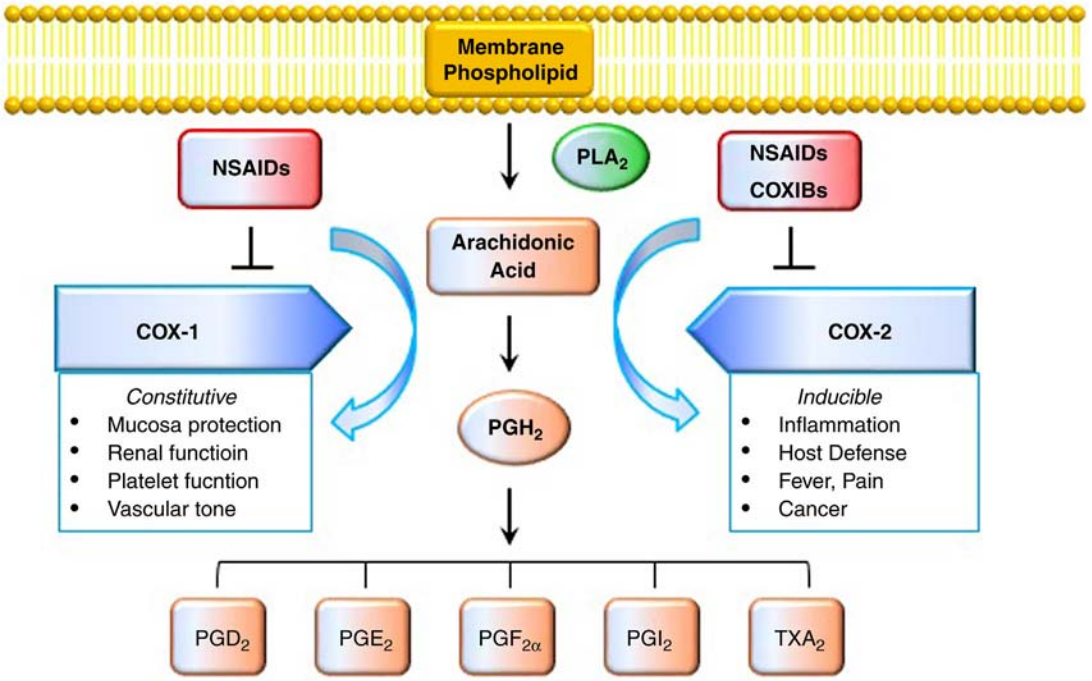

FIG. 6.2 **COX pathway.** Prostaglandins are signaling molecules produced from oxidation of membrane fatty acids. Membrane phospholipid is first converted into arachidonic acid by the enzyme, phospholipase A2 (PLA2). Next, COX catalyzes the rate-limiting step in the conversion of arachidonic acid to the intermediate, prostaglandin H2 (PGH_2) that can then be converted into prostaglandins D_2, E_2, and $F_{2\alpha}$ (PGD_2, PGE_2, $PGF_{2\alpha}$), prostacyclin I_2 (PGI_2), and thromboxane A_2 (TXA_2). There are two known COX isoforms (COX-1 and COX-2). COX-1 is ubiquitously expressed in most cells and plays a role in gastrointestinal mucosa homeostasis, renal function, platelet aggregation, and vascular tone. COX-2 is not normally expressed in most tissues but is induced in response to physiologic stress. COX-2 expression is upregulated by cytokines and is a major source of prostaglandins produced during inflammation and cancer. NSAIDs, including aspirin, inhibit both COX-1 and COX-2. Selective COX-2 inhibitors (COXIBs) preferentially inhibit COX-2 with minimal COX-1 inhibition. The overproduction of PGE_2 is associated with neoplastic effects on tumor growth, apoptosis inhibition, tumor angiogenesis, and immunosuppression. Abbreviation: COX, cyclooxygenase.

a transcription factor involved in the upregulation of pro-inflammatory genes, including inducible *COX-2*, which generates prostaglandins [93].

NF-κB plays a pivotal role in the regulation of cellular proliferation and immune responses, as well as the production of inflammatory mediators that contribute to the pathogenesis of inflammation and cancer [94]. In addition, NF-κB can increase the expression of anti-apoptotic cellular proteins, including members of the Bcl-2 family, which can confer chemotherapy resistance in cancer cells [95]. Several lines of evidence have suggested that aspirin and other NSAIDs, such as sulindac, block NF-κB activity, through inhibition of I-κB phosphorylation, resulting in reduced levels of COX-2 and PGE_2 [96,97]. In fact, sulfasalazine, an anti-inflammatory agent that includes a nonsteroidal anti-inflammatory moiety, 5-aminosalicyclic acid, is a potent inhibitor of NF-κB and has been one of the most commonly prescribed medications for patients with IBD [98].

6.7 ASPIRIN USE IN *PIK3CA*-MUTATED CRCs

A significant effort has been made to understand the role of COX in CRC progression and the clinical benefit of COX inhibition by NSAIDs for CRC prevention and treatment. Although NSAIDs are some of the most commonly used drugs for their analgesic, anti-inflammatory, and potential anticancer properties, the prolonged used of nonselective NSAIDs is associated with adverse events including dyspepsia, gastrointestinal bleeding, and gastroduodenal ulceration. These unwanted side effects are largely attributed to inhibition of COX-1, which plays a key role in the production of prostaglandins protective of gastrointestinal mucosal integrity and induction of platelet aggregation [99]. The development of COX-2-selective inhibitors (COXIBs) such as celecoxib and rofecoxib have made it possible to minimize adverse gastrointestinal complications associated with nonselective NSAIDs while maintaining their therapeutic properties [100].

COX-2, which mediates prostaglandin synthesis during inflammation and is overexpressed in CRC tumors and premalignant polyps, is thought to play an important role in CRC carcinogenesis [83]. COX-2 upregulation in CRCs has been associated with increased tumor growth, angiogenesis, and metastasis [3,87,101]. Emerging animal, epidemiologic, and clinical data have indicated that NSAIDs and COXIBs are promising chemopreventive agents for CRC and other malignancies. Early animal studies from the 1980s demonstrated beneficial effects of NSAIDs on inflammation and tumor regression in carcinogen-induced rat CRC models [5,6,102]. Later, prospective epidemiology studies in humans found an association between regular NSAID use, in particular, aspirin, and decreased CRC risk and cancer-specific mortality. This benefit of aspirin was found to be exclusive in patients with CRC tumors overexpressing COX-2, whereas regular aspirin use did not appear to influence tumors with weak or absent COX-2 [103]. In another study, regular low-dose aspirin use was shown to reduce the risk of fatal CRC [14]. A similar investigation discovered a link between aspirin use and reduction in fatal cancers from other cancers of the gastrointestinal tract including esophageal and gastric adenocarcinoma [104].

Clinical trials evaluating the role of NSAIDs in humans have revealed a notable clinical benefit in the primary and secondary prevention of CRC. Of particular interest, NSAIDs have been shown to diminish the overproduction of colonic polyps in genetically susceptible individuals. For example, treatment with the NSAID sulindac was found to be associated with a significant reduction in the number and size of polyps and overall CRC risk in patients with FAP and HNPCC [7,105]. Mechanisms proposed for the chemopreventive effect of sulindac include induction of the intrinsic apoptosis pathway and inhibition of Wnt/β-catenin pathway [105,106]. Similarly, in nonhereditary CRC, aspirin was found to reduce the recurrence of adenomatous polyps after surgical resection in patients with previous CRC (relative risk: 0.65; 95% CI: 0.46–0.91) [15]. In addition, findings from two large cohorts (Nurses' Health Study and Health Professionals Follow-up Study) showed that following surgical removal of a primary tumor, regular aspirin use significantly reduced the risk of CRC recurrence [103]. Final follow-up of 135,965 health-care professionals (88,084 women and 41,881 men) from these two studies demonstrated that long-term (6 years) regular aspirin use (0.5–1.5 standard aspirin tablets per week) was associated with a modest but significantly reduced risk for overall cancer, particularly CRCs [107]. Furthermore, in a collective analysis of 35,535 patients

from six randomized clinical trials, low-dose daily aspirin use resulted in marked reduction in the risk of developing metastatic CRCs [108].

Pivotal studies by Liao et al. [109] and Domingo et al. [110] identified canonical *PIK3CA* mutations as a predictive marker of aspirin therapeutic benefit in CRC. Indeed, regular aspirin use after CRC diagnosis in patients with mutated *PIK3CA* yielded a remarkable improvement in CRC-specific mortality and overall survival (hazards ratio 0.18; 95% CI: 0.06–0.61) [109]. On the other hand, CRC tumors with wild-type *PIK3CA* did not show a clinical benefit with aspirin. Interestingly, the study by Domingo et al. [110] failed to observe a greater benefit of the COX2-selective inhibitor rofecoxib vs placebo in patients with *PIK3CA*-mutated CRC. Nonetheless, the findings from these randomized clinical trials, along with epidemiologic evidence, provide a strong rationale for prospective evaluation of NSAIDs therapy in other malignancies characterized by *PIK3CA* alterations.

6.8 ASPIRIN USE IN *PIK3CA*-MUTATED BREAST CANCER

The production of PGE_2 in breast cancer has been shown to upregulate Wnt signaling and promotes cellular proliferation and survival, angiogenesis, and invasion/metastasis [113]. Elevated levels of COX-2 and PGE_2, which mediate inflammation in the tumor microenvironment, are indicators of poor prognosis in breast cancer [114]. Importantly, COX-2 is upregulated in up to 72% (46 of 64 cases) of breast cancers and associated with larger tumor size, nodal metastasis, and advanced clinical stage [115]. Mounting evidence has described a link between inflammation and breast cancer and stimulated strong interest in targeting COX enzymes, in particular, COX-2, for chemoprevention and breast cancer therapy. Clinical trials and findings from the Women's Health Initiative have reported that women who were diagnosed with breast cancer and were regular aspirin users experienced a decreased risk of distant tumor metastasis and death [116,117]. Furthermore, another study reported aspirin's clinical benefit in decreasing breast cancer-specific mortality and all-cause mortality among women with breast cancer [118].

Since dysregulated COX-2/PGE_2 activity likely contributes to multiple facets of tumorigenesis, targeting this pathway with COX inhibitors offers considerable promise for the prevention and treatment of breast cancer. Preclinical *in vitro* studies have evaluated the cytotoxic mechanism of aspirin and other NSAIDs in breast cancer cells [119,120]. For instance, aspirin was shown to trigger apoptosis in MCF-7 breast cancer cells through increased nuclear translocation and phosphorylation of anti-apoptotic factor, B-cell lymphoma 2 (Bcl-2), leading to its inactivation [119]. Similarly, MDA-MB-453 breast cancer cells treated with aspirin demonstrated increased release of cytochrome c from mitochondria and subsequent activation of caspase-3 [119,120]. Beyond activation of apoptosis, aspirin has also been shown to downregulate NF-κB and inhibit mTOR signaling, leading to cell-cycle arrest and induction of autophagy [121,122]. Likewise, other NSAIDs, such as the selective COX-2 inhibitor celecoxib, have been shown to suppress tumor angiogenesis and growth through inhibition of vascular endothelial growth factor (VEGF) [123], enhance the efficacy of aromatase inhibitors to inhibit mammary carcinoma formation [124], and increase sensitivity to chemotherapy in MCF7 breast cancer cells [125].

Recent preclinical studies have linked *PIK3CA* mutations in breast cancer to enhanced growth suppression by aspirin. Turturro et al. [126] reported that a nontumorigenic breast epithelial cell line harboring canonical *PIK3CA* mutations in either exon 9 or exon 20 demonstrated increased sensitivity to physiologic doses of aspirin. Another study by Henry et al. [127] showed that breast cancer cells expressing mutant *PIK3CA* that were treated daily with aspirin exhibited greater growth inhibition and lower viability than *PIK3CA*-wild-type cancer cells. This effect was attributed to the ability of aspirin to activate the enzyme AMPK, a known inhibitor of mTORC1 signaling. In addition, findings from this study provided a mechanistic rational for the use of aspirin as an adjuvant therapy to augment the efficacy of PI3K inhibitors against breast cancer. Taken together, these data provide evidence that alterations in the PI3K signaling pathway, in particular, *PIK3CA* mutations, sensitize breast cancer cells to aspirin. Prospective clinical trials will be needed to fully evaluate the impact of *PIK3CA* mutations in response to NSAIDs in breast cancer patients.

6.9 CONCLUSIONS

Activating *PIK3CA* mutations is a frequent event in the development and progression of numerous human cancers, including CRCs and breast cancer. Recent epidemiological, preclinical, and clinical studies have propelled NSAIDs, including aspirin, to the forefront of cancer research. The compelling finding that the presence of canonical *PIK3CA* mutations in CRC predicts response to aspirin has given impetus to ongoing clinical trials to validate this observation in other human cancers. Future investigations should also incorporate patients with noncanonical *PIK3CA* mutations, as well as PTEN loss, to potentially broaden the pool of patients who may benefit from NSAID therapy. Moreover, experiments are needed to elucidate the mechanism of COX-2 regulation by PI3K signaling, and the influence of NSAID-mediated COX-2 inhibition on the tumor microenvironment and antitumor immune response. Overall, NSAIDs, including aspirin, have far-reaching clinical benefits beyond their analgesic, anti-inflammatory, and cardiovascular benefits. As they are readily available, low-cost, and relatively safe, NSAIDs and aspirin hold promise for making a major impact on the global burden of human cancers.

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