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NSAID Therapy for *PIK3CA*-Altered Colorectal, Breast, and Head and Neck Cancer

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Abstract

Epidemiologic evidence indicates that regular use of nonsteroidal anti-inflammatory drugs (NSAIDs) provides a protective effect against the development of colorectal, breast, and head and neck cancers. Genomic characterization of these cancers has lent considerable insight into the subpopulations of cancer patients who are most likely to benefit from NSAID therapy. The *PIK3CA* gene encodes the catalytic subunit of phosphatidylinositol 3-kinase (PI3K) and is among the most frequently mutated genes in solid tumor malignancies. Cancer-associated mutations in *PIK3CA* promote signaling via the PI3K pathway and stimulate tumor cell growth. In addition, activation of the PI3K pathway leads to induction of cyclooxygenase-2 (COX-2) enzyme and production of immunosuppressive prostaglandin E₂ (PGE₂). Notably, in both colorectal cancer and head and neck cancer the subpopulation of patients that benefit from NSAID use is restricted to those whose tumors exhibit *PIK3CA* genomic alterations. Preclinical studies, particularly in models of head and neck cancer, support the hypothesis that the chemopreventive impact of NSAIDs may be due, in part, to inhibition of COX-2 and reduction of PGE₂ levels in the tumor microenvironment.

Keywords

Colorectal cancer; breast cancer; head and neck squamous cell carcinoma; HNSCC; *PIK3CA*; NSAIDs

Introduction

Members of the phosphoinositide 3-kinase (PI3K) family of intracellular kinases are activated by external stimuli and play key roles in promoting cell survival, proliferation, motility, and differentiation. PI3Ks exert their effects by phosphorylating the inositol ring structure of inositol phospholipids.

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Competing interests

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PI3K holoenzymes are comprised of two subunits, a catalytic subunit and a regulatory subunit, with the regulatory subunit acting to inhibit the kinase activity of the catalytic subunit. In humans, there are eight catalytic subunit isoforms and they are classified into 3 classes based on their specificity for different substrates (Bilanges et al., 2019; Hawkins and Stephens, 2015; Jean and Kiger, 2014; Vadas et al., 2011). Among them, Class I enzymes are involved in phosphorylation of phosphatidylinositol (4,5)-bisphosphate to phosphatidylinositol (3,4,5)-trisphosphate (PIP₃), which is an important secondary messenger for activating downstream signaling components such as AKT. There are four distinct isoforms of Class I catalytic subunits (p110 α , β , δ , or γ) and at least 4 distinct Class I regulatory subunits (p85 α , p85 β , p55 γ , p101). As depicted in Figure 1, stimulation of cells with growth factors or cytokines results in the activation of membrane-spanning growth factor receptors with associated tyrosine kinase activities. This leads to recruitment of Class I PI3Ks to the receptor cytoplasmic domains and relief of the inhibition imposed by the PI3K regulatory subunit, resulting in activation of the PI3K kinase activity and generation of PIP₃. The PIP₃ moiety serves as a recruitment locus for proteins containing pleckstrin homology domains, including PDK1 and the serine/threonine kinase AKT. PDK1-mediated phosphorylation of AKT leads to AKT activation and subsequent activation of mTOR signaling (Dibble and Cantley, 2015). Negative regulation of PI3K/AKT signaling is mediated, in large part, by phosphatase and tensin homolog deleted on chromosome ten (PTEN), which dephosphorylates PIP₃ to PIP₂, shutting down AKT activation.

While signaling via the PI3K/AKT/mTOR pathway is crucial in multiple cellular functions that impact growth and motility, dysregulation of this pathway also plays an important role in carcinogenesis (Fruman et al., 2017; Saxton and Sabatini, 2017). The PI3K/AKT/mTOR signaling pathway is one of the most commonly mutated pathways in many types of cancers, including colorectal cancer (CRC), breast cancer, and head and neck squamous cell carcinoma (HNSCC) (Danielsen et al., 2015; Lui et al., 2013; Noorolyai et al., 2019; Yuan and Cantley, 2008). Thus, components of this pathway have emerged as attractive targets for cancer treatment. A broad spectrum of inhibitors targeting this pathway have been developed and are being evaluated in clinical trials (Bauer et al., 2015; Cai et al., 2017; Janku, 2017; Janku et al., 2018).

Preclinical and clinical studies have shown that the use of nonsteroidal anti-inflammatory drugs (NSAIDs) has a role in prevention and treatment of cancers such as breast cancer and CRC (Hua et al., 2019; Patrignani and Patrono, 2016; Zhang et al., 2018). Furthermore, preclinical and clinical studies in CRC have shown that the mutational status of *PIK3CA* (which encodes p110 α) can affect tumor response to NSAIDs (Liao et al., 2012a). Recent evidence has also elucidated an important role for NSAIDs in the treatment breast cancer and HNSCC. In this review, we describe the current evidence supporting NSAID use in CRC, breast cancer, and HNSCC, with a focus on the mechanisms underlying the benefit of NSAIDs in *PIK3CA*-mutated cancers.

***PIK3CA* mutations and NSAID use in CRC and breast cancer**

The *PIK3CA* gene is among the most commonly mutated genes in CRC and breast cancer (Cai et al., 2017). Mutations in *PIK3CA* have been reported in 15-32% of CRCs and 32-38%

of breast cancers (Barault et al., 2008; Cancer Genome Atlas, 2012b; Ciriello et al., 2015; De Roock et al., 2010; Hamada et al., 2017; Liao et al., 2012b; Lievre et al., 2010; Mao et al., 2012). Several studies have also reported similar frequencies of *PIK3CA* mutations between sporadic and inherited CRC, an increased prevalence of *PIK3CA* mutations in women with CRC, and an increased risk of recurrence and poorer prognosis associated with *PIK3CA* mutations (Benvenuti et al., 2008; Kato et al., 2007; Miyaki et al., 2007). Data from The Cancer Genome Atlas (TCGA) has revealed *PIK3CA* mutations in 20% of sequenced CRC tumor samples, making it the fifth most common mutation found in CRC (Cancer Genome Atlas, 2012a). Over 80% of these *PIK3CA* mutations were “hotspot” missense mutations located in exons 9 and 20, resulting predominantly in the p110 α canonical mutations E542K (5%), E545K (20%), and H1047R (12.5%). Functional studies have shown that these canonical mutations activate the kinase activity of the p110 α enzyme (Kang et al., 2005; Zhao et al., 2005; Zhao and Vogt, 2010). The presence of hotspot mutations in *PIK3CA* is similar to what has been observed for other commonly mutated oncogenes in CRC, such as *KRAS* and *BRAF* (Cancer Genome Atlas, 2012a).

Similar findings related to *PIK3CA* mutation have been reported for breast cancer. Studies from the TCGA have shown that *PIK3CA* mutations occur in 28-47% of estrogen receptor (ER)/progesterone receptor (PR)-positive breast cancers, 25% of HER2-positive breast cancers, and 8% of triple-negative breast cancers. (Lee et al., 2015). Again, the majority of *PIK3CA* mutations occur in exons 9 and 20. (Lee et al., 2015) In the largest TCGA breast cancer study (977 patients), E542K, E545K, and H1047R mutations accounted for 11%, 18%, and 35%, respectively, of p110 α mutations. Further studies have demonstrated an association between *PIK3CA* mutations and nodal metastasis, poorer survival, and increased resistance to trastuzumab (a monoclonal antibody against HER2 receptors) (Berns et al., 2007; Li et al., 2006; Saal et al., 2005).

Given the prevalence of *PIK3CA* activating mutations in CRC and breast cancer, numerous studies have evaluated the impact of drugs that target the PI3K/AKT/mTOR pathway, with several showing therapeutic benefit. Notably, *PIK3CA* activating mutations have been shown to induce the expression and cellular activity of COX-2 enzyme, leading to increased production of prostaglandin E₂ (PGE₂) (Di Popolo et al., 2000). Thus, COX-2 inhibitors such as NSAIDs have been used to inhibit this arm of the PI3K pathway. Approximately 85% of CRC and 72% of breast cancers have also been shown to overexpress COX-2, with overexpression being associated with larger tumor size, disease progression, and worsened survival (Eberhart et al., 1994; Gupta and Dubois, 2001; Kochel et al., 2016; Nie and Honn, 2002; Ogino et al., 2008; Shim et al., 2003; Tsujii and DuBois, 1995).

Aspirin use was first shown to be associated with a decreased incidence of CRC in 1988 (Kune et al., 1988). Since then, numerous studies have reported that regular use of aspirin has a protective effect on the development of CRC, including several observational case-control studies and 4 randomized controlled trials (RCTs), all of which supported this effect (Algra and Rothwell, 2012; Andreotti et al., 2017; Cole et al., 2009; Kune et al., 1988; Liao et al., 2012a; Patrignani and Patrono, 2016). A meta analysis of 17 case-control studies showed that regular use of aspirin was associated with a reduced risk of CRC (95% confidence interval: 0.58 – 0.67; p<0.0001) (Algra and Rothwell, 2012). Each RCT showed

consistent results, with a 17-40% relative risk reduction in any adenoma recurrence and a 28% relative risk reduction in the recurrence of advanced lesions associated with 3-year treatment with an NSAID (Cole et al., 2009; Patrono and Rocca, 2008). Further post hoc analysis of these RCTs showed that daily aspirin use for 5 years reduced mortality due to CRC by 30-40% after 20 years of follow-up (Rothwell et al., 2011). This benefit was again shown in 6 primary prevention trials demonstrating that daily aspirin use led to a decrease in overall cancer incidence starting at 3 years (CI: 0.66 – 0.99; $p=0.008$) (Rothwell et al., 2012). Multiple large epidemiological studies have also shown a long-term decrease in CRC incidence with aspirin use (Giovannucci et al., 1995; Soriano et al., 2018). Recently the United States Preventive Services Task Force (USPSTF) issued a class B recommendation of daily aspirin use for the prevention of CRC in adults aged 50-59 years old who have a predicted life expectancy of 10 or more years.

Further analysis in CRC patients showed that the effect of aspirin on CRC patient outcomes may depend on *PIK3CA* mutational status. In a study of 964 CRC patients by Liao *et al.*, aspirin use after diagnosis in *PIK3CA*-mutated CRC patients was associated with superior cause-specific survival (CSS) (disease stage-adjusted HR 0.18, 95% CI 0.06-0.61, $p<0.001$) and overall survival (OS) (disease stage-adjusted hazard ratio (HR) 0.54, 95% Confidence Interval (CI) 0.31-0.94, $p=0.01$), while there was no association in CRC patients with wild-type *PIK3CA* (Liao et al., 2012a). These results suggested that *PIK3CA* mutation in CRC may be predictive of response to adjuvant aspirin therapy. In addition, two recent meta-analyses also indicate a benefit to aspirin use specific to *PIK3CA*-mutated CRC. In one study, post-diagnosis aspirin use was associated with reduced total mortality (HR 0.71, 95% CI 0.51-0.99) and a beneficial trend in CSS (HR 0.37, 95% CI 0.11-1.32) (Paleari et al., 2016). In the other study, post-diagnosis aspirin use was associated with both a marked reduction in CSS (HR 0.45, 95% CI 0.28-0.71) and a significant reduction in total-cause mortality (HR 0.69, 95% CI 0.52-0.93) in *PIK3CA*-mutated CRC (Elwood et al., 2016).

Similar results have been shown with aspirin use and breast cancer. Epidemiological studies have shown that aspirin use is associated with a reduced risk of breast cancer progression (Fraser et al., 2014; Harris et al., 2003; Holmes et al., 2010; Holmes et al., 2014). To date, 3 meta-analyses have examined prospective observational studies on aspirin use and breast cancer. Each of these studies showed similar results with an approximately 30% reduced risk of death due to breast cancer associated with aspirin use (Elwood et al., 2016; Huang et al., 2015; Zhong et al., 2015). On the other hand, there is limited RCT data on NSAID use and breast cancer recurrence. Many of the RCTs of NSAID use and breast cancer have employed celecoxib and were either small RCTs or were terminated early due to cardiovascular risks associated with celecoxib (Chow et al., 2008; Falandry et al., 2009; Goss et al., 2013; Martin et al., 2010). Despite these limitations, a few of these studies have shown an increase in progression-free survival in patients taking celecoxib (Falandry et al., 2009; Martin et al., 2010).

***PIK3CA* mutations in HNSCC**

Head and neck squamous cell carcinoma (HNSCC) is a common cancer with high morbidity and mortality. HNSCC patients who receive curative-intent therapy for a primary tumor are

at high risk for the development of second primary malignancies, a leading cause of mortality (Khuri et al., 2001). As in CRC and breast cancers, mutations in the PI3K pathway are frequently observed in HNSCC tumors. Alterations in genes encoding major components of the PI3K pathway were found in 183 out of 279 HNSCC tumors from data deposited to the Cancer Genome Atlas (TCGA) (Vander Broek et al., 2015). Among them, the *PIK3CA* gene was the most frequently altered (altered in 34% and 56% of HPV negative and positive tumors, respectively). Genomic alterations of *PIK3CA* found in HNSCC include both amplification and mutation. With respect to mutation, *PIK3CA* was reported to be mutated in 8% of HPV-negative HNSCC tumors and 21% of HPV-positive tumors (Cancer Genome Atlas, 2015). Subsequent analysis of 504 HNSCC tumors by the TCGA indicates an overall *PIK3CA* mutation rate of 13.7% and an amplification rate of 16.5%, with 4.4% of tumors exhibiting both mutation and amplification. Mutation at canonical sites (E542, E545, and H1047) account for roughly 65% of *PIK3CA* mutations in HNSCC tumors.

Clinically, alteration of PI3K pathway has been associated with poor outcome and high risk of recurrence. *PIK3CA* amplification was significantly associated with shorter medium disease-specific survival time (16 months versus 23 months for wild-type patients, $p = 0.037$) (Brauswetter et al., 2016) and earlier recurrence in HNSCC patients without lymph node metastasis (31% with 2-year disease free status versus 90% for patients without amplification) (Suda et al., 2012). Activation of the PI3K pathway has also been shown to contribute to treatment resistance (Burris, 2013). Yes-associated protein (YAP) is a downstream effector of the Hippo pathway that controls organ size by regulating cell survival, proliferation and apoptosis (He et al., 2018). It has been proposed as a marker of poor prognosis and resistance to therapy (Segrelles et al., 2018). In a recent analysis of 243 primary HNSCC tumors from the TCGA cohort, overexpression of *PIK3CA* was associated with decreased expression of phosphorylated YAP (inactive) and induction of YAP-mediated gene expression (Garcia-Escudero et al., 2018). These results were confirmed with an independent cohort from the University Hospital of A Coruña ($n=62$). Not surprisingly, molecular targeting of the PI3K pathway has been a priority in solid tumor malignancies, including HNSCC. Multiple inhibitors of the PI3K inhibitors have been developed and are currently being evaluated in clinical trials (Cai et al., 2017; Jung et al., 2018).

NSAID use in HNSCC risk reduction

A beneficial effect of NSAID use on HNSCC was first described by Panje in 1981, where 5 cases of regression and 2 cases of stabilization of HNSCC with commonly recommended dosages of indomethacin were reported (Panje, 1981). Subsequently, several case-control studies have been conducted to investigate the impact of NSAID use in head and neck cancer. In an analysis of 3 case-control studies between 1992-2000 in Italy, 37 of 965 upper aerodigestive tract (UADT) cancer cases and 87 of 1779 controls were found to regularly use aspirin. The multivariate odds ratio for UADT decreased from 0.89 (95% CI 0.56-1.43) to 0.33 (95% CI 0.13-0.82) for aspirin users of ≥ 5 years and the reduced head and neck cancer risk was observed in all included subsites (oropharyngeal, esophageal, and laryngeal) (Bosetti et al., 2003). In the Alcohol-Related Cancers and GEnetic Susceptibility (ARCAGE) project, 235/1779 UADT SCC cases and 321/1993 controls reported regular aspirin use (Macfarlane et al., 2012). An association between aspirin use and reduced risk of

head and neck cancer approached significance for cancer of the hypopharynx (Odds Ratio (OR) 0.53, 95% CI 0.28–1.02) and larynx (OR 0.74, 95% CI 0.54–1.01) (Macfarlane et al., 2012). In another case-control study, data from a comprehensive epidemiologic questionnaire of 529 patients with primary head and neck cancer and 529 healthy subjects showed an association between head and neck cancer risk reduction and aspirin use (adjusted odds ratio, 0.75; 95% CI 0.58–0.96) (Jayaprakash et al., 2006). The risk reduction was also associated with frequency and duration of aspirin use. Similar results were seen in a recent case-control study that focused on head and neck cancer patients with SCC. In 71 HNSCC patients and 71 healthy controls matched by age (within 10 years), gender, and history of tobacco use, there was significant HNSCC risk reduction with NSAID use (OR 0.25; 95% CI 0.09–0.67) and the risk reduction was associated with the frequency of NSAID use (Ahmadi et al., 2010).

Other studies have investigated the effects of different types of NSAIDs on HNSCC. In a study of data from the National Cancer Institute Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screen Trial, 151 head and neck cancer patients and 69,729 controls used aspirin (Wilson et al., 2013). Interestingly, significant reduction of head and neck cancer risk was associated with weekly and monthly aspirin use (adjusted hazard ratio (AHR) 0.69, 95% CI 0.51–0.93) but not daily aspirin use (AHR 0.85, 95% CI 0.65–1.11). Meanwhile, ibuprofen use was reported in 82 head and neck cancer patients and 41,946 controls, and there was no association with head and neck cancer risk (AHR 0.97, 95% CI 0.75–1.27). In another study, 232 daily users of COX inhibitors and 87 nonusers were identified among HNSCC patients from the cancer registry at the Ralph H. Johnson VA Medical Center (Gillespie et al., 2007). There were no significant differences between COX inhibitor users and nonusers in terms of recurrence (OR 0.94, 95% CI 0.42–2.10, $p=0.88$) or survival (OR 1.47, 95% CI 0.66–3.25, $p=0.34$). Kaplan-Meier curve analyses in this study also showed no significant differences in disease-free survival ($p=0.27$) or overall survival ($p=0.44$).

Macfarlane *et al.* also investigated the use of aspirin, COX-2 selective inhibitors, and other NSAIDs in a nested case-control study of UADT cancer (which included head and neck) patients using the Primary Care Clinical Informatics Unit (PCCIU) database (Macfarlane et al., 2014). In the study, 594 UADT cancer patients and 1768 controls used aspirin and aspirin use was associated with a non-significant reduction in UADT cancer risk (adjusted OR 0.90, 95% CI 0.78–1.04). Similar results were obtained when UADT cancer was stratified into head and neck cancer (adjusted OR 0.93, 95% CI 0.76–1.15) and esophageal cancer (adjusted OR 0.87, 95% CI 0.72–1.05). In the same study, the use of other NSAIDs, excluding COX-2 inhibitors, in 780 UADT cancer patients was associated with significant risk reduction for UADT cancer (adjusted OR 0.83, 95% CI 0.75–0.93) as well as head and neck cancer (adjusted OR 0.82, 95% CI 0.70–0.96). Data from these same patients were also used to investigate the effects of aspirin, COX-2 selective inhibitors, and other NSAIDs on survival (Macfarlane et al., 2015). For head and neck cancer patients, COX-2 selective inhibitor use did not improve survival but use of aspirin (AHR 0.56, 95% CI 0.44–0.71) and other NSAIDs (adjusted HR 0.74, 95% CI 0.60–0.90) did. In comparison, for esophageal cancer patients, use of aspirin (AHR 0.54, 95% CI 0.45–0.64), COX-2 selective inhibitors (AHR 0.78, 95% CI 0.62–0.98), and other NSAIDs (AHR 0.67, 95% CI 0.56–0.80) were all associated with improved survival.

While the above studies have shown that aspirin/NSAID use may reduce HNSCC risk and/or improve OS and DSS, other investigations have reported conflicting results. A study of the Danish population between 1991 and 2002 identified 75 NSAID users and 110 nonusers amongst oral cancer patients and failed to demonstrate an association between NSAID use and development of oral cancers (Friis et al., 2006). In another study of South Korean patients (n = 1392) with newly diagnosed HNSCC (between 2000 and 2013), Kim *et al.* defined 81 patients as low-dose aspirin users and 89 as other NSAID users (Kim et al., 2018). Even after adjusting for confounding factors in multivariate analyses, the results did not identify a significant association between use of aspirin, a different NSAID, or both aspirin and another NSAID with disease-free survival (DFS), recurrence, non-cancer survival (NCS), or OS.

Of note, the above studies did not stratify head and neck cancer into distinct subpopulations, which could impact the conclusions. For instance, the effects of aspirin and other NSAIDs may differ with HNSCC disease stage. Lumley *et al.* conducted a retrospective study of 584 veterans with HNSCC treated at the Washington DC Veterans Affairs Medical Center between 1995 and 2015 (Lumley et al., 2019). Among 329 patients included in the analysis, 84 had used aspirin for at least one year after HNSCC diagnosis. After a median follow-up of 40 (aspirin users) and 25 months (aspirin nonusers), aspirin users exhibited better OS (HR 0.53, 95% CI 0.38-0.75, $p < 0.001$) and DSS (HR 0.31, 95% CI 0.17-0.56, $p < 0.001$). These results may be limited by significant differences in the prevalence of stage I disease (34.5% and 13.1% for users and nonusers, respectively) and stage IV disease (28.6% and 55.5% for users and nonusers, respectively) between the aspirin and non-aspirin user groups (Lumley et al., 2019). (The study included T and N stage in multivariate regression analysis but not disease stage.) However, the authors also examined the effect of aspirin use among patients with different stages of disease. In early stage disease, aspirin remained a significant predictor of OS ($p = 0.013$) and DSS ($p = 0.006$); meanwhile, in advanced stage disease, aspirin use was significantly associated with improved DSS ($p = 0.041$), but only trended toward significance for OS ($p = 0.072$).

In addition, NSAID effects may depend on the genomic profile of HNSCC tumors, as has been shown in CRC. In a recently published study, Hedberg *et al.* analyzed aspirin use in HNSCC patients harboring either wild-type *PIK3CA* or *PIK3CA* altered by amplification or mutation. In patients with *PIK3CA* alterations they found that regular NSAID use was associated with a dramatically prolonged DSS (HR 0.23, $P = 0.0032$, 95% CI 0.09–0.62) and OS (HR 0.31, $P = 0.0043$, 95% CI 0.14–0.69) when compared to non-regular NSAID use (Hedberg et al., 2019). On the other hand, HNSCC patients whose tumors had unamplified, wild-type *PIK3CA* demonstrated no statistically significant change in DSS (HR 0.86, 95% CI 0.48–1.54) or OS (HR 0.98, 95% CI 0.60–1.62) with NSAID use. These results were subsequently validated in preclinical models. Specifically, mice harboring patient-derived xenografts (PDXs) endogenously expressing either wild-type *PIK3CA* or mutant *PIK3CA* were treated with saline or the NSAIDs sulindac, celecoxib, or aspirin, followed by assessment of tumor growth. NSAID treatment markedly inhibited the growth of the *PIK3CA*-mutated PDX tumors, but had no significant impact on the growth of PDX tumors harboring wild-type *PIK3CA*. Analogous results were seen in cell line models, where

NSAID treatment inhibited proliferation and induced apoptosis in *PIK3CA* mutant cell lines, but not cell lines with wild-type *PIK3CA* (Hedberg et al., 2019).

Plausible mechanism of NSAID-associated risk reduction in *PIK3CA*-altered cancers

While the mechanisms underlying NSAID-related risk reduction in CRC, breast cancer, and HNSCC remain to be fully characterized, recent studies suggest a role for inhibition of cyclooxygenase (COX) and prostaglandin (PG) production. Aspirin is known to inhibit the two isoforms of COX enzymes, COX-1 and COX-2 (Figure 1) (Chandrasekharan and Simmons, 2004). COX-1 is constitutively expressed in various tissues and plays an important role in tissue homeostasis. While normally absent in most cells, COX-2 expression can be stimulated by an inflammatory response and is known to be associated with tumorigenesis (Echizen et al., 2018; Hashemi Goradel et al., 2019) in CRC (Liu et al., 2017; Tougeron et al., 2014) and breast cancer (Chen and Holmes, 2017). The COX enzymes play a key role in the metabolism of membrane phospholipids, following cleavage of these lipids by phospholipase A₂ enzymes to release fatty acids, including arachidonic acid (Vasquez et al., 2018). Of note, COX-1 and -2 catalyze a rate-limiting step in biosynthesis of PGs from arachidonic acid (Figure 2) (Chandrasekharan and Simmons, 2004). These enzymes convert arachidonic acid to prostaglandin H₂ (PGH₂) and PGH₂ is further converted by various enzymes to other PGs that are involved in cellular proliferation, migration, and invasion (Figure 2) (Ulrich et al., 2006). Among these PGs, prostaglandin E₂ (PGE₂) plays an important role in the tumor growth-promoting effects of COX-2 (Hashemi Goradel et al., 2019; Karpisheh et al., 2019; Tougeron et al., 2014).

Chan *et al.* characterized COX-2 levels in 636 paraffin-embedded CRC specimens from the Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS). Among them, 423 (67%) exhibited moderate or strong COX-2 expression and regular aspirin use was associated with a significant risk reduction in the cases that overexpressed COX-2 (multivariate risk ratio 0.64, 95% CI 0.52-0.78) but not cases with weak or absent expression of COX-2 (Chan et al., 2007). In addition, aspirin use in CRC patients with COX-2 overexpression was associated with lower risks of cancer-specific mortality (multivariate-adjusted HR 0.39, 95% CI 0.20-0.76) and overall mortality (multivariate-adjusted HR 0.62, 95% CI 0.42-0.93). However, these risk reductions in mortality were not seen in CRC patients who used aspirin, but whose tumors were COX-2-negative (Triadafilopoulos, 2010). These results suggest that the beneficial effects of aspirin in CRC are associated with inhibition of overexpressed COX-2.

Interaction between the PI3K/AKT/mTOR pathway and COX-2 may also underlie the NSAID-associated risk reduction in CRC and HNSCC. Some studies suggest COX-2 may activate the PI3K pathway. For instance, the PI3K pathway is activated by PGE₂ in cells expressing PGE₂ receptor 4 (EP₄) (Fujino et al., 2002) and COX-2 activation was associated with AKT phosphorylation and poor survival in breast cancer (Glynn et al., 2010). In addition, COX-2 was found to be overexpressed in 60% of epithelial ovarian cancers and was significantly associated with activated AKT in ovarian cancer and CRC (Chang et al.,

2019; Uddin et al., 2010). In hepatocellular carcinoma, COX-2 was shown to also activate the PI3K pathway by suppressing PTEN (Chen et al., 2017). Conversely, other studies suggest the PI3K pathway may activate COX-2. For example, the PI3K pathway has been shown to activate COX-2 through induction of NF- κ B when PTEN is inhibited by miR - 221/222 (Li et al., 2017). Furthermore, when the PI3K pathway is inhibited by suppression of EGFR, COX-2 has been shown to be down-regulated through blockade of NF- κ B (Ramu et al., 2018).

In the setting of HNSCC, work by Hedberg *et al.* suggests that NSAID sensitivity in *PIK3CA*-mutated HNSCC is mediated via inhibition of COX-2-dependent production of PGE₂ (Hedberg et al., 2019). They found that mice harboring PDX tumors with mutant *PIK3CA* exhibited elevated levels of circulating PGE₂ relative to mice harboring PDX tumors with wild-type *PIK3CA*. Furthermore, treatment with celecoxib, sulindac, and aspirin resulted in dramatic reduction in PGE₂ levels in the mutant *PIK3CA* PDX group when compared to the wild-type group. Thus, taken together, the above data suggests a role for NSAIDs in *PIK3CA*-altered HNSCC through inhibition of COX-2 and thus lowering PGE₂ levels (Figure 1). NSAID-mediated reduction of PGE₂ levels in mice with *PIK3CA*-altered tumors may be critically important, since PGE₂ is a known immunosuppressive molecule. In future studies it will be valuable to investigate whether *PIK3CA*-mutant tumors are characterized by a more immunosuppressive immune profile, and whether NSAID treatment serves to reverse the immunosuppressive stature of the tumor microenvironment.

Conclusions

Several studies have shown that NSAIDs can provide a protective effect against the development of CRC, breast, and head and neck cancers. First shown in CRC and breast cancer, long term NSAID use has been shown to decrease the incidence and mortality of both colorectal and breast cancer. Further analyses have indicated that this effect was most prevalent in patients with a mutated *PIK3CA* gene. Mutation of the *PIK3CA* gene leads to constitutive activation of the PI3K/AKT/mTOR pathway, upregulation of COX-2 enzyme and enhanced production of PGE₂. PGE₂ is a known immunosuppressive molecule and its overproduction may lead to a more immunosuppressive tumor microenvironment in CRC and breast cancers exhibiting genetic alterations of *PIK3CA*. As NSAIDs can be used to inhibit COX-2 activity and reduce PGE₂ levels, further studies are needed to determine whether NSAIDs reverse immunosuppressive profiles in the subpopulation of CRC and breast cancer patients that harbor *PIK3CA*-altered tumors.

NSAID use in HNSCC has also shown potential benefit. Early epidemiological studies in HNSCC showed mixed results with some studies finding improved DSS and OS while other studies observed no differences. However, a recent retrospective study has determined that regular use of NSAIDs is associated with a dramatic improvement of DSS and OS in HNSCC. This positive benefit of NSAIDs was restricted to patients whose tumors exhibited *PIK3CA* alterations, and was not seen in patients with wild-type (*PIK3CA*) tumors. These findings support the need for prospective evaluation of NSAIDs in *PIK3CA*-altered HNSCC. If confirmation is obtained, NSAIDs therapy should be strongly considered as a strategy to

prevent HNSCC development in patients whose initial primary tumor contained *PIK3CA* alteration and who are at high risk for the development of second primary tumors.

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Figure 1. Signaling via the PI3K pathway and inhibition of PGE₂ production by NSAIDs. Activation of the PI3K/AKT pathway leads to stimulation mTORC1, as well as induction of COX enzymes. The induction of COX enzymes leads to production of immunosuppressive PGE₂. NSAIDs, including aspirin, can inhibit COX-2 enzyme and reduce production of PGE₂. PDK1, phosphoinositide-dependent kinase 1; PIP₂, phosphatidylinositol 4,5-bisphosphate; PIP₃, phosphatidylinositol 3,4,5-trisphosphate.

Figure 2:
COX-mediated production of PGE₂ from arachidonic acid.