Anti-Inflammatory Drugs Decrease the PD-L1 Expression and Increase the CD8$^+$ T-Cell Infiltration

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ABSTRACT

In this issue of *Cancer Prevention Research*, Cecil and colleagues show that nonsteroidal anti-inflammatory drugs (NSAID), celecoxib and naproxen, decrease the expression of programmed death-ligand 1 (PD-L1) and increase the influx of Type I tumor-infiltrating lymphocytes in colonic tumors. Importantly, both decrease of PD-L1 expression and increase of CD8$^+$ T cells were associated with the inhibition of COX-2/PGE_2 pathway *in vitro* and syngeneic colonic tumor xenograft models. This study clearly suggests that NSAIDs regulate the intratumoral immunity multiple ways, including suppression of expression of immune checkpoint blockade. Thus, NSAIDs should be considered as chemopreventive for patients with PD-L1–positive colonic polyp.

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Globally, colorectal cancer is a major health problem with more than 1.9 million new cases diagnosed and over 915,900 deaths worldwide every year (1). In the United States, deaths from colorectal cancer account for approximately 9% of all cancer-related deaths. Despite proven prevention strategies, colorectal cancer remains the second leading cause of cancer-related mortality in the United States and worldwide. In spite of effective targeted chemo- and immunotherapies, the 5-year survival rate of patients with stage IV colorectal cancer remains <10% and many patients still fail to respond to these novel therapies. Moreover, life-threatening toxicities and immune-related adverse events are often developed in patients treated with these therapies. In addition, targeted and immune therapies are very costly. Therefore, there is a need to better understand colonic tumor progression mechanisms and develop combinational regimens to prevent and treat colorectal cancer.

Tumors are infiltrated by a diverse group of immune and nonimmune cells, and immune evasion drives the progression of premalignant lesions to malignant tumors. Immune evasion mechanisms are complex and well studied. Compelling evidence from both animal tumor models and human cancers suggest that immune suppression in the tumor microenvironment (TME), mediated by CD4$^+$CD25$^+$FoxP3$^+$ regulatory T cells (Treg), myeloid-derived suppressor cells (MDSC), and activated macrophages have been identified as major factors in the tumor immune escape and tumor progression (2). In the TME, inflammatory mediators secreted by MDSCs and M2 macrophages drive the differentiation of CD4+ T cells into suppressive Tregs and functionally impaired dendritic cells with high indoleamine 2,3-Dioxygenase 1. In addition, the overexpression of immune inhibitory checkpoints (CTLA4, PD-1/PD-L1) override the capacity of the immune system to eradicate tumor cells (2). Although targeting immune inhibitory checkpoints through the blockade of programmed cell death protein 1/programmed death-ligand 1 (PD-1/PD-L1) and CTLA4 has led to unprecedented and durable responses in multiple cancer types, there are still many concerns as stated above.

Among the tumor inflammatory mediators, prostaglandin E_2 (PGE_2) plays the central role in the immune evasion. In the 1970s, it has been established that PGE_2 inhibits T-cell activation in part through the suppression of IL2 (3, 4). Thus, in the 1980s, IL2 was considered as a candidate for cancer immunotherapy and was approved by the FDA as treatment for a few cancers. However, no aggressive approaches had been taken to use PGE_2 inhibitors for cancer therapy. In this context, aspirin and other NSAIDs as inhibitors of COX-2/PGE_2 have consistently shown preventive and survival benefit effects on patients with different organ-site cancers, particularly colorectal cancer (5). A number of epidemiologic studies and randomized controlled trials have shown a clear reduction risk of colorectal cancer for individuals using aspirin/NSAIDs for 5 or more years (5). In addition, equivocal evidence from laboratory experiments show the dose-dependent chemopreventive potential of aspirin/other NSAIDs in animal models of colon cancer (6). Importantly, we and others have shown that COX-2 selective inhibitors, such as celecoxib, have superior tumor-inhibitory effects as compared with COX-1–selective NSAIDs in animal models (6). This relative effectiveness is understandable given the high PGE_2 levels predominantly derived from COX-2 overexpression in colonic tumors. In addition, NSAID-induced colon tumor inhibitory effects are not only limited to the reduction of PGE_2 levels. This depends on

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the status of metabolic enzyme 15-PGDH levels, EP2/4 receptor expression, and the mutation status of PIK3CA, BRAFV600, etc. based on the evidence from prospective cohort trials (5, 6).

Over the past five decades, studies on aspirin/NSAIDs support their primary mechanisms of anti-inflammatory action as an inhibition of PGE_2. However, many studies support other possible mechanisms in this context to tumor inhibition. In this issue, Cecil and colleagues (7), it is reported that COX-2 inhibitors decrease expression of PD-L1 in colon tumors and increase the influx of Type I infiltrating lymphocytes as an additional mechanism for naproxen and celecoxib. In this study, authors show that dietary administration of COX-2 selective inhibitor, celecoxib and nonselective inhibitor naproxen inhibited intestinal polyp growth in APC mice and tumor inhibition is significantly associated with decreased PD-L1 expression with influx of $CD8^+$ T cells. To understand the mechanisms by which NSAIDs decrease the PD-L1 expression and whether it is associated with COX-2 or COX-1 and/or with PGE_2 levels, the author used siRNA knockdown of COX-1 or COX-2 and measured PGE_2 levels *in vitro* and *in vivo* models of colonic tumor xenografts. Overall, the author's experiments support that COX-2 expression is positively correlated with PD-L1 expression. Furthermore, naproxen treatment resulted in a significant reduction in MC38 growth (syngeneic colon tumor xenograft) as compared with control with an associated increase of Tbet+ CD4 and CD8 tumor-infiltrating T cells (TIL), a concurrent decrease of PD-L1 expression in tumors and a significant reduction in Tregs. In summary, the author's data demonstrates that COX-2 inhibitors significantly decrease PD-L1 expression in colonic lesions and favorably impact the phenotype of tumor-infiltrating lymphocytes to control tumor growth.

The above results have significant implications for colorectal cancer prevention and treatment. In support, recent studies suggest that PD-L1 is overexpressed in colonic adenomas of APC^{-min} mice, and in AOM/DSS-induced colonic adenoma and carcinoma (8). Importantly, human colonic adenomas (tumor cells, >92%, and tumor-infiltrating cells, 100%) show a significant overexpression of PD-L1, even when compared with lymph node and liver metastases from patients with colorectal cancer (8). Thus, targeting PD-L1 is a valid approach for colorectal cancer prevention and treatment. Optimized direct targeting of PD-L1 by immunoprevention strategy is considered to be superior. To date, no significant efforts have been made to apply PD-L1 mABs in human clinical trials for the immunoprevention of colorectal cancer, mainly due to three major challenges: (i) lack of critical evidence on the effectiveness of PD-L1 mAbs in colon tumor models; (ii) repeated dosing with mAbs may cause serious side effects in immunoprevention trials, which require long-term use; and (iii) current manufacturing processes and patient delivery result in a high cost for mAbs (8). Thus, there is a pressing need to develop more effective PD-L1 inhibitors without side effects and with low manufacturing costs for use in cancer immunoprevention. NSAIDs alone or a combinational regimen of chemopreventive drug treatments will benefit high-risk cohorts such FAP and HNPCC with PD-L1–positive polyp patients for colorectal cancer prevention.

In the past decade, a number of clinical studies have shown that a relationship between the use of aspirin or other NSAIDs on the incidence and survival outcomes of patients with colorectal cancer is associated with expression levels of PD-L1 and the number of tumor-infiltrating lymphocytes (5). In addition, some studies support that the concomitant use of NSAIDs along with immune checkpoint inhibitors have more beneficial outcomes of therapy (9). Recently, this notion is fully supported by Pelly and colleagues (9), who showed that the inhibition of PGE_2 synthesis by COX-2 inhibitor, celecoxib, synergizes with immune checkpoint blockade therapy by triggering a potent intratumoral IFNγ response in mouse models and in fresh surgical human tumor explants. In all these experiments, COX-2/PGE_2 levels were positively associated with tumor PD-L1/PD-1 expression and provided a solid basis to develop clinical strategies for cancer prevention and treatment. However, the exact mechanisms that led COX-2/PGE_2 inhibition to PD-L1/PD-1 needs further investigation. Furthermore, recent clinical observational studies suggest that commonly used medications such as statins, in addition to aspirin/other NSAIDs, may also have immune checkpoint blockade therapy outcomes (10).

In summary, the usefulness of COX-2/PGE_2 inhibitors to overcome the immune evasion in TME and improve PD-L1 therapies is well supported. The use of NSAIDs for colon cancer prevention is not new. In 2015, low-dose aspirin use was approved by the United States Preventive Services Task Force for colorectal cancer prevention. Also, celecoxib was approved for FAP patients but was later withdrawn due to increased association with cardiovascular side effects. Similarly, the chronic use of aspirin and naproxen is associated with GI toxicities and individuals >65 years of age have no survival benefit due to increased hemorrhages and stroke. Thus, a caution should be taken on individual risk–benefit ratios of NSAIDs use and/or alternative approaches should be taken for the safer use of existing NSAIDs for colorectal cancer prevention. Developing novel NSAIDs without side effects based on the alternative mechanistic pathways to inhibit the PGE_2 in the TME is urgent.

Authors' Disclosures

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