Published in final edited form as:
Cancer Prev Res (Phila). 2010 November ; 3(11): 1379–1381. doi:10.1158/1940-6207.CAPR-10-0251.

Breaking the NF-κB and STAT3 Alliance Inhibits Inflammation and Pancreatic Tumorigenesis

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Abstract

This perspective on Liby et al. (beginning on page XXX in this issue of the journal) discusses the importance of the finding that two synthetic triterpenoids prolonged survival in a pancreatic cancer mouse model. This finding is significant because pancreatic cancer is one of the deadliest human cancers. These compounds inhibited the interaction between nuclear factor-kappa B and signal transducer and activator of transcription 3, and determining the mechanisms underlying this inhibition will help to rapidly move these compounds into phase-I clinical trials.

Despite many recent advances in treatment and surgery, pancreatic cancer has one of the worst prognoses of all cancers. Only 20% of patients have localized, potentially curable tumors at the initial diagnosis (1), and diagnosis at an advanced stage, as often occurs, makes pancreatic cancer difficult to treat. This disease has a complex etiology that involves both environmental and genetic factors. Although cigarette smoking has been linked to at least 25% of cases, recent studies reveal that obesity and type II diabetes are two major modifiable risk factors for this highly lethal disease (2). A better understanding of the mechanistic effects of obesity and diabetes on the pancreas would pave the way for new strategies for prevention or therapy of pancreatic cancer (2).

Over the past decade, at least a dozen molecular pathways implicated in pancreatic carcinogenesis have been unraveled. Moreover, global gene-expression profiling and the use of microarray databases have facilitated the identification of hundreds of genes that are differentially expressed in pancreatic cancer (3). Validation of these genes as biomarkers for early diagnosis, prognosis, or treatment efficacy, however, is still incomplete. Although several studies indicated the plausible contribution of some genetic factors to the development and progression of pancreatic cancer, common genetic variants associated with this disease remain poorly understood. A Japanese genome-wide association study (GWAS) in 991 cases of invasive pancreatic ductal adenocarcinoma and 5,209 controls identified single-nucleotide polymorphisms (SNPs) present in the three chromosomal loci 6p25.3, 12p11.21, and 7q36.2 that were significantly associated with increased risk of pancreatic cancer (4).

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Disclosure of Potential Conflicts of Interest No potential conflicts of interest were disclosed.

The relatively low survival rate of patients with pancreatic cancer is primarily due to a late diagnosis and the absence of effective treatments. Standard current pancreatic-cancer therapies, such as gemcitabine or erlotinib, are not very effective, emphasizing the need for novel chemopreventive, as well as better therapeutic strategies, for this disease. Synthetic and naturally occurring substances have been evaluated in cell culture and *in vivo* animal models for their pancreatic-cancer chemopreventive potential (5). Some chemopreventive agents, such as curcumin or resveratrol, were reported to sensitize pancreatic-cancer cells to standard chemotherapeutic drugs (e.g., gemcitabine or erlotinib). However, only a few clinical trials of these agents have been completed or initiated in this setting, and more are needed. Pancreatic cancer risk increases with age, but genetic and environmental factors also can increase the risk. Premalignant epithelial lesions of the pancreas have been used for screening. Development of chemopreventive agents is particularly needed for individuals with the aforementioned risk factors and for patients with premalignant pancreatic lesions (5).

Inflammation is implicated in the majority of human malignancies, including pancreatic cancer (6–9), and chronic inflammation is estimated to contribute to about 15% to 20% of all human cancers. Pro-inflammatory enzymes, such as cyclooxygenase-2 and inducible nitric oxide synthase, and cytokines, including tumor necrosis factor-alpha (TNF-α), are overexpressed and/or overproduced in inflammation-associated carcinogenesis. The expression of these pro-inflammatory proteins is regulated primarily by the transcription factor nuclear factor-kappa B (NF-κB). Because NF-κB is highly active in both inflammatory cells, such as macrophages, and in cells found in inflamed tissues, it is recognized as a key mediator of inflammation (10). Moreover, constitutive activation of this redox-sensitive transcription factor is frequently observed in many human tumor specimens and is associated with a poor prognosis. Cells having abnormally elevated NF-κB activity are more resistant to drug and radiation therapies. A high level of NF-κB contributes to a cell's impaired ability to undergo apoptosis, which would eliminate defective or damaged cells. NF-κB normally is sequestered in the cytoplasm in an inactive complex with the inhibitor of NF-κB alpha (IκBα). Phosphorylation and subsequent ubiquitination of IκBα render this inhibitory protein inactive through proteasome-mediated degradation and thereby release NF-κB for translocation into the nucleus. The key enzyme that is involved in IκBα phosphorylation is IκB kinase (IKK), especially IKKβ (11,12).

In addition to NF-κB, signal transducer and activator of transcription 3 (STAT3) is recognized as an important mediator of inflammation associated with tumor promotion. Persistently activated STAT3 stimulates proliferation, survival, and invasion of tumor cells and suppresses antitumor immune responses (13,14). Recent attention has focused on the interplay or crosstalk between NF-κB and STAT3 in controlling the crosstalk or physiologic interactions of malignant cells with the tumor microenvironment, especially with inflammation and immune cells that infiltrate tumors (15). Thus, the IKK/NF-κB and STAT3 pathways seem to be central signaling hubs in inflammation-mediated tumor promotion and progression (16). Furthermore, maintenance of constitutively elevated NF-κB activity requires STAT3, which is also frequently activated in cancer. STAT3 prolongs retention of NF-κB in the nucleus, which occurs through p300-mediated acetylation of RelA/p65 (17). The interplay between NF-κB and STAT3, however, does not appear to be unidirectional. Therefore, NF-κB might also control the activation of STAT3, specifically in intestinal epithelial cells. This control can be achieved by recruiting bystander cells (i.e., myeloid cells) that secrete STAT3-activating cytokines such as interleukin-6 (IL-6) and TNF-α or by inducing the transcription of genes that encode these pro-inflammatory cytokines (16).

The suggestion has been made that IL-6 released by either myeloid cells or T lymphocytes would promote epithelial-cell proliferation through STAT3 activation. In support of this speculation, deletion of the gene encoding IL-6 and the intestinal epithelial–cell restricted deletion of STAT3 both suppressed the development of colitis-associated cancer (ref. (16) and

refs. therein). In this context, the inflammation microenvironment is as important as the tumor-cell population, even in the formation of tumors that are not caused by chronic inflammation (18). This idea led to the suggestion that the tumor microenvironment is the seventh hallmark of cancer (accompanying growth signal autonomy; not paying attention to the stop signs; evasion of apoptosis; angiogenesis; unlimited replicative potential; and invasion and metastasis; ref. (19). Whereas the activation of NF-κB is controlled by STAT3, NF-κB upregulates expression of IL-6 and other pro-inflammatory cytokines, which can act in a paracrine manner on initiated or premalignant cells and promote neoplastic transformation. Besides playing a role in the promotion stage of tumorigenesis, these inflammatory circuits also affect the progression of cancer (16). The expression of proteins responsible for invasion (matrix metalloproteinase-2 and −9), cell adhesion (E-cadherin), and angiogenesis (vascular endothelial growth factor and hypoxia-inducible factor-1α) is controlled by NF-κB and STAT3. Although NF-κB and STAT3 are an Achilles' heel of tumors, they also serve as essential master regulators of many critical signal transduction pathways in normal cells. Therefore, new preventive or therapeutic intervention strategies should consider specific delivery of inhibitors targeting these transcription factors in, or in the vicinity of, the tumor or premalignant tissue and also focus on the identification of downstream molecules whose levels are abnormally altered in cancer or premalignancy compared with surrounding normal tissue.

Chronic pancreatitis accounts for part of the overall pancreatic-cancer picture, supporting inflammation as a valuable target for preventing and treating pancreatic cancer. Even non-infectious agents, such as cigarette smoke, heavy alcohol consumption, obesity, and diabetes, can cause inflammation and represent risk factors for pancreatic and certain other human cancers (2). An attractive anti-inflammatory preventive and/or treatment approach would be to target, or inhibit IKK/NF-κB and STAT3, which might cooperate to promote the development and progression of pancreatic cancer, as has been observed for colon and liver tumors. In this respect, inhibition of IKK/NF-κB and STAT3 might represent a promising approach to combat cancer including pancreatic cancer. Two promising such inhibitors are the synthetic oleanane triterpenoids 2-cyano-3,12-dioxoolen-1,9-dien-28-oic acid (CDDO) methyl ester (CDDO-ME) and CDDO ethyl amide (CDDO-EA). These agents inhibited activation of STAT3 and NF-κB by suppressing both phosphorylation of STAT3 and degradation of the NF-κB inhibitory protein IκBα (5). CDDO-ME and CDDO-EA are Michael reaction acceptors and therefore can react with cellular nucleophiles, such as the sulfhydryl (-SH) groups of cysteines on target proteins.

In this issue of the journal, Liby, Sporn, and colleagues report that CDDO-ME or CDDO-EA significantly increased survival in the KPC (*K-ras, p53,* Cre recombinase) transgenic mouse model of pancreatic cancer (20). This mouse harbors a point mutation in both the *K-ras* oncogene and *p53* tumor suppressor gene under the control of the pancreas-specific promoter Pdx-1. Because this triplet transgenic mouse model mimics both the genetic and histologic changes observed in human pancreatic cancer, it is considered to be one of the most-relevant animal models for preclinical evaluation of new drugs for preventing and treating human pancreatic cancer. By using biotinylated CDDO-ME and CDDO-EA, Liby et al. (20) nicely demonstrated the direct binding of these synthetic triterpenoids to IKK and STAT3. This binding might explain the subsequent inactivation of NF-κB and STAT3 in several different cell lines derived from solid pancreatic carcinoma or abdominal ascites in KPC mice (20). Furthermore, both compounds inhibited the constitutive secretion of IL-6, a pro-inflammatory cytokine that plays an essential role in linking STAT3 and NF-κB signaling.

CDDO-ME and CDDO-EA are highly effective for preventing and/or treating cancer in some animal models (5,21,22) including KPC transgenic mice, which recapitulate the genetic mutations, clinical symptoms, and histopathology found in human pancreatic cancer. KPC transgenic mice fed a diet containing each triterpenoid exhibited a significantly prolonged

lifespan (20). A salient finding of this study is that the aforementioned synthetic triterpenoids are well-tolerated at the doses used, without causing any weight-loss during the experiment. We look forward to seeing whether synthetic triterpenoids such as CDDO-ME and CDDO-EA as single agents or combined with each other or with other chemopreventive agents such as tea polyphenols, to inhibit NF-κB (23) or combined with retinoic acid to inhibit STAT3 (24) can perform well in the clinic without any serious side effects. We also look forward to future investigation of the mechanisms underlying this approach's potential clinical effects, especially of molecular effects on the unholy alliance between NF-κB and STAT3. Considering the lethality of pancreatic cancer and the ability of CDDO-ME and CDDO-EA to prolong survival in a pancreatic-cancer mouse model, phase-I clinical testing would be a logical choice in developing these agents for pancreatic-cancer prevention.

Acknowledgments

This work was supported by National Institutes of Health grants CA11536, CA077646 and CA120388 (Z. Dong) World Class University project grant (R31-008-000-10103-0) from the Ministry of Education, Science and Technology, Republic of Korea (Y. J. Surh and Z. Dong).

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