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Inflammation in Lung Carcinogenesis: New Targets for Lung Cancer Chemoprevention and Treatment

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Abstract

Lung carcinogenesis is a complex process involving the acquisition of genetic mutations that confer cancer development and the malignant phenotype, and is critically linked to apoptosis resistance, unregulated proliferation, invasion, metastasis, and angiogenesis. Epithelial mesenchymal transition in cancer is an unregulated process in a host environment with deregulated inflammatory response that impairs cell-mediated immunity and permits cancer progression. Given the immunosuppressive

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tumor environment, strategies to reverse these events by stimulating host immune responses are an important area of investigation. COX-2 and its downstream signaling pathways are potential targets for lung cancer chemoprevention and therapy. Clinical trials are underway to evaluate COX-2 inhibitors as adjuvants to chemotherapy in patients with lung cancer and to determine efficacy in prevention of bronchogenic carcinoma. The understanding of molecular mechanisms involved in inflammation and lung carcinogenesis provide insight for new drug development that target reversible, non-mutational events in the chemoprevention and treatment of lung cancer.

Keywords

inflammation; lung cancer; cyclooxygenase 2; COX-2; NSCLC; chemoprevention; targeted therapy; bronchogenic carcinoma

1. Introduction

Lung cancer is the leading cause of cancer-related mortality in the United States and is responsible for more deaths than prostate, colon, and breast cancers combined [1]. The overall 5-year survival rate is less than 15% for patients with lung cancer, which has remained largely unchanged for the last three decades. Understanding the molecular mechanisms involved in the pathogenesis of lung cancer can provide opportunities to develop innovative therapies for non-small cell lung cancer (NSCLC). The acquisition of genetic mutations facilitates cancer development and malignant phenotype. These mutations are critically linked to acquiring cellular properties associated with apoptosis resistance, unregulated proliferation, invasion, metastasis, and angiogenesis. Inflammation has been postulated to play a key role in lung carcinogenesis. There is a growing body of evidence to suggest that smoking induced pulmonary inflammation increases lung cancer development in smokers [2,3]. In addition, the regular use of aspirin and other non-steroidal anti-inflammatory drugs is associated with reduced risk of developing lung cancer in animal models and in smokers [3,4]. Cyclooxygenase 2 (COX-2) has been implicated in apoptosis resistance, angiogenesis, decreased host immunity, and enhanced invasion and metastasis, and thus has a critical involvement in carcinogenesis. COX-2 is one of the novel targets being studied for lung cancer therapy and chemoprevention [5,6].

2. COX-2

Cyclooxygenase (also referred to as prostaglandin endoperoxidase or prostaglandin G hydroperoxide synthase) is the ratelimiting enzyme for the production of eicosanoids prostaglandins (PGs) and thromboxanes (TX) from free arachidonic acid, which is released from the membrane phospholipids by phospholipase A2 [7]. Cyclooxygenase is bound to the cytosolic side of the endoplasmic reticulum and cell membrane [8]. It is a bifunctional enzyme, with fatty acid cyclooxygenase (COX) activity producing PGG2 from arachidonic acid and two O_2 molecules and PG hydroperoxidase (HOX) activity in which PGG2 undergoes a two-electron reduction to PGH2 [9,10]. PGH2 is converted to final products by isomerases and individual prostaglandin (PG) synthases that are often expressed in a cell type-dependent manner. Three forms of COX have now been described [11–13]. COX-1 is constitutively expressed in most cells and tissues; its activity appears to depend entirely on substrate availability. Alternatively, an inducible isoenzyme, COX-2, acts as an immediately early gene expressed in response to cytokines, growth factors, and other stimuli. All COX isoforms share the same structural features including a hydrophobic channel that allows the arachidonic acid bearing a constrained hairpin configuration to access the COX catalytic site [9,10].

Thromboxanes and prostacyclins are short-lived molecules with the half-lives on the order of seconds, whereas prostaglandins (PGs) have half-lives within the range of tens of minutes to hours [14,15]. Interacting with their cell surface G protein (heterotrimeric GTP-binding protein)-coupled receptors (GPCR), PGs serve as autocrine and paracrine mediators of "housekeeping" functions, including the regulation of renal water and sodium metabolism, stomach acid secretion, parturition, and homeostasis. It has been shown that in certain experimental settings some PGs, especially PGJ2, are able to bind nuclear receptors such as PPAR-gamma [16]. At least 9 PG receptors have been identified to date, four of which bind PGE2 and two bind PGD2. There are individual receptors for PGF2-alfa, PGI2 and TxA2 [8]. Among other PGs, PGE2 is a major COX-2 metabolite abundantly present in the cancer microenvironment, and it is an important mediator of immune regulation [17], epithelial cell growth and invasion [18] as well as epithelial survival [19].

3. COX-2 and Lung Cancer

Several studies have demonstrated high-level constitutive COX-2 expression in human NSCLC [20–29]. In the initial report describing COX-2 in human lung cancer, Huang et al. assessed COX-2 expression in NSCLC and normal adjacent lung tissue of resected specimens by immunohistochemistry [20]. All of the 15 tumor specimens (8 adenocarcinomas and 7 squamous cell carcinomas) showed cytoplasmic staining for COX-2 in tumor cells. In contrast, adjacent normal lung showed no COX-2 staining in the alveolar lining epithelium, but demonstrated positive cytoplasmic staining often in alveolar macrophages and occasionally in bronchiolar epithelium. Wolff et al. showed with immunohistochemistry that COX-2 was expressed in 19 of 21 adenocarcinomas and in all 11 squamous cell carcinomas studied [28]. Hida et al. reported that COX-2 overexpression was seen in approximately 70% of lung adenocarcinomas [21]. The level of staining appeared to be less in squamous cell carcinomas than in the adenocarcinomas. Hida et al. reported that COX-2 expression was documented in one-third of atypical adenomatous hyperplasias and carcinoma *in situ* which supports the role of COX-2 throughout the progression from pre-malignant lesion to the metastatic phenotype [21]. In addition, the same study demonstrated a greater proportion of lung cancer cells staining positively in lymph node metastases compared to the corresponding primary tumor [21]. In the report from Tsubochi and colleagues, there was a significant association between COX-2 expression and lymph node metastasis in patients with adenocarcinomas, but evaluation of squamous cell carcinomas did not demonstrate this relationship [22].

Other studies have corroborated and expanded on these initial findings further documenting the importance of COX-2 in lung cancer [23–27]. Khuri et al. evaluated COX-2 expression in specimens from 160 stage I NSCLC patients by *in situ* hybridization and reported that COX-2 overexpression appears to portend a shorter survival among patients with early stage NSCLC [27]. The strength of COX-2 expression was associated with both a decreased overall survival rate ($p = 0.001$) and a diminished disease-free survival rate ($p = 0.022$) [27]. Tsubochi et al. showed the relationship between COX-2 expression and poor prognosis in stage I adenocarcinomas [22]. Other reports have associated tumor COX-2 overexpression with poor prognosis as well independent of TNM stage in surgically resected NSCLC [27]. These reports, together with other studies documenting an increase in COX-2 expression in precursor lesions [28,29], a common polymorphism in the COX-2 gene associated with increased risk of lung cancer [30], and epidemiological studies that indicate a decreased incidence of lung cancer in patients who regularly take aspirin [31], all support the involvement of COX-2 in the pathogenesis of lung cancer.

Mounting evidence indicates that tumor COX-2 activity has a multi-faceted role in conferring the malignant and metastatic phenotype of lung cancer. Although multiple genetic alterations are necessary for lung cancer invasion and metastasis, COX-2 may be a central element in

orchestrating this process, [21–23,32] and has been implicated in apoptosis resistance [19, 33], angiogenesis [34,35], decreased host immunity [36,37], and enhanced invasion and metastasis [38,39]. These newly discovered molecular mechanisms in the pathogenesis of lung cancer provide novel opportunities for targeted therapies in NSCLC carcinogenesis [40,41]. COX-2 is one of the targets under investigation for lung cancer therapy and chemoprevention [5,6].

4. COX-2 Down-Stream Signaling: Prostanoid Receptors

The prostanoid receptors are in the superfamily of G-protein coupled receptors (GPCR). PGE2 exerts its multiple effects through four GPCR designated as EP1, EP2, EP3 and EP4 [11]. Studies of the receptor subtypes have shown that the EP1 receptor acts via G_q protein and upon activation increases cellular Ca^{2+} level. Studies indicate EP1 receptors can be localized not only on the cell membrane but also on the nuclear membrane [42]. The EP2 and EP4 receptor signaling is mediated by G_s G-proteins and leads to activation of adenylate cyclase and elevated cAMP synthesis. In contrast, EP3 signaling trough G_i inhibits adenylate cyclase and cAMP synthesis [43].

The EP_4 receptor is critically involved in inducing the expression of COX-2 and PGE_2 synthase [44]. We have previously demonstrated the importance of PGE2 and its signaling through the EP4 receptor in mediating NSCLC invasiveness, and shown that genetic inhibition of tumor COX-2 led to diminished matrix metalloproteinase (MMP)-2, CD44, and EP4 receptor expression and invasion [38]. These findings indicate that PGE2 regulates COX-2-dependent, CD44- and MMP-2-mediated invasion in NSCLC via EP receptor signaling [38]. Yang and colleagues revealed in a murine model that tumor metastasis to the lung was significantly reduced when treated with a specific EP4 antagonist or when EP4 receptor expression was knocked down in the tumor cells using RNA interference technology [45]. In addition, the host EP4 receptors contribute to tumor metastasis and tumor growth with decreased metastasis and tumor growth in EP4 receptor knockout animals [45]. Further evidence supporting the role of prostanoid receptors in lung carcinogenesis was shown by the fibronectin mediated stimulation of human lung carcinoma cell proliferation through the PGE_2 receptor subtype EP4 [46]. Thus, blocking the COX-2 dependent PGE2 production or activity by targeting the downstream signaling pathway of COX-2, such as EP4 receptor, may produce more profound anti-cancer effects than COX-2 inhibition alone. This could be the basis for new approaches in chemoprevention or treatment of NSCLC.

5. Complicity of Host Cellular Networks in Lung Tumorigenesis

The pulmonary environment presents a unique milieu in which lung carcinogenesis proceeds in complicity with the host cellular network. The pulmonary diseases that are associated with the greatest risk for lung cancer are characterized by abundant and deregulated inflammation [47–49]. Pulmonary disorders such as chronic obstructive pulmonary disease (COPD)/ emphysema and pulmonary fibrosis are characterized by profound abnormalities in inflammatory–fibrotic pathways [50–52]. For example, among the cytokines, growth factors, and mediators released in these lung diseases and the developing tumor microenvironment, IL-1β, PGE2, and TGF-β have been found to have deleterious properties that simultaneously pave the way for both epithelial mesenchymal transition (EMT) and destruction of specific host cell mediated immune (CMI) responses against tumor antigens [53–56]. EMT is the developmental shift from a polarized, epithelial phenotype to a highly motile mesenchymal phenotype [57]. While this process is essential in embryogenesis and organ development, EMT is also critically involved in much adult pathology, including cancer, chronic inflammation, and fibrosis [57,58]. Although EMT is a tightly regulated process during embryonic development [59], in cancer progression, EMT is unregulated with selective elements of the

process amplified and other aspects circumvented [60]. Thus lung cancer develops in a host environment in which the deregulated inflammatory response both degrades CMI and promotes tumor progression. Investigators have attempted to reverse these events by stimulating host immune responses against tumor antigens in lung cancer.

Polymorphisms of genes coding for inflammatory pathway signaling molecules may be involved in promoting lung cancer development. Engels et al. systematically assessed lung cancer risk in relation to a large number of candidate polymorphisms in inflammation-related genes [61]. The study revealed an association of increased lung cancer risk with specific polymorphisms of IL-1A and IL-1B genes, especially among older subjects and those with a history of heavy smoking [61]. These cytokines are secreted by macrophages in response to tissue injury or infection, and are known to be critical mediators in the initiation of inflammation [61]. These findings further support the role of a deregulated inflammatory response to an environmental exposure, such as tobacco smoke, in lung carcinogenesis.

6. Reversal of Epithelial Mesenchymal Transition

EMT requires alterations in the cell morphology, adhesion, and migration [58]. These cellular changes result in variable expression of proteins which define EMT markers. Decreased e-cadherin level is a hallmark feature of EMT, which allows reduction in cell to cell adhesion and enhances migratory capacity [58]. We have previously shown a COX-2 dependent transcriptional regulation of e-cadherin expression and cellular agregation in NSCLC, and a reciprocal relationship between COX-2 and e-cadherin, as well as, ZEB1 and e-cadherin [54]. COX-2 and PGE2 expression resulted in significant reduction in e-cadherin via a ZEB1 and SNAIL transcriptional factor mediated mechanism, and inhibition of COX-2 resulted in rescue of e-cadherin expression [54]. Thus, therapies targeting the COX pathway may diminish the propensity for tumor metastasis in NSCLC by blocking the PGE_2-mediated induction of e-cadherin transcriptional repressors. This newly defined pathway for transcriptional regulation of e-cadherin in NSCLC has important implications for chemoprevention and treatment of NSCLC using COX-2 inhibitors in combination with other agents. For example, e-cadherin expression in NSCLC has recently been implicated as a marker of sensitivity to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKI) [62]. Thus, COX-2 inhibitors enhance tumor e-cadherin expression, and may therefore augment sensitivity to EGFR TKI therapy [63].

Histone deacetylase (HDAC) inhibitors may be another strategy to increase e-cadherin and overcome EGFR inhibitor resistance in patients with lung cancer. Transcriptional repressor, ZEB1, inhibits e-cadherin expression by recruiting HDAC. Witta et. al. have shown that e-cadherin transfection into a gefitinib-resistant line increased its sensitivity to gefitinib, and pretreating resistant cell lines with an HDAC inhibitor, induced e-cadherin and EGFR [64]. This resulted in enhanced growth-inhibition and apoptosis effect of gefitinib similar to that in gefitinib-sensitive NSCLC cell lines [64]. Thus, combined HDAC inhibitor and gefitinib treatment may represent a potential strategy to overcome resistance to EGFR TKI.

Bone morphogenetic protein-7 (BMP-7), also known as osteogenic protein-1, is a member of the transforming growth factor-β (TGF-β) superfamily [65–67]. It is expressed during embryonic development and plays an important role in organogenesis [65,66]. BMP-7 production is highest in the kidney, and its genetic deletion in murine studies revealed severe impairment in eye, skeletal, and kidney development [67]. In the embryonic lung, BMP-5 and BMP-7 expression has been detected in the mesenchyme and endoderm, respectively, and BMP-4 expression has been restricted to the distal epithelial cells and the adjacent mesenchyme [68]. TGF-β is a major regulator and inducer of EMT [58]. Zeisberg et. al. have reported that BMP-7 reverses the TGF-β1 induced EMT by re-induction of e-cadherin through a Smad

dependent mechanism in renal tubular epithelial cells and mammary ductal epithelial cells [69]. In addition, administration of BMP-7 led to repair of severely damaged renal tubular epithelial cells and reversal of chronic renal injury [69]. These results provide evidence of the complex interaction between BMP-7 and TGF-β1 in the regulation of EMT, and imply a potential role of BMP-7 as a therapeutic target in reversing EMT in carcinogenesis.

7. Immunosuppression

It was originally hypothesized more than thirty years ago that specialized T cell subpopulations existed that functioned to suppress immune responses [70]. North and others pursued this avenue of investigation within the context of tumor immunity [71,72]. However, these early studies in the field of suppressor T cells were stymied by an inability to characterize the cellular and molecular mechanisms responsible for the observed suppressive phenomena. There has been a renewed interest in the study of T cell mediated suppression of immunity that has been accompanied by the identification regulatory T cells. Although a variety of T regulatory cells have been described [73], much attention has focused on the specific activities of those that have been referred to as "naturally occurring" $CD4+CD25^{high}$ T regulatory cells [74,75], and hereafter refer to these as CD4+CD25+ T reg cells. Although investigators had pursued this topic for many years, the ground-breaking studies of Sakaguchi et al. [76] have been viewed as initiating a renaissance in T reg cell research; these, as well as more recent results have led to the characterization of the CD4+CD25+ T cell population as "professional suppressor cells" [75]. These studies revealed that transfer of CD25-depleted CD4 cells to nude mice recipients resulted in the spontaneous development of autoimmune disease [76]. Reconstitution of CD4+CD25+ cells within a limited period after transfer of CD4+CD25− cells prevented the autoimmune disease in a dose-dependent fashion. These initial studies indicated that CD4 +CD25+ cells contribute to the maintenance of self-tolerance by down-regulating immune response to self and non-self antigens; elimination or reduction of CD4+CD25+ cells ablated this general suppression, and thereby not only enhanced immune responses to non-self antigens, but also elicited autoimmune responses to certain self-antigens [76]. Subsequent studies have revealed that these cells are both hyporesponsive and suppressive and can act through an APC independent pathway [76–79]. The CD4+CD25+ cells were found to require TCR-dependent activation for induction of suppressor activity [77]. The thymic origin of CD4 +CD25+ T reg cells has been documented [80,81]. As originally hypothesized by Shevach [82] and subsequently demonstrated by Jordan et al. [83], the derivation of T reg cells in the thymus appears to occur through a process referred to as "altered negative selection." More recently it has been appreciated that T reg cells can differentiate from activated human PBL CD4+CD25− cells in the periphery [84,85]. Although many aspects of this peripheral T reg cell differentiation pathway have not yet been defined, it may be pivotal in limiting immune responses to human cancer.

The active immune suppression induced by the tumor has been well documented in lung cancer and other malignancies [86]. Tumor-reactive T cells have been shown to accumulate in lung cancer tissues but fail to respond [87,88]. In fact, a high proportion of NSCLC tumor-infiltrating lymphocytes (TIL) are $CD4+CD25^{high}$ T regulatory (T reg) cells [89]. Tumor cells may contribute to promoting immune suppression by directing surrounding inflammatory cells to release suppressive cytokines in the tumor milieu, augmenting the trafficking of suppressor cells to the tumor site, and/or promoting differentiation of effector lymphocytes to a T reg cell phenotype [32,90]. Liu et al. recently demonstrated that tumor cells could directly convert CD4 +CD25− T cells to Treg cells through the production of high levels of TGF-β, suggesting a possible mechanism through which tumor cells evade the immune system [91].

One major impediment to effective therapy is our inadequate understanding of how lung cancer cells escape immune surveillance and inhibit anti-tumor immunity [92]. In previous studies an

immune suppressive network in NSCLC that is due to over-expression of tumor COX-2 has been defined. COX-2 isoenzyme activity is significantly increased in cancerous tissues compared to their normal counterparts in several malignancies and studies document this over-expression in human lung cancer [93]. In murine lung cancer models specific genetic or pharmacological inhibition of COX-2 *in vivo* led to significant tumor regression [36]. Although COX-2 metabolites have been identified as mediators of immunosuppression, the specific molecular and cellular pathways in the COX-2-dependent immune suppressive network are now being defined. Particular attention has recently focused on defining the pathways whereby COX-2 and its metabolite PGE2 inhibit immune responses in lung cancer by promoting T regulatory cell activity. PGE2 promotes the CD4+CD25+ T regulatory phenotype and increases the expression of the forkhead transcription factor FOXP3 that is known to program the development and function of T reg cells. This pivotal relationship is currently under investigation in the laboratory utilizing human cells in vitro as well as in patients with lung cancer. Based on the results of pre-clinical murine models [94] and human cells in vitro [37], clinical studies are now evaluating the optimal biological dose of a COX-2 inhibitor, celecoxib, to decrease FOXP3 and T regulatory function in patients with lung cancer.

Tumor progression has been associated with alteration in normal hematopoiesis. The ability of cancer to evade immune recognition and resist immune mediated injury can also be mediated by shifting the hematopoietic process to accumulation of cells of the myelo-monocytic lineage at the primary tumor site and at lymphoid organs [95,96]. Myeloid suppressor cells (MSCs) are one of these cell populations and have potent ability to inactivate CD4+ and CD8+ cells [95,96]. MSCs produce high levels of arginase I which depletes L-arginine and mediates inhibition of T cell function [97]. Inhibition of COX-2 blocked arginase I induction, and elicited a lymphocyte-mediated antitumor response. Furthermore, signaling through the PGE_2 receptor EP4, expressed in MSCs, induced arginase I [97]. These results demonstrate another pathway of prostaglandin-induced immune dysfunction, and further substantiate the potential cancer prevention and therapeutic effects of COX-2 inhibitors.

8. Interaction Between COX-2 and EGFR Signaling

Studies demonstrating that EGFR and COX-2 have related signaling pathways that can interact to regulate cellular proliferation, migration and invasion [98–102] have triggered interest in evaluating the combination of COX-2 and EGFR inhibition in NSCLC. Coffey, et al. [100] demonstrated that the activation of EGFR by transforming growth factor alpha stimulates COX-2 production resulting in increased release of PGE2 and increased mitogenesis. They also showed that COX-2 inhibition in a human colon cancer cell line led to attenuation of TGF-α activity. Another study [99] evaluated the effects of PGE2 on EGFR activation in a colon cancer cell line. PGE2 induced increased phosphorylation of EGFR and Erk 1/2, leading to cell proliferation. Inactivation of EGFR TK with selective inhibitors resulted in decreased PGE2-related Erk activation, decreased c-fos mRNA production and decreased cell proliferation. In addition, EGFR inhibitors have been associated with a decrease in the production of angiogenic factors such as IL-8 and VEGF [103,104]. This has also been found to be a mechanism of angiogenesis inhibition by COX-2 inhibitors [105,106]. When studied in combination in a familial adenomatous polyposis (FAP) mouse model, treatment with EKB-785 (an EGFR TKI) and sulindac (a COX inhibitor) resulted in a 95–97% reduction in the incidence of colonic polyps [107]. Consistent with these findings, the co-expression of EGFR and COX-2 in human cervical cancer specimens portends a poor prognosis with increased recurrences [108]. Recently, Chen et al [109] reported that the combination of an EGFR TKI with celecoxib either additively or synergistically inhibited growth of squamous cell carcinoma of the head and neck (SCCHN), significantly induced G1 arrest and apoptosis, and suppressed capillary formation of endothelium. Furthermore, the combination showed strong reduction of EGFR, Erk1/2, and Akt phosphorylation in SCCHN cells as compared with

the single agents [109]. Importantly, we have recently found a novel mechanism of PGE2-induced EGFR TKI resistance in NSCLC mediated through an EGFR-independent activation of the MAPK/Erk signaling pathway [98]. In these investigations, we demonstrate that PGE2 is able to completely overcome the growth inhibitory activity of EGFR TKIs in approximately 40% of NSCLC cell lines.

9. COX-2 Clinical Trials

Based on these findings, recent studies have been conducted evaluating combined inhibition of the EGFR and COX-2 pathways in patients with NSCLC. Gadgeel et al. [110] reported a Phase II study of gefitinib and celecoxib in patients with platinum refractory NSCLC. Patients received gefitinib 250 mg daily and celecoxib 400 mg twice daily. The response rate to the combination of celecoxib and gefitinib was similar to that observed with gefitinib alone. O'Byrne [111] recently reported a phase I/II trial of combination therapy with gefitinib (250 mg/day) and rofecoxib (50 mg/day) in patients with platinum-pretreated relapsed NSCLC. Gefitinib combined with rofecoxib was found to provide disease control rates equivalent to that expected with single-agent gefitinib. The lack of beneficial effect of combined EGFR TKI and COX-2 inhibitor therapy from these studies raise the question of whether higher dosage may have a critical effect on efficacy.

Reckamp et. al. conducted a Phase I trial evaluating escalating doses of celecoxib (200–800 mg twice daily) in combination with a fixed dose of erlotinib (150 mg/day) in late stage NSCLC patients and established an optimal biological dose (OBD) of 600 mg twice daily, as defined by the maximal decrease in urinary prostaglandin E-M (PGE-M) [63]. This study revealed an acceptable toxicity profile with combination therapy and demonstrated a disease control rate above that expected for erlotinib alone. Based on these results, a Phase II trial is planned to assess combination therapy with celecoxib at 600 mg twice daily and erlotinib versus single agent erlotinib. The use of COX-2 inhibitors at the optimal biological dose may improve efficacy of combination therapy and may explain the lack of benefit in some trials in which a lower dose of COX-2 inhibitors were used. Also, selection of patients with high expression of COX-2 may reveal a beneficial effect of COX-2 inhibitors used in combination with targeted agents or chemotherapy in clinical trials.

Although the use of COX-2 inhibitors at the optimal biological dose may promote responses to combination therapy, there may be associated toxicities with the use of COX-2 inhibitors. Gridelli et al. [112] evaluated the addition of rofecoxib (50 mg/day) to cisplatin and gemcitabine in stage IV or IIIB NSCLC subjects. The groups receiving rofecoxib were closed early due to safety issues surrounding the higher frequency of cardiac ischemia in subjects that received Rofecoxib at 50 mg/day [112]. In a cumulative meta-analysis of 18 randomized controlled trials and 11 observational studies, Juni et al. reported on the increased risk of myocardial infarction in subjects who received rofecoxib [113]. Other reports have shown that rofecoxib exhibits a greater risk of cardiovascular toxicity as compared to celecoxib and may be dose dependent [114]. Solomon et al. found that rofecoxib was associated with a greater incidence of cardiovascular toxicity compared to celecoxib and NSAIDS, and that patients taking rofecoxib at >25 mg doses were associated with higher risk than lower doses [114]. These studies suggest that COX-2 inhibitors may have differing cardiovascular risk and dose may also determine safety profile. It is unclear if cardiac ischemia will occur at a higher risk with short-term usage of COX-2 inhibitors alone or in combination with targeted therapies or conventional chemotherapy.

Several ongoing clinical trials are evaluating COX-2 inhibitors as adjuvants to chemotherapy in patients with advanced NSCLC. Lilenbaum and colleagues reported a phase II trial of irinotecan/docetaxel or irinotecan/gemcitabine with or without celecoxib to determine if

COX-2 inhibition may enhance the efficacy of these chemotherapeutic agents [115]. Patients were randomly assigned to receive irinotecan 60 mg/m2 and docetaxel 35 mg/m2, or irinotecan 100 mg/m2 and gemcitabine 1,000 mg/m2, with or without celecoxib 400 mg twice daily, for four cycles [115]. The median survival was 6.31 months for patients treated with celecoxib and 8.99 months for those treated with chemotherapy alone, and the one-year survival rates were 24% and 36%, respectively [115]. COX-2 inhibition did not appear to enhance efficacy of this chemotherapeutic regimen.

Other studies are focused on the potential role of COX-2 inhibitors in chemoprevention. Mao et al. reported on the feasibility of celecoxib as a chemopreventive agent for lung cancer by administering heavy current smokers with a 6 month course of oral celecoxib and performing serial bronchoscopies with bronchoalveolar lavage and biopsy [116]. Treatment with celecoxib significantly reduced the Ki-67 labeling index in smokers by 35% ($p = 0.016$), and increased the expression of nuclear survivin by 23% ($p = 0.036$) without significantly changing that of cytoplasmic survivin [116]. These findings support the hypothesis that oral administration of celecoxib is capable of modulating Ki-67 labeling index in the bronchial tissue of active smokers at high risk for developing lung cancers [116]. Larger randomized, placebo-controlled clinical trials are underway to determine efficacy of COX-2 inhibitors in preventing the development of bronchogenic carcinoma [6,117].

10. Conclusion

Lung carcinogenesis is a complex process involving the acquisition of genetic mutations that lead to cancer development and the malignant phenotype. These mutations are critically linked to cellular changes, such as apoptosis resistance, unregulated proliferation, invasion, metastasis, and angiogenesis. Elucidation of the molecular mechanisms involved in these cellular changes provides opportunities to develop innovative therapies. COX-2 has been implicated in apoptosis resistance, angiogenesis, decreased host immunity, and enhanced invasion and metastasis, and thus has a pivotal role in carcinogenesis. COX-2 is one of the targets under investigation for lung cancer therapy and chemoprevention. Furthermore, targeting the downstream signaling pathways of COX-2 may produce more profound effects than COX-2 inhibition alone, and thus strategies to antagonize the prostanoid receptors, such as EP4, are potential candidate targets in cancer prevention and therapy.

EMT in cancer is an unregulated process in a host environment with deregulated inflammatory response that degrades CMI and that permits lung cancer progression. Understanding transcriptional regulation of key features in EMT, such as down regulation of e-cadherin, has important implications for chemoprevention and treatment of NSCLC using COX-2 inhibitors in combination with other agents. COX-2 inhibition enhances tumor E-cadherin expression, and may therefore augment sensitivity to other anti-tumor agents, such as EGFR TKI therapy. Based on these observations, several ongoing clinical trials are currently evaluating COX-2 inhibitors as adjuvants to chemotherapy in patients with advanced NSCLC and to determine efficacy of COX-2 inhibitors in prevention of bronchogenic carcinoma. In addition, the reversal of EMT has been a focus of intense investigation. As further understanding of the complex interaction between BMP-7 and TGF-β in the regulation of EMT is acquired, strategies to enhance BMP-7 expression are potential therapeutic targets to reverse EMT in lung carcinogenesis.

Given the immunosuppressive environment in the tumor, investigators are attempting to reverse these events by stimulating host immune responses against tumor antigens in lung cancer. Both TGF-β and PGE2 are among the mediators that promote the CD4+CD25+ T regulatory phenotype and increase the expression of the forkhead transcription factor FOXP3 that is known to program the development and function of T reg cells. These pivotal

relationships are currently under investigation in the laboratory, and clinical studies are underway currently to evaluate the optimal biological dose of a COX-2 inhibitor, celecoxib, to decrease FOXP3 and T regulatory function in patients with lung cancer.

In summary, the understanding of molecular mechanisms involved in inflammation and lung carcinogenesis provide insight for new drug development that target reversible, non-mutational events in the chemoprevention and treatment of lung cancer.

11. References

1. Jemal A, Siegel R, Ward E, Murray T, Xu J, Thun MJ. CA Cancer J Clin 2007;57:43–66. [PubMed: 17237035]
2. Brody JS, Spira A. State of the art. Chronic obstructive pulmonary disease, inflammation, and lung cancer. Proc Am Thorac Soc 2006 Aug;3(6):535–537. [PubMed: 16921139]
3. Smith CJ, Perfetti TA, King JA. Perspectives on pulmonary inflammation and lung cancer risk in cigarette smokers. Inhal Toxicol 2006 Aug;18(9):667–677. [PubMed: 16864557]
4. Moysich KB, Menezes RJ, Ronsani A, Swede H, Reid ME, Cummings KM, Falkner KL, Loewen GM, Bepler G. Regular aspirin use and lung cancer risk. BMC Cancer 2002 Nov 26;2:31. [PubMed: 12453317]
5. Dubinett S, Sharma S, Huang M, Dohadwala M, Pold M, Mao J. Cyclooxygenase-2 in lung cancer. Prog Exp Tumor Res 2003;37:138–162. [PubMed: 12795053]
6. Lee JM, Mao JT, Krysan K, Dubinett SM. Significance of cyclooxygenase-2 in prognosis, targeted therapy and chemoprevention of NSCLC. Future Oncol 2007 Apr;3(2):149–153. [PubMed: 17381414]
7. Katori M, Majima M. Cyclooxygenase-2: its rich diversity of roles and possible application of its selective inhibitors. Inflamm Res 2000 Aug;49(8):367–392. [PubMed: 11028754]
8. Funk CD. Prostaglandins and leukotrienes: advances in eicosanoid biology. Science 2001 Nov 30;294 (5548):1871–1875. [PubMed: 11729303]
9. Malkowski MG, Ginell SL, Smith WL, Garavito RM. The productive conformation of arachidonic acid bound to prostaglandin synthase. Science 2000 Sep 15;289(5486):1933–1937. [PubMed: 10988074]
10. FitzGerald GA. COX-2 and beyond: Approaches to prostaglandin inhibition in human disease. Nat Rev Drug Discov 2003 Nov;2(11):879–890. [PubMed: 14668809]
11. Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, Van De Putte LB, Lipsky PE. Cyclooxygenase in biology and disease. FASEB J 1998 Sep 12;12(12):1063–1073. [PubMed: 9737710]
12. Smith WL, DeWitt DL, Garavito RM. Cyclooxygenases: structural, cellular, and molecular biology. Annu Rev Biochem 2000;69:145–182. [PubMed: 10966456]
13. Chandrasekharan NV, Dai H, Roos KL, Evanson NK, Tomsik J, Elton TS, Simmons DL. COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression. Proc. Natl. Acad. Sci. U S A 2002 Oct 15;99(21):13926–13931. [PubMed: 12242329]
14. Ishihara O, Sullivan MH, Elder MG. Differences of metabolism of prostaglandin E2 and F2 alpha by decidual stromal cells and macrophages in culture. Eicosanoids 1991;4(4):203–207. [PubMed: 1789996]
15. Aoyama T, Yui Y, Morishita H, Kawai C. Prostaglandin I2 half-life regulated by high density lipoprotein is decreased in acute myocardial infarction and unstable angina pectoris. Circulation 1990 Jun;81(6):1784–1791. [PubMed: 2111741]
16. Kliewer SA, Lenhard JM, Willson TM, Patel I, Morris DC, Lehmann JM. A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor gamma and promotes adipocyte differentiation. Cell 1995 Dec 1;83(5):813–819. [PubMed: 8521498]
17. Riedl K, Krysan K, Pold M, Dalwadi H, Heuze-Vourc'h N, Dohadwala M, Liu M, Cui X, Figlin R, Mao JT, Strieter R, Sharma S, Dubinett SM. Multifaceted roles of cyclooxygenase-2 in lung cancer. Drug Resist. Updat 2004 Jun;7(3):169–184. [PubMed: 15296859]

18. Krysan K, Reckamp K, Sharma S, Dohadwala M, Dubinett S. PGE2 activates MAPK/Erk pathway in non-small cell lung cancer cells in an EGF receptor-independent manner. Cancer Res 2005 Jul 15;65(14):6275–6281. [PubMed: 16024629]
19. Tsujii M, Dubois R. Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase-2. Cell 1995 Nov 3;83(3):493–501. [PubMed: 8521479]
20. Huang M, Stolina M, Sharma S, et al. Non-small cell lung cancer cyclooxygenase-2-dependent regulation of cytokine balance in lymphocytes and macrophages: up-regulation of interleukin 10 and down-regulation of interleukin 12 production. Cancer Res 1998 Mar 15;58(6):1208–1212. [PubMed: 9515807]
21. Hida T, Yatabe Y, Achiwa H, et al. Increased expression of cyclooxygenase 2 occurs frequently in human lung cancers, specifically in adenocarcinomas. Cancer Res 1998 Sep 1;58(17):3761–3764. [PubMed: 9731479]
22. Tsubochi H, Nobuyuki S, Hiyama M, et al. Combined analysis of cyclooxygenase-2 expression with p53 and Ki-67 in nonsmall cell lung cancer. Ann Thorac Surg 2006 Oct;82(4):1198–1204. [PubMed: 16996907]
23. Achiwa H, Yatabe Y, Hida T, et al. Prognostic significance of elevated cyclooxygenase 2 expression in primary, resected lung adenocarcinomas. Clin. Cancer Res 1999 May;5(5):1001–1005. [PubMed: 10353732]
24. Brabender J, Park J, Metzger R, et al. Prognostic significance of cyclooxygenase 2 mRNA expression in non-small cell lung cancer. Ann Surg 2002 Jun;235(3):440–443. [PubMed: 11882767]
25. Hasturk S, Kemp B, Kalapurakal SK, et al. Expression of cyclooxygenase-1 and cyclooxygenase-2 in bronchial epithelium and nonsmall cell lung carcinoma. Cancer 2002 Feb 15;94(4):1023–1031. [PubMed: 11920472]
26. Soslow RA, Dannenberg AJ, Rush D, et al. COX-2 is expressed in human pulmonary, colonic, and mammary tumors. Cancer 2000 Dec 15;89(12):2637–2645. [PubMed: 11135226]
27. Khuri FR, Wu H, Lee JJ, et al. Cyclooxygenase-2 overexpression is a marker of poor prognosis in stage I non-small cell lung cancer. Clin. Cancer Res 2001 Apr;7(4):861–867. [PubMed: 11309334]
28. Wolff H, Saukkonen K, Anttila S, et al. Expression of cyclooxygenase-2 in human lung carcinoma. Cancer Res 1998 Nov 15;58(22):4997–5001. [PubMed: 9823297]
29. Hosomi Y, Yokose T, Hirose Y, et al. Increased cyclooxygenase 2 (COX-2) expression occurs frequently in precursor lesions of human adenocarcinoma of the lung. Lung Cancer 2000 Nov;30(2): 73–81. [PubMed: 11086200]
30. Campa D, Zienolddiny S, Maggini V, et al. Association of a common polymorphism in the cyclooxygenase 2 gene with risk of non-small cell lung cancer. Carcinogenesis 2004 Feb;25(2):229–235. [PubMed: 14604894]
31. Schreinemachers DM, Everson RB. Aspirin use and lung, colon, and breast cancer incidence in a prospective study. Epidemiology 1994 Mar;5(2):138–146. [PubMed: 8172988]
32. Huang M, Sharma S, Mao JT, Dubinett SM. Non-small cell lung cancer-derived soluble mediators and prostaglandin E_2 enhance peripheral blood lymphocyte IL-10 transcription and protein production. J. Immunol 1996 Dec 15;157(12):5512–5520. [PubMed: 8955201]
33. Krysan K, Dalwadi H, Sharma S, et al. Cyclooxygenase 2-dependent expression of survivin is critical for apoptosis resistance in non-small cell lung cancer. Cancer Res 2004 Sep 15;64(18):6359–6362. [PubMed: 15374938]
34. Gately S. The contributions of cyclooxygenase-2 to tumor angiogenesis. Cancer Metastasis Rev 2000;19(1–2):19–27. [PubMed: 11191059]
35. Leahy K, Koki A, Masferrer J. Role of cyclooxygenases in angiogenesis. Curr. Med. Chem 2000 Nov;7(11):1163–1170. [PubMed: 11032965]
36. Stolina M, Sharma S, Lin Y, Dohadwala M, Gardner B, Luo J, Zhu L, Kronenberg M, Miller PW, Portanova J, Lee JC, Dubinett SM. Specific inhibition of cyclooxygenase 2 restores antitumor reactivity by altering the balance of IL-10 and IL-12 synthesis. J Immunol 2000 Jan 1;164(1):361–370. [PubMed: 10605031]
37. Baratelli F, Lin Y, Zhu L, Yang SC, Heuze-Vourc'h N, Zeng G, Reckamp K, Dohadwala M, Sharma S, Dubinett SM. Prostaglandin E2 induces FOXP3 gene expression and T regulatory cell function in human CD4+ T cells. J Immunol 2005 Aug 1;175(3):1483–1490. [PubMed: 16034085]

38. Dohadwala M, Batra RK, Luo J, Lin Y, Krysan K, Pold M, Sharma S, Dubinett SM. Autocrine/paracrine prostaglandin E2 production by non-small cell lung cancer cells regulates matrix metalloproteinase-2 and CD44 in cyclooxygenase-2-dependent invasion. J Biol Chem 2002 Dec 27;277(52):50828–50833. [PubMed: 12393872]
39. Dohadwala M, Luo J, Zhu L, et al. Non small cell lung cancer cylooxygenase-2-dependent invasion is mediated by CD44. J. Biol. Chem 2001 Jun 15;276(24):20809–20812. [PubMed: 11320076]
40. Dy GK, Adjei AA. Novel targets for lung cancer therapy: part I. J. Clin. Oncol 2002 Jun 15;20(12):2881–2894. [PubMed: 12065566]
41. Dy GK, Adjei AA. Novel targets for lung cancer therapy: part II. J. Clin. Oncol 2002 Jul 1;20(13):3016–3026. [PubMed: 12089232]
42. Bhattacharya M, Peri KG, Almazan G, Ribeiro-da-Silva A, Shichi H, Durocher Y, Abramovitz M, Hou X, Varma DR, Chemtob S. Nuclear localization of prostaglandin E2 receptors. Proc. Natl. Acad. Sci. U S A 1998 Dec 22;95(26):15792–15797. [PubMed: 9861049]
43. Breyer RM, Kennedy CR, Zhang Y, Breyer MD. Structure-function analyses of eicosanoid receptors. Physiologic and therapeutic implications. Ann. N Y Acad. Sci 2000 Apr;905:221–231. [PubMed: 10818456]
44. Fujino H, Regan JW. Prostanoid receptors and phosphatidylinositol 3-kinase: a pathway to cancer. Trends Pharmacol. Sci 2003 Jul;24(7):335–340. [PubMed: 12871665]
45. Yang L, Huang Y, Porta R, Yanagisawa K, Gonzalez A, Segi E, Johnson DH, Narumiya S, Carbone DP. Host and Direct Antitumor Effects and Profound Reduction in Tumor Metastasis with Selective EP4 Receptor Antagonism. Cancer Res 2006 October 1;66(19):9665–9672. [PubMed: 17018624]
46. Han S, Ritzenthaler JD, Wingerd B, Rivera HN, Roman J. Extracellular matrix fibronectin increases prostaglandin E2 receptor subtype EP4 in lung carcinoma cells through multiple signaling pathways: the role of AP-2. J Biol Chem 2007 Mar 16;282(11):7961–7972. [PubMed: 17237224]
47. Rennard SI. Chronic obstructive pulmonary disease: linking outcomes and pathobiology of disease modification. Proc Am Thorac Soc 2006;3:276–280. [PubMed: 16636098]
48. O'Donnell R, Breen D, Wilson S, Djukanovic R. Inflammatory cells in the airways in COPD. Thorax 2006;61:448–454. [PubMed: 16648353]
49. Sevenoaks MJ, Stockley RA. Chronic obstructive pulmonary disease, inflammation and co-morbidity – a common inflammatory phenotype? Respir Res 2006;7:70. [PubMed: 16669999]
50. Reynolds PR, Cosio MC, Hoidal JR. Cigarette smoke-induced Egr-1 upregulates pro-inflammatory cytokines in pulmonary epithelial cells. Am J Respir Cell Moll Biol 2006 Sep;35(3):314–319.
51. Tan RJ, Fattman CL, Niehouse LM, et al. Matric metalloproteinases promote inflammation and fibrosis in asbestos-induced lung injury in mice. Am J Respir Cell Mol Biol 2006 Sep;35(3):289–297. [PubMed: 16574944]
52. Soberman RJ, Christmas P. Revisiting prostacyclin-new directions in pulmonary fibrosis and inflammation. Am J Physiol Lung Cell Mol Physiol 2006 Aug;291(2):L142–L143. [PubMed: 16581828]
53. Baratelli F, Lin Y, Zhu L, et al. Prostaglandin E2 induces FOXP3 gene expression and T regulatory cell function in human CD4+ T cells. J Immunol 2005;175:1483–1490. [PubMed: 16034085]
54. Dohadwala M. Cyclooxygenase-2-dependent regulation of E-cadherin: prostaglandin E2 induces transcriptional repressors ZEB1 and Snail in non-small cell lung cancer. Cancer Res 2006 May 15;66(10):5338–5345. [PubMed: 16707460]
55. Keshamouni VG, Michailidis G, Grasso CS, et al. Differential protein expression profiling by iTRAQ-2DLC-MS/MS of lung cancer cells undergoing epithelial-mesenchymal transition reveals a migratory/invasive phenotype. J Proteome Res 2006;5:1143–1154. [PubMed: 16674103]
56. Leng Q, Bentwich Z, Borkow G. Increased TGF-β, Cbl-b and CTLA-4 levels and immunosuppression in association with chronic immune activation. Int Immunol 2006;18:637–644. [PubMed: 16608902]
57. Huber MA, Kraut N, Beug H. Molecular requirements for epithelial-mesenchymal transition during tumor progression. Curr Opin Cell Biol 2005;17:548–558. [PubMed: 16098727]
58. Lee JM, Dedhar S, Kalluri R, Thompson EW. The epithelial-mesenchymal transition: new insights in signaling, development, and disease. J Cell Biol 2006 Mar 27;172(7):973–981. [PubMed: 16567498]

59. Thiery JP. Epithelial-mesenchymal transitions in development and pathologies. Curr Opin Cell Biol 2003;15:740–746. [PubMed: 14644200]
60. Dasari V, Gallup M, Lemjabbar H, Maltseva I, McNamara N. Epithelial-mesenchymal transition in lung cancer: is tobacco the “smoking gun”? Am J Respir Cell Mol Biol. 2006
61. Engels EA, Wu X, Gu J, Dong Q, Liu J, Spitz MR. Systematic evaluation of genetic variants in the inflammation pathway and risk of lung cancer. Cancer Res 2007 Jul 1;67(13):6520–6527. [PubMed: 17596594]
62. Lippman SM, Gibson N, Subbaramaiah K, Dannenberg AJ. Combined targeting of the epidermal growth factor receptor and cyclooxygenase-2 pathways. Clin Cancer Res 2005;11:6097–6099. [PubMed: 16144906]
63. Reckamp KL, Krysan K, Morrow JD, et al. A phase I trial to determine the optimal biological dose of celecoxib when combined with erlotinib in advanced non-small cell lung cancer. Clin Cancer Res 2006;12:3381–3388. [PubMed: 16740761]
64. Witta SE, Gemmill RM, Hirsch FR, Coldren CD, Hedman K, Ravdel L, Helfrich B, Dziadziuszko R, Chan DC, Sugita M, Chan Z, Baron A, Franklin W, Drabkin HA, Girard L, Gazdar AF, Minna JD, Bunn PA Jr. Restoring E-cadherin expression increases sensitivity to epidermal growth factor receptor inhibitors in lung cancer cell lines. Cancer Res 2006 Jan 15;66(2):944–950. [PubMed: 16424029]
65. Kopp JB. BMP-7 and the proximal tubule. Kidney Int 2002 Jan;61(1):351–352. [PubMed: 11786119]
66. Okada H, Kalluri R. Recapitulation of kidney development paradigms by BMP-7 reverses chronic renal injury. Clin Exp Nephrol 2005 Jun;9(2):100–101. [PubMed: 15980942]
67. Hogan BL. Bone morphogenetic proteins in development. Curr. Opin. Genet. Dev 1996;6:432–438. [PubMed: 8791534]
68. Bellusci S, Henderson R, Winnier G, Oikawa T, Hogan BL. Evidence from normal expression and targeted misexpression that bone morphogenetic protein (Bmp-4) plays a role in mouse embryonic lung morphogenesis. Development 1996;122:1693–1702. [PubMed: 8674409]
69. Zeisberg M, Hanai J, Sugimoto H, Mammoto T, Charytan D, Strutz F, Kalluri R. BMP-7 counteracts TGF-beta1-induced epithelial-to-mesenchymal transition and reverses chronic renal injury. Nat Med 2003 Jul;9(7):964–968. [PubMed: 12808448]
70. Gershon RK, Kondo K. Cell interactions in the induction of tolerance: the role of thymic lymphocytes. Immunology 1970 May;18(5):723–737. [PubMed: 4911896]
71. Rakhmilevich AL, North RJ. Elimination of CD4+ T cells in mice bearing an advanced sarcoma augments the antitumor action of interleukin-2. Cancer Immunol Immunother 1994 Feb;38(2):107–112. [PubMed: 7905789]
72. DiGiacomo A, North RJ. T cell suppressors of antitumor immunity. The production of Ly-1-,2+ suppressors of delayed sensitivity precedes the production of suppressors of protective immunity. J Exp Med 1986 Oct 1;164(4):1179–1192. [PubMed: 2944983]
73. Antony PA, Restifo NP. Do CD4+ CD25+ immunoregulatory T cells hinder tumor immunotherapy? J Immunother 2002 May–Jun;25(3):202–206. [PubMed: 12000861]
74. Maloy KJ, Powrie F. Regulatory T cells in the control of immune pathology. Nat Immunol 2001 Sep; 2(9):816–822. [PubMed: 11526392]
75. Shevach EM. Certified professionals: CD4(+)CD25(+) suppressor T cells. J Exp Med 2001 Jun 4;193 (11):F41–F46. [PubMed: 11390442]
76. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol 1995 Aug 1;155(3):1151–1164. [PubMed: 7636184]
77. Thornton AM, Shevach EM. CD4+CD25+ immunoregulatory T cells suppress polyclonal T cell activation in vitro by inhibiting interleukin 2 production. J Exp Med 1998 Jul 20;188(2):287–296. [PubMed: 9670041]
78. Takahashi T, Kuniyasu Y, Toda M, Sakaguchi N, Itoh M, Iwata M, Shimizu J, Sakaguchi S. Immunologic self-tolerance maintained by CD25+CD4+ naturally anergic and suppressive T cells: induction of autoimmune disease by breaking their anergic/suppressive state. Int Immunol 1998 Dec; 10(12):1969–1980. [PubMed: 9885918]

79. Thornton AM, Shevach EM. Suppressor effector function of CD4+CD25+ immunoregulatory T cells is antigen nonspecific. J Immunol 2000 Jan 1;164(1):183–190. [PubMed: 10605010]
80. Papiernik M, de Moraes ML, Pontoux C, Vasseur F, Penit C. Regulatory CD4 T cells: expression of IL-2R alpha chain, resistance to clonal deletion and IL-2 dependency. Int Immunol 1998 Apr;10(4): 371–378. [PubMed: 9620592]
81. Nomura T, Sakaguchi S. Foxp3 and Aire in thymus-generated T reg cells: a link in self-tolerance. Nature Immunology 2007 Apr;8(4):333–334. [PubMed: 17375092]
82. Shevach EM. Regulatory T cells in autoimmmunity*. Annu Rev Immunol 2000;18:423–449. [PubMed: 10837065]
83. Jordan MS, Boesteanu A, Reed AJ, Petrone AL, Holenbeck AE, Lerman MA, Naji A, Caton AJ. Thymic selection of CD4+CD25+ regulatory T cells induced by an agonist self-peptide. Nat Immunol 2001 Apr;2(4):301–306. [PubMed: 11276200]
84. Walker MR, Kasprowicz DJ, Gersuk VH, Benard A, Van Landeghen M, Buckner JH, Ziegler SF. Induction of FoxP3 and acquisition of T regulatory activity by stimulated human CD4+CD25− T cells. J Clin Invest 2003 Nov;112(9):1437–1443. [PubMed: 14597769]
85. Sakaguchi S. The origin of FOXP3-expressing CD4+ regulatory T cells: thymus or periphery. J Clin Invest 2003 Nov;112(9):1310–1312. [PubMed: 14597756]
86. Sogn JA. Tumor immunology: the glass is half full. Immunity 1998 Dec;9(6):757–763. [PubMed: 9881966]
87. Batra RK, Lin Y, Sharma S, Dohadwala M, Luo J, Pold M, Dubinett SM. Non-small cell lung cancer-derived soluble mediators enhance apoptosis in activated T lymphocytes through an I kappa B kinase-dependent mechanism. Cancer Res 2003 Feb 1;63(3):642–646. [PubMed: 12566308]
88. Yoshino I, Yano T, Murata M, Ishida T, Sugimachi K, Kimura G, Nomoto K. Tumor-reactive T-cells accumulate in lung cancer tissues but fail to respond due to tumor cell-derived factor. Cancer Res 1992 Feb 15;52(4):775–781. [PubMed: 1737336]
89. Woo EY, Yeh H, Chu CS, Schlienger K, Carroll RG, Riley JL, Kaiser LR, June CH. Cutting edge: Regulatory T cells from lung cancer patients directly inhibit autologous T cell proliferation. J Immunol 2002 May 1;168(9):4272–4276. [PubMed: 11970966]
90. Alleva DG, Burger CJ, Elgert KD. Tumor-induced regulation of suppressor macrophage nitric oxide and TNF-alpha production: role of tumor-derived IL-10, TGF-beta and prostaglandin E2. J Immunol 2007 Mar 1;178(5):2883–2892. [PubMed: 17312132]
91. Liu VC, Wong LY, Jang T, Shah AH, Park I, Yang X, Zhang Q, Lonning S, Teicher BA, Lee C. Tumor Evasion of the Immune System by Converting CD4+CD25− T Cells into CD4+CD25+ T Regulatory Cells: Role of Tumor-Derived TGF-beta. J Immunol 2007 Mar 1;178(5):2883–2892. [PubMed: 17312132]
92. Dubinett, S.; Sharma, S.; Huang, M.; Mao, J.; Batra, R. Lung Cancer and Immune Dysfunction. In: Finke, J.; Bukowski, R., editors. Current Clinical Oncology: Cancer Immunotherapy at the Crossroads: How Tumors Evade Immunity and What Can be Done. Vol. 18. New Jersey: Human Press Inc.; 2004. p. 335
93. Huang M, Stolina M, Sharma S, Mao J, Zhu L, Miller P, Wollman J, Herschman H, Dubinett S. Non-small cell lung cancer cyclooxygenase-2-dependent regulation of cytokine balance in lymphocytes and macrophages: up-regulation of interleukin 10 and down-regulation of interleukin 12 production. Cancer Res 1998 Mar 15;58(6):1208–1216. [PubMed: 9515807]
94. Sharma S, Yang SC, Zhu L, Reckamp K, Gardner B, Baratelli F, Huang M, Batra RK, Dubinett SM. Tumor cyclooxygenase-2/prostaglandin E2-dependent promotion of FOXP3 expression and CD4+ CD25+ T regulatory cell activities in lung cancer. Cancer Res 2005 Jun 15;65(12):5211–5220. [PubMed: 15958566]
95. Serafini P, Borrello I, Bronte V. Myeloid suppressor cells in cancer: recruitment, phenotype, properties, and mechanisms of immune suppression. Semin Cancer Biol 2006 Feb;16(1):53–65. [PubMed: 16168663]
96. Serafini P, De Santo C, Marigo I, Cingarlini S, Dolcetti L, Gallina G, Zanovello P, Bronte V. Derangement of immune responses by myeloid suppressor cells. Cancer Immunol Immunother 2004 Feb;53(2):64–72. [PubMed: 14593498]

97. Rodriguez PC, Hernandez CP, Quiceno D, Dubinett SM, Zabaleta J, Ochoa JB, Gilbert J, Ochoa AC. Arginase I in myeloid suppressor cells is induced by COX-2 in lung carcinoma. J Exp Med 2005 Oct 3;202(7):931–939. [PubMed: 16186186]

98. Krysan K, Reckamp K, Sharma S, Dohadwala M, Dubinett SM. PGE2 activates MAPK/Erk pathway in non-small cell lung cancer cells in an EGF receptor-independent manner. Cancer Res 2005;65(14): 6275–6281. [PubMed: 16024629]

99. Pai R, Soreghan B, Szabo IL, Pavelka M, Baatar D, Tarnawski AS. Prostaglandin E2 transactivates EGF receptor: a novel mechanism for promoting colon cancer growth and gastrointestinal hypertrophy. Nat Med 2002;8(3):289–293. [PubMed: 11875501]

100. Coffey RJ, Hawkey CJ, Damstrup L, et al. Epidermal growth factor receptor activation induces nuclear targeting of cyclooxygenase-2, basolateral release of prostaglandins, and mitogenesis in polarizing colon cancer cells. Proc Natl Acad Sci U S A 1997;94(2):657–662. [PubMed: 9012840]

101. Buchanan FG, Wang D, Bargiacchi F, DuBois RN. Prostaglandin E2 regulates cell migration via the intracellular activation of the epidermal growth factor receptor. J Biol Chem 2003;278(37): 35451–35457. [PubMed: 12824187]

102. Shao J, Evers BM, Sheng H. Prostaglandin E2 synergistically enhances receptor tyrosine kinase-dependent signaling system in colon cancer cells. J Biol Chem 2004;279(14):14287–14293. [PubMed: 14742435]

103. Hirata A, Ogawa S, Kometani T, et al. ZD1839 (Iressa) induces antiangiogenic effects through inhibition of epidermal growth factor receptor tyrosine kinase. Cancer Res 2002;62:2554. [PubMed: 11980649]

104. Yang XD, Jia XC, Corvalan JR, Wang P, Davis CG. Development of ABX-EGF, a fully human anti-EGF receptor monoclonal antibody, for cancer therapy. Crit Rev Oncol Hematol 2001;38(1): 17–23. [PubMed: 11255078]

105. Williams CS, Tsujii M, Reese J, Dey SK, DuBois RN. Host cyclooxygenase-2 modulates carcinoma growth. J Clin Invest 2000;105(11):1589–1594. [PubMed: 10841517]

106. Pold M, Zhu LX, Sharma S, et al. Cyclooxygenase-2-dependent expression of angiogenic CXC chemokines ENA-78/CXC Ligand (CXCL) 5 and interleukin-8/CXCL8 in human non-small cell lung cancer. Cancer Res 2004;64(5):1853–1860. [PubMed: 14996749]

107. Torrance CJ, Jackson PE, Montgomery E, et al. Combinatorial chemoprevention of intestinal neoplasia. Nat Med 2000;6(9):1024–1028. [PubMed: 10973323]

108. Kim G, Kim Y, Cho N, et al. Synchronous coexpression of epidermal growth factor receptor and cyclooxygenase-2 in carcinomas of the uterine cervix: a potential predictor of poor survival. Clin Cancer Res 2004;10:1366. [PubMed: 14977838]

109. Chen Z, Zhang X, Li M, et al. Simultaneously targeting epidermal growth factor receptor tyrosine kinase and cyclooxygenase-2, an efficient approach to inhibition of squamous cell carcinoma of the head and neck. Clin Cancer Res 2004;10(17):5930–5939. [PubMed: 15355926]

110. Gadgeel SM, Ruckdeschel JC, Heath EI, Heilbrun LK, Venkatramanamoorthy R, Wozniak A. Phase II study of gefitinib, an epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI), and celecoxib, a cyclooxygenase-2 (COX-2) inhibitor, in patients with platinum refractory non-small cell lung cancer (NSCLC). J Thorac Oncol 2007 Apr;2(4):299–305. [PubMed: 17409801]

111. O'Byrne KJ, Danson S, Dunlop D, Botwook N, Taguchi F, Carbone D, Ranson M. Combination therapy with gefitinib and rofecoxib in patients with platinum-pretreated relapsed non-small cell lung cancer. J Clin Oncol 2007 Aug 1;25(22):3266–3273. [PubMed: 17664473]

112. Gridelli C, Gallo C, Ceribelli A, Gebbia V, Gamucci T, Ciardiello F, et al. Factorial phase III randomized trial of rofecoxib and prolonged constant infusion of gemcitabine in advanced non-small cell lung cancer: the GEmcitabine-COxib in NSCLC (GECO) study. Lancet Oncol 2007;8:500–512. [PubMed: 17513173]

113. Juni P, Nartey L, Reichenbach S, Sterchi R, Dieppe PA, Egger M. Risk of cardiovascular events and rofecoxib: cumulative meta-analysis. Lancet 2004;364:2021–2029. [PubMed: 15582059]

114. Solomon D, Schneeweiss S, Glynn RJ, Kiyota Y, Levin R, Mogun H, Avorn J. Relationship between selective cyclooxygenase-2 inhibitors and acute myocardial infarction in older adults. Circulation 2004;109:2068–2073. [PubMed: 15096449]

115. Lilenbaum R, Socinski MA, Altorki NK, Hart LL, Keresztes RS, Hariharan S, Morrison ME, Fayyad R, Bonomi P. Randomized phase II trial of docetaxel/irinotecan and gemcitabine/irinotecan with or without celecoxib in the second-line treatment of non-small-cell lung cancer. J Clin Oncol 2006 Oct 20;24(30):4825–4832. [PubMed: 17050867]

116. Mao JT, Fishbein MC, Adams B, et al. Celecoxib Decreases Ki-67 Proliferative Index in Active Smokers. Clin Cancer Res 2006;12:314–320. [PubMed: 16397057]

117. Mao JT, Cui X, Reckamp K, et al. Chemoprevention strategies with cyclooxygenase-2 inhibitors for lung cancer. Clin Lung Cancer 2005;7(1):30–39. [PubMed: 16098242]

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