

Chapter 2

Inflammation and Lung Cancer: The Role of Epithelial–Mesenchymal Transition

Tonya C. Walser, Stacy J. Park, Jane Yanagawa and Steven M. Dubinett

Abstract Epithelial–mesenchymal transition (EMT) is a type of cellular plasticity by which epithelial cells acquire the form and function of mesenchymal cells. Physiologic EMT is an essential part of normal embryonic development and an adult organism’s ability to overcome acute injury. However, chronic injury and inflammation can yield dysregulated or pathologic EMT that drives organ fibrosis and cancer development. In this chapter, we review seminal work and recent findings regarding the molecular, cellular, microenvironmental, and environmental factors that drive inflammation-induced EMT-dependent lung carcinogenesis. We also discuss potential approaches for treating or perhaps preventing lung cancer by targeting the inflammation-EMT-cancer axis.

Introduction

To maintain the tissue integrity that is essential for adult organ homeostasis and architecture, epithelial cells have the capacity for conversion to mesenchymal cells via the process known as epithelial–mesenchymal transition (EMT). EMT and the reverse process, mesenchymal–epithelial transition (MET), are physiologic processes that are essential during normal embryonic development and wound heal-

S. M. Dubinett (✉) · T. C. Walser · S. J. Park
Division of Pulmonary & Critical Care Medicine, David Geffen School of Medicine at UCLA,
Los Angeles, CA 90095, USA
e-mail: twalser@mednet.ucla.edu

S. M. Dubinett
e-mail: sdubinett@mednet.ucla.edu

S. J. Park
e-mail: sjpark@mednet.ucla.edu

J. Yanagawa
Division of Thoracic Surgery, David Geffen School of Medicine at UCLA, Los Angeles,
CA 90095, USA
e-mail: jyanagawa@mednet.ucla.edu

ing. However, when the stimuli inducing these processes are sustained and their downstream signaling pathways are constitutively activated, EMT/MET can lead to serious pathologies, such as fibrosis and cancer. This is the case with chronic inflammation and EMT in the setting of the lung. In this chapter, we describe leading mechanisms by which chronic inflammation leads to constitutive or memorized EMT and ultimately lung carcinogenesis and cancer progression. Specifically, we review the key molecular, cellular, microenvironmental, and environmental factors that drive inflammation-induced EMT-dependent lung carcinogenesis. We also discuss potential approaches for treating or perhaps preventing lung cancer by targeting the inflammation-EMT-cancer axis.

Inflammation

Chronic inflammation in the lung microenvironment, as often observed in smokers [1–3], can lead to increased tumor initiation, cancer progression, invasion, and metastasis [1–7]. The link between unresolved inflammation and cancer has been well established with current estimates indicating that as many as 25 % of all cancers are associated with chronic inflammation [6]. Evidence for the inflammation-to-cancer link includes the following: (a) numerous inflammatory diseases are associated with increased risk of cancer development [3, 4, 8, 9]; (b) inflammatory mediators are present within and surrounding most tumors [4–6, 9]; (c) nonsteroidal anti-inflammatory drugs (NSAIDs) have been found to decrease cancer incidence and delay progression in patients with breast, prostate, colorectal, and lung cancers [10–13]; (d) overexpression of inflammatory cytokines increases cancer development and progression in murine studies [4, 14–16]; and (e) inhibition of inflammatory mediators decreases tumor incidence, burden, and progression in murine studies [4, 14–16]. In fact, the link between unresolved inflammation and cancer is now so well substantiated in cell culture, mice, and man that it is considered an enabling characteristic, if not a full-fledged hallmark, of cancer development [9].

EMT and MET

EMT is an important process during embryogenesis, fetal development, and wound healing because it facilitates the cell motility necessary for tissue remodeling [17–19]. In response to both intrinsic and extrinsic inflammatory signals (discussed below), epithelial cells transition from a highly polarized “epithelial” phenotype with intact cell–cell junctions and both barrier and proliferative functions to a fibroblast-like “mesenchymal” phenotype with minimal adhesion to neighboring cells and both motility and connective functions. During the dynamic EMT process, there is a switch from E-cadherin-mediated adhesion to integrin-based adhesion, cell–cell

junctions are dissolved, the cytoskeleton is reorganized, and the basement membrane is protolytically degraded. Fully transitioned mesenchymal cells are endowed with motility, migration, and invasion capacities that precipitate the critical shift from the epithelial “grow” program to the mesenchymal “go” program [20–21]. As a part of embryonic development and wound healing, this EMT process is tightly regulated and finite. Where there is unresolved inflammation, however, EMT signaling pathways are dysregulated and the EMT process persists, giving rise to organ fibrosis and/or tumor development.

Main Features of Epithelial and Mesenchymal Cells

Cells are characterized as epithelial or mesenchymal based on their own unique cellular morphology and the morphology of the multicellular structures they form in aggregate [17–19]. Epithelial cells typically have cobblestone-like morphology with apical–basal polarity, and these uniformly shaped epithelial cells typically arrange themselves into well-organized sheets that also have apical–basal polarity. Largely due to the abundance and localization of E-cadherin, epithelial cells associate tightly with their neighbors, inhibiting their movement and dissociation from the sheet. Neighboring epithelial cells are also adjoined by specific junctional complexes—adherens junctions, tight junctions, desmosomes, and gap junctions—that give the sheet structural integrity. The highly organized actin cytoskeleton of epithelial cells also contributes to the integrity of the cell sheet. This confers the epithelium with barrier function, whereby the sheet is able to contain space and volume, effectively lining the surfaces of organs and creating glands. Importantly, epithelial cells are typically found in the grow program; thus, they are capable of proliferating rapidly to replace damaged epithelial cells [20–21].

In contrast, mesenchymal cells do not form regular layers, and they do not have abundant E-cadherin or specialized intercellular adhesion complexes that characterize epithelial cells [17–19]. Mesenchymal cells are elongated or spindle shaped (fibroblast-like), and are entirely devoid of the apical–basal polarity that typifies epithelial cells. Instead of E-cadherin, mesenchymal cells typically express N-cadherin, and there is an abundance of focal adhesions collected at their furthest protrusions that establish end-to-end polarity when the cells are engaged in movement or interacting with neighbors. Furthermore, the actin cytoskeleton is disassembled and rearranged, such that there is an increased abundance of F-actin structures, including stress fibers, actin arcs, and actin spikes. This particular cellular and molecular configuration is conducive to cell motility, migration, and invasion. Because the barrier function of the epithelial sheet is lost when the cells undergo EMT, cell-secreted products access and protolytically degrade the basement membrane, further facilitating the movement program. Taken together, mesenchymal cells are typically found in the go program, and they can move readily within tissues either as individual cells or as islands of discohesive cells [20, 21].

Molecular Markers of Epithelial and Mesenchymal Cells

Investigations in the EMT field have not found consensus regarding universal markers indicative of the epithelial state, the mesenchymal state, and the EMT process, because most markers appear to be tissue and/or context specific. As previously described, EMT requires alterations in cellular morphology, adhesion, and migratory capacity, thus the majority of EMT markers are related to these signaling programs. Below, we provide a brief description of the molecular markers of EMT that are relevant in a preponderance of tissues/contexts [18, 22].

The hallmark molecular marker of epithelial cells is E-cadherin, and there is a prototypical shift to N-cadherin and expression of integrins during EMT. The exact repertoire of cadherins and integrins specific to mesenchymal cells and the mechanisms of the E-to-N “cadherin switch” appear to be tissue specific and are still under active investigation. Generally, however, E-cadherin is downregulated, as are the claudins, occludins, and desmosomes, while N-cadherin and several isoforms of integrins are upregulated during EMT. Cytoplasmic molecular markers of EMT are primarily those associated with the disassembly of the cytoskeleton and the arrangement of intermediate filaments to form the contractile apparatus. These changes provide the strength and integrity required for traction forces and the movement program activated following EMT. Specifically, the cytokeratins, vimentin, and α -smooth muscle actin (α -SMA) are all upregulated in mesenchymal cells. β -catenin is another potential molecular marker of EMT, because it is quiescent in epithelial cells, due to its cytoplasmic localization, and active in mesenchymal cells following its translocation to the nucleus. β -catenin is pleiotropic in nature, acting as a cytoplasmic anchor of cadherins while the cell is in epithelial configuration and then as a transcriptional activator of Snail once translocated to the nucleus while the cell is undergoing EMT. Extracellular matrix (ECM) proteins are also common markers of EMT, and the switch is from markers of proteins comprising the basement membrane to markers of ECM fibril disposition, which facilitates the movement of newly transitioned mesenchymal cells to distant sites. The matrix metalloproteinases (MMPs), for example, are upregulated during EMT providing a mechanism of degrading the movement-restrictive basement membrane, while there is increased production of the ECM fibril-related proteins, fibroblast-specific protein 1 (FSP-1) and fibronectin, during EMT. Finally, transcriptional repressors of the epithelial state are upregulated as cells undergo EMT. Direct repressors of E-cadherin include Snail (Snail and Slug), basic helix-loop-helix (bHLH; Twist1), and zinc finger-E-box binding (Zeb and Sip1) family members. The lesser known indirect transcriptional repressors, E47 and forkhead box C2 (FOXC2), also facilitate EMT, and each is expressed at elevated levels by mesenchymal cells. The list of molecular markers summarized here is not exhaustive, and the basal and post-EMT levels of each molecular marker are often tissue and context dependent.

Phenotypic Changes and Functional Outcomes of EMT

The cobblestone morphology, contact inhibition, and apical–basal polarity characterizing epithelial cells afford epithelial sheets their barrier function. Their abundant intercellular junctions and well-organized cytoskeleton also provide the structural integrity needed to form the linings of organs and glands. In addition to being conducive for barrier function, the largely symmetrical shape of epithelial cells facilitates cell proliferation. There is a rapid turnover of the epithelial lining of most organs, including the lung. As cells in the epithelial sheet age and slough off, cells in an epithelial state are able to rapidly proliferate and replace the old cells. Conversely, the asymmetrical elongated spindle morphology of mesenchymal cells prohibits them from rapid symmetric division into daughter cells. Furthermore, the basement membrane and surrounding tissue are degraded during EMT, and epithelial barrier function is lost. However, the production of ECM fibril proteins, along with the shift to an integrin-based adhesion program and production of filopodia stress fibers that arise with EMT completion, allows mesenchymal cells to enter the movement program.

Collectively, the molecular marker alterations and the intracellular and micro-environmental reorganization described above allow epithelial cells to alter their shape and function. The structural constraints that limit the movement of epithelial cells are eliminated after EMT, as the newly transitioned mesenchymal cells attain a shape that facilitates movement. The cytokines, growth factors, proteases, and ECM proteins produced throughout the EMT process further promote movement. There is general agreement that this movement comes at the price of the cells' barrier function and proliferation capacity—the “go versus grow” hypothesis. Thus, the functional outcome of EMT is deactivation of the cellular proliferation program and activation of its motility, migration, and invasion program. As discussed below, the shape change and functional shift inherent in EMT can serve a physiologically necessary purpose, or it can initiate a pathologic cascade of events, depending upon the duration of the inflammatory stimulus and the EMT signals present in the microenvironment.

Physiologic EMT

Embryonic development and an adult organism's ability to respond to acute inflammation and injury are dependent upon the processes of EMT and MET. While it was long thought that terminal differentiation was required for cells to carry out their specialized functions, this concept was challenged by the observation of mammary gland development, wound healing, fibrosis and carcinogenesis. These processes suggest that epithelial cells are plastic in adult tissues, and EMT is one mechanism by which an organism creates morphologically and functionally distinct cell types. Physiologic EMT involves the transition of specialized proliferative epithelial cells

into specialized motile mesenchymal cells, and it is associated with the generation of diverse cell types required during development and wound healing. The resulting mesenchymal cells either undergo MET to give rise to secondary epithelial cells, as is the case during embryonic development and organogenesis, or they transition into resident tissue fibroblasts, as is the case during wound healing and tissue regeneration. EMT associated with development is programmed, tightly regulated, and finite. EMT associated with wound healing may be induced in response to inflammation, but quite critically, it is also tightly regulated and finite, such that EMT stops once the acute inflammation is attenuated. Conversely, chronic inflammation yields sustained or memorized EMT, and the resulting EMT program shifts from physiologic to pathologic in nature.

Pathologic EMT

Unresolved or chronic inflammation can result in sustained or memorized EMT that can result in tissue destruction, tissue fibrosis, and chronic diseases that are the cause of significant morbidity and mortality worldwide. Additionally, this injury–fibrosis–disease state and the deleterious microenvironment they create place individuals at increased risk for cancer development. As it pertains to cancer development, inflammation-induced EMT was once thought to be a late event impacting only established cancers by facilitating their movement to distant sites and their resistance to certain therapies. As discussed in detail below, inflammation-induced EMT is now recognized to play a role throughout all stages of cancer development, including tumor initiation. Regardless of the timing, pathologic EMT is driven by signals derived from the epithelium (at risk or transformed), the microenvironment, and the environment. Recognition that carcinogenesis is impacted by both intrinsic (tumor epithelial, cell derived) and extrinsic (from all other sources) stimuli is a relatively recent development. In the not-so-distant past, malignant epithelial cells were considered the tumor and the adjacent histologically normal appearing epithelium, immune effector cells, inflammatory mediators, and stroma were all considered irrelevant bystanders that had no impact on cancer development. We now understand that inflammatory signals derived from any source can serve as the stimulus for epithelial cells to convert to mesenchymal cells, affording them the ability to detach from their neighbors, invade surrounding tissue, migrate through the bloodstream, and form tumors at distant sites. Physiologic and pathologic EMT obviously have distinct outcomes, but whether the triggers and signaling events that precipitate each type of EMT are distinct or simply sustained remains to be determined. In the sections that follow, we discuss key EMT effectors common in the chronically inflamed lung that play known roles in lung carcinogenesis.

Inflammation-Related Induction and Regulation of EMT

EMT Effectors Common in the Chronically Inflamed Lung

As stated previously, chronic inflammation often characterizes the lung microenvironment of smokers [1–3]. Here, we describe key drivers of EMT that are nearly ubiquitous in the airways of smokers with chronically inflamed lungs. Space constraints do not permit us to describe all of the EMT mediators known to be present in the developing lung tumor microenvironment (TME). Thus, we have selected those that are: (a) present most uniformly, (b) present in high concentrations, and (c) linked most definitively to EMT and lung cancer development. Importantly, the list of EMT effectors described below includes cytokines (TGF- β , IL-6, TNF- α , and IL-1 β), growth factors (HGF), enzymes (COX-2), master regulators of signaling (NF- κ B), and transcriptional repressors (Snail). The broad class/function range of these proteins and the high degree of cross talk and redundancy that characterize these EMT effectors indicate the evolutionary importance of this EMT program and the problematic nature of subversion of the EMT signaling program by the pulmonary lung carcinogenesis process.

TGF- β

The transforming growth factor- β (TGF- β) cytokine and its cognate receptors, TGF- β receptors type I and type II (T β RI and T β RII), are expressed in most tissues. Under normal conditions, TGF- β regulates tissue homeostasis, including differentiation, survival, and adhesion. In normal epithelial cells, TGF- β can induce growth arrest or apoptosis via its ability to increase expression of cyclin-dependent kinase inhibitors, such as p15 and p21, and via its repression of c-MYC [23]. However, chronic overexpression of TGF- β in the pulmonary microenvironment often leads to fibrosis [24, 25]. Where an established lung tumor exists, the TGF- β signaling cascade is typically dysregulated, such that elevated TGF- β production contributes to potent induction of EMT, immune evasion, and apoptosis resistance [23, 26].

For example, alveolar epithelial cells have been shown to undergo EMT following chronic exposure to TGF- β 1 in vitro and in vivo, leading to loss of epithelial proteins, such as the cytokeratin and occludens, and acquisition of mesenchymal proteins, including vimentin and collagen type I [25]. Studies of non-small cell lung cancer (NSCLC) progression have also revealed that TGF- β promotes EMT via high-mobility group AT-hook 2 (HMGA2) induction [23, 27], which leads to upregulation of the transcriptional repressors Snail and Twist [28]. TGF- β -induced translocation of β -catenin is also involved in the transcription of EMT target genes [29]. Many reports now indicate that high levels of TGF- β characterize most tumor tissues, and they indicate that this TGF- β is primarily produced by the tumor itself to exacerbate the pro-tumorigenic lung TME and further bolster the tumor's metastatic potential [30]. However, even minor production of TGF- β by the TME

is likely sufficient to initiate feed-forward signaling cascades that launch the pathologic EMT program. One such feed-forward loop involves interleukin 6 (IL-6; discussed below), which is primarily produced by the TME. IL-6 enhances epithelial cell EMT and stimulates tumor progression by enhancing TGF- β signaling. Thus, a TGF- β /IL-6 paracrine loop exists that sustains EMT and exacerbates lung carcinogenesis [31]. Via a similar feed-forward mechanism, Snail (which is typically induced by TGF- β) activates TGF- β and extracellular signal-regulated kinase (ERK) signaling that then downregulates microRNA (miR)-29b and upregulates secreted protein, acidic and rich in cysteine (SPARC), an effector of EMT-mediated invasion [32]. The observation of this phenomenon in models of established lung cancer and lung premalignancy suggests that TGF- β may be pro-tumorigenic much earlier during carcinogenesis than previously thought, perhaps facilitating EMT and the movement program before the epithelial cells are fully malignant.

More recent studies of TGF- β have strengthened the connection between inflammation, EMT, and immune evasion. For example, in a spontaneous murine model of melanoma, macrophage-derived suppressor cells (MDSCs) preferentially infiltrated the primary tumor and promoted cancer cell dissemination by inducing EMT [33]. The MDSCs were attracted to the tumor by CXCL5 and, upon arrival, produced the TGF- β , epidermal growth factor (EGF), and hepatocyte growth factor (HGF) that induced EMT of the cancer cells and their subsequent immune system escape. Similarly, in vitro and patient-derived xenograft (PDX) models of lung cancer indicate that TGF- β -mediated EMT induces resistance to EGF receptor (EGFR) inhibitors by suppressing the miR-200 family which then upregulates expression of the mitogen-inducible gene 6 (*MIG6*), a known negative regulator of EGFR [34]. Another study implicates the miR-134/487b/655 cluster in membrane-associated guanylate kinase inverted 2 (*MAGI2*) repression that mediates phosphatase and tensin (PTEN) instability and NSCLC resistance to gefitinib following TGF- β -induced EMT [35]. Taken together, the literature supports a role for TGF- β as a potent inducer of EMT- and EMT-mediated malignant phenotypes that are robustly supportive of lung carcinogenesis.

IL-6

IL-6 is another multifunctional cytokine that can act as both a pro-inflammatory and an anti-inflammatory mediator. It is secreted by T cells and macrophages to stimulate immune responses, and increased levels of IL-6 have been associated with trauma, infection, and elevated cancer risk. The function of IL-6 is primarily mediated through the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway, and elevated IL-6 has been shown to increase the production of collagen and alpha-actin, which together induce interstitial lung disease. In the cancer setting, high levels of IL-6 are also responsible for EMT activation, enhanced neo-angiogenesis, apoptosis resistance, and drug resistance [36]. Interestingly, IL-6 has also been associated with poor prognosis and many of the debilitating symptoms that often plague late-stage lung cancer patients, such as fatigue, thromboembolism, cachexia, and anemia.

In a head and neck squamous cell carcinoma (HNSCC) examination of EMT with in vitro and in vivo study arms, IL-6 induced EMT in both immortalized epithelial cells and cancer cells via JAK/STAT3/Snail signaling [37]. The EMT was associated with cell scattering and motility, and all cancer-related endpoints were reversed by STAT3 knockdown due to attenuation of IL-6 function and focal adhesion kinase (FAK) activation. Using fibroblasts from human patients with benign prostatic hyperplasia or aggressive carcinoma, cancer cell-produced IL-6 was also shown to activate fibroblasts, which in turn secreted MMPs, elicited EMT, and enhanced cancer “stemness,” leading to both tumor formation and metastatic behavior [38]. Similarly, groups studying the deleterious effects of hypoxia both in vitro and in vivo have demonstrated that it induces expression of IL-6, vascular endothelial growth factor (VEGF), and a repertoire of cancer stem cell (CSC) markers, along with their downstream malignant phenotypes. Treatment of pancreatic or prostate cancer cells in this setting with a curcumin-derived synthetic analogue yielded anti-tumor results mediated by IL-6 signaling interference [39, 40]. Finally, in the setting of lung cancer, investigators have demonstrated that COX-2 activates STAT3 via induction of IL-6 expression [41]. Furthermore, COX-2 pathway-derived IL-6 was shown to promote lung tumor progression via induction of VEGF production and survivin-dependent apoptosis resistance.

IL-6 secretion by lung cancer cells has also been shown to mediate resistance to molecularly targeted therapies. For example, IL-6 frees previously “addicted” lung tumor cells from their EGFR dependency [42]. Similarly, IL-6 induction has been linked to initiation of EMT and expansion of a drug-resistant breast CSC population. Targeting IL-6 disrupted the inflammatory loop responsible for activation of the stemness program, resulting in decreased tumor growth and metastasis of the previously resistant cells [43]. Similar results were observed in HNSCC, where IL-6-induced EMT was responsible for erlotinib resistance mediated by the receptor tyrosine kinase Axl [44].

In perhaps one of the most important studies of IL-6 and EMT, investigators studying the metastasis of colorectal cancer cells to the lungs of mice determined that IL-6 drives EMT via suppression of miR-34a [45, 46]. Specifically, they determined that activation of the IL-6R/STAT3/miR-34a feedback loop by IL-6-induced EMT shifts tumor cells toward a mesenchymal state conducive to metastasis. Furthermore, disruption of the loop—a result of less IL-6 available at the new location—induces MET and facilitates metastatic colonization and outgrowth. This report provided in vitro and in vivo evidence for the EMT-to-MET switch and the first description of a central role of IL-6 in maintenance of the balance between EMT and MET and regulation of the lung carcinogenesis process.

TNF- α

Another cytokine capable of acting in both an anti-tumor and pro-tumor manner is tumor necrosis factor- α (TNF- α). TNF- α can be secreted by a number of cell types, including macrophages, T lymphocytes, and B lymphocytes. Originally described as reducing tumor burden, it is a potent pro-inflammatory mediator that can induce

tumor cell death [47]. However, in an inflammatory environment, TNF- α is often overexpressed and its downstream signaling cascade is often dysregulated. TNF- α has been associated with a number of degenerative pulmonary diseases, such as asthma, chronic bronchitis, and chronic obstructive pulmonary disease (COPD), in addition to smoking-induced emphysema [48]. TNF- α overexpression in the TME can lead to EMT, recruitment of leukocytes, and depletion of antioxidants from the microenvironment, which can lead to cellular oxidative stress [49].

Wu et al. provided one of the first comprehensive demonstrations of TNF- α -mediated EMT via their report of TNF- α stabilization of the Snail protein in a nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B)-dependent manner [50]. Investigators of macrophage-derived TNF- α described a similarly important role for the cytokine in launching the TGF- β and NF- κ B signaling cascades that drive EMT and malignant phenotypes [51]. Importantly, most of the deleterious effects of the EMT were blocked with TNF- α neutralization. Another group subsequently reported that TNF- α induces EMT and stemness of breast epithelial cells and breast cancer cells via the upregulation of Twist [52]. Specifically, TNF- α rapidly induces Twist mRNA and protein in an NF- κ B-dependent manner, driving EMT, stemness, migration, and invasion. In a set of in vitro lung cancer studies conducted by two separate groups, one group of investigators implicated TNF- α , not TGF- β , as the major driver of EMT and malignant phenotypes, in part mediated by Claudin 1 [53]. The other group cited TGF- β as the major driver of EMT, but TNF- α was reported to be a potent accelerator of the process, in part due to miR-23a [54]. While the literature supports an important role for TNF- α in EMT-dependent lung carcinogenesis, its exact role and mechanism of action are complex and require further delineation.

IL-1 β

The cytokine interleukin-1 β (IL-1 β) is another important mediator of inflammation and EMT that can be secreted by immune, stromal, and malignant cells [55]. It is one of the first responders following injury and induces a number of other inflammatory signaling events. While IL-1 β mRNA expression is not normally seen in healthy tissues, it is increased in a number of malignancies, including melanoma, colon cancer, and lung cancer [55]. Elevated IL-1 β levels are associated with increased tumor aggressiveness, an invasive phenotype, and poor patient prognosis [56]. A role for IL-1 β in the progression of established tumors has been argued via a series of well-designed murine studies [57, 58]. But more recently, murine models have been used to establish a role for IL-1 β in early tumorigenesis. For example, overexpression of IL-1 β in parietal cells of the stomach has been linked to the development of chronic gastritis, metaplasia, and high-grade dysplasia/carcinoma [59]. In studies utilizing 3-methylcholanthrene, a known chemical initiator and promoter of tumorigenesis, mice deficient in IL-1 β had delayed and diminished tumor development compared to wild-type mice [60]. Conversely, when IL-1 receptor antagonist expression was depleted, the mice had a greater number of tumors and faster tumor development than wild-type mice.

One of the first lung cancer studies linking IL-1 β and EMT demonstrated that IL-1 β expression leads to downregulation of E-cadherin, increased tumor aggressiveness, and enhanced invasion [56]. IL-1 β was subsequently shown to induce production of factors involved in EMT and invasion, such as the MMPs [61]. IL-1 β -induced EMT of NSCLC cell lines is also reportedly dependent on c-Jun N-terminal kinase (JNK) and mitogen-activated protein kinase/extracellular signal-regulated kinase (MEK/ERK) signaling pathway activation of Fos-related antigen-1 (Fra-1) [62]. In an examination of IL-1 β in the setting of HNSCC, IL-1 β modulation of Snail was found to regulate COX-2-dependent E-cadherin expression [63]. Another group confirmed and extended these findings by implicating Slug in the repression of EMT and cancer progression [64]. Similarly, IL-1 β has been shown to enhance Zeb1 binding to E-cadherin at the chromatin level, and an inverse relationship between Zeb1 and E-cadherin was confirmed in immunostained human HNSCC tissues [65].

Those studying the IL-1 β -EMT-cancer axis have begun to delineate the mechanisms connecting IL-1 β to carcinogenesis via EMT activation. For example, L1 cell adhesion molecule (L1CAM) expression was shown to be associated with poor prognosis in a variety of human cancers. Investigators determined that L1CAM promotes a motile and invasive tumor cell phenotype that is dependent upon IL-1 β -induced integrin signaling [66]. Using a murine oral squamous cell carcinoma (OSCC) model induced by tobacco-related carcinogens, investigators linked expression of pro-IL-1 β to the severity of malignant transformation [67]. They determined that IL-1 β drove the invasiveness via downregulation of E-cadherin and up-regulation of Snail, Slug, and Vimentin, along with obvious alterations in the cell's morphology. In this study, IL-1 β was linked to the aggressiveness of both early and late stage OSCC via the EMT program. Another group made the observation that non-transformed breast epithelial cells adjacent to tumor cells are constantly exposed to IL-1 β and TNF- α [68]. They then made the novel determination that IL-1 β and TNF- α induce dissemination of non-transformed breast epithelial cells and their reseeding away from the primary tumor site. This suggested that if these migratory cells were exposed to transforming events, they may form secondary malignant foci and eventual disease recurrence; a finding of critical clinical importance. Finally, in a study of colon cancer development, investigators determined that IL-1 β promotes tumor growth and invasion via activation of EMT and CSC self-renewal programs, both of which were dependent on Zeb1 for their cancer-associated effects [69]. Analogous relationships in the pathogenesis of lung cancer remain to be determined.

HGF

HGF was first identified as “scatter factor” due to its ability to induce epithelial cell scattering in cell culture. It is a potent growth factor secreted by mesenchymal cells that begins as a pro-form. The pro-form of HGF is activated via cleavage by serine proteases, like the plasminogen activators [70]. This allows HGF to form a fully ac-

tivated heterodimer capable of signaling via its receptor tyrosine kinase, MET. Because HGF has a limited capacity to diffuse *in vivo*, the mesenchymal cells expressing HGF normally reside within close proximity to the target MET expressing cells.

HGF/MET signaling is functional in both embryonic and adult tissues, and it typically acts to increase epithelial cell proliferation and motility. During cancer progression, HGF can be induced by several inflammatory mediators, including TGF- β and TNF- α , and it can be released from the ECM and activated by serine proteases secreted by infiltrating inflammatory cells and the tumor stroma [71]. HGF/MET signaling activates a number of downstream pathways that are implicated in cancer progression, the most prominent of which are NF- κ B, mitogen-activated protein kinases (MAPK), and phosphatidylinositol 3'-kinase (PI3K)/AKT [70]. During development, the HGF/MET axis is a potent inducer of EMT, and during tissue injury, HGF levels increase, precipitating EMT and wound healing [71, 72]. HGF is also elevated in the serum of lung cancer patients, where it serves as an indicator of poor prognosis independent of tumor stage [73, 74].

In the cancer setting, HGF/MET signaling induces angiogenesis, EMT, motility, and cell survival [70]. Recent studies demonstrated that HGF contributes to EMT via upregulation of Snail and subsequent downregulation of E-cadherin through the MAPK pathway [75]. Similarly, HGF/MET signaling through the MAPK and PI3K pathways is known to stimulate the disassembly of epithelial junctional complexes by downregulating Desmoglein 1 in melanoma, as well as by mediating methylation of the Claudin 7 gene in human breast cancer cells [76, 77]. In a more recent study of small cell lung cancer (SCLC) drug resistance, investigators determined that the activation of the MET receptor through HGF induced expression of mesenchymal markers, an aggressive phenotype, and chemoresistance [78]. Blockade of this process with a MET inhibitor re-sensitized the SCLC cells to chemotherapy both *in vitro* and *in vivo*. Additionally, they discovered that mesenchymal marker expression by the SCLC cells: (a) was associated with MET activation, (b) predicted worse survival, and (c) was upregulated in chemorefractory disease [78]. In a study of hepatocellular carcinoma (HCC), the use of HGF neutralizing antibodies abolished previously observed EMT, CSC marker expression, and cisplatin resistance of HCC cells [79]. In a similar study, investigators determined that cancer cells undergo EMT under the influence of increased expression of HGF, thereby becoming circulating tumor cells (CTCs) [80]. The process involved upregulation of MET via promoter demethylation at six CpG sites, and it suggests that targeting the HGF/MET axis in CTCs may be a novel approach by which to impact the clinical outcome of HCC patients. Furthermore, this study highlights an emerging area of investigation—epigenetic regulation of EMT and EMT-driven cancer-associated phenotypes that may be clinically targetable.

COX-2

Cyclooxygenase-2 (COX-2) is the rate-limiting enzyme in the conversion of free arachidonic acid into prostaglandin H₂, a substrate for prostaglandin and throm-

boxane synthases. Prostaglandin production impacts multiple biological processes, including wound healing, blood vessel tone, and immune response, in an autocrine and paracrine manner [81]. COX-2 is induced by stimuli such as cytokines, growth factors, and inflammatory mediators, like TGF- β , IL-1 β , EGF, as well as by cigarette smoke [82]. The major COX-2 metabolite, prostaglandin E2 (PGE2), signals through four G protein coupled receptors (EP1, EP2, EP3, and EP4), triggering downstream signaling cascades, such as MAPK/ERK [81, 83]. PGE2 also signals in a receptor-independent manner through a family of ligand-dependent transcription factors (peroxisome proliferator-activated receptor α , γ , and δ ; PPAR α , PPAR γ , and PPAR δ), leading to the activation of downstream gene expression. Elevated PGE2 can be found throughout NSCLC progression, including in some premalignant lesions [84], and it is associated with proliferation, EMT induction, invasion, apoptosis resistance, and immune suppression [83, 85–87]. High tumor COX-2 and PGE2 expression is associated with poor patient prognosis independent of cancer stage.

In the initial report on COX-2-mediated EMT in lung cancer, overexpression of COX-2 by NSCLC cells induced EMT via the downregulation of E-cadherin by the transcriptional repressors Snail and Zeb1 [88]. More recently, most groups have focused on identifying (a) inducers of EMT that act via COX-2 and (b) identification of COX-2 inhibitors that limit tumor progression by attenuating EMT and restoring E-cadherin to the cell surface. For example, in a study of bladder cancer, FGFR1 was identified as an inducer of COX-2- and PGE2-dependent EMT. Full EMT, including changes in cell size, morphology, migration, and invasion, required both MAPK and phospholipase C gamma (PLC γ) pathway activation [89]. Investigators studying COX-2 inhibitors in human bladder, pancreatic, and head and neck cancer have reported similar findings: selective COX-2 inhibitors (e.g., etodolac, celecoxib, NS-398, apricoxib, and SC-791) upregulate E-cadherin, attenuate EMT, and facilitate anti-tumor effects [90–93]. In HNSCC, the restoration of E-cadherin was further linked to downregulation of the transcriptional repressors Smad interacting protein 1 (SIP1), Snail, and Twist [92]. In HNSCC, apricoxib prevented EMT, including tumor cell three-dimensional growth, anchorage-independent growth, and migratory capacity, by inhibiting COX-2 and upregulating 15-hydroxyprostaglandin dehydrogenase (15-PGDH) and the prostaglandin transporter (PGT) [93]. Taken together, modulation of the eicosanoid pathway continues to be an attractive approach by which to target dysregulated inflammation and EMT and may have potential if combined with one or more other molecularly targeted approaches.

NF- κ B

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) is a protein complex that controls the transcription of DNA, thus it is often referred to as “the master regulator.” Members of the NF- κ B transcription factor family are ubiquitously expressed, but in most cell types, they are transiently activated by cytokines and growth factors, including TGF- β , IL-6, TNF- α , IL-1 β , and HGF. The down-

stream effects of each of these NF- κ B-dependent signaling cascades are tissue and context specific. Aberrant NF- κ B activation has been reported in a number of tumor types, including lung, and has been linked to increased proliferation, angiogenesis, invasion, metastasis, and cell survival [47, 94]. NF- κ B can also directly activate the expression of potent inducers of EMT, including Snail and Zeb family members [95]. The connection between inflammation, NF- κ B, and EMT is reinforced by the finding that TNF- α and IL-1 β (which are crucial for the induction of NF- κ B) stabilize the Snail protein. Under standard conditions (homeostasis or physiologic EMT), Snail is unstable and targeted for degradation by phosphorylation and ubiquitylation mediated by glycogen synthase kinase 3 beta (GSK-3 β) and Skp, Cullin, F-box containing complex (SCF), respectively [96, 97]. However, TNF- α induces Snail stabilization by activating expression of COP9 signalosome 2 (CNS2), which inhibits the binding of Snail to GSK-3 β and β -transducin repeat containing protein 1 (β -TRCP1) [50]. Transcriptional and post-transcriptional mechanisms regulate this Snail stabilization, and they act via TNF- α induction of NF- κ B. Oxidative stress is another major activator of NF- κ B that also facilitates EMT. Additionally, “the field” appears to contribute to the deleterious lung TME through NF- κ B; NF- κ B is often over-active in the field, which mediates the release of potent pro-inflammatory cytokines and inducers of EMT [95]. Thus, many of intrinsic and extrinsic factors that drive inflammation-induced EMT-dependent lung carcinogenesis converge at NF- κ B activation.

In more recent investigations, NSCLC cells grown in 3D culture and stimulated with TGF- β and TNF- α were found to have elevated expression of Snail, Slug, Twist, and Sip1 and to be highly invasive [98]. Furthermore, they displayed stemness features, including expression of the induced pluripotency stem (iPS) cell Yamanaka factors, that contributed to the formation of lung metastases in mice using limiting cell dilution. The authors of this study determined that NF- κ B was constitutively activated in concert with the EMT induction and malignant phenotypes. Furthermore, inhibition of NF- κ B reversed the phenotypes and resulted in loss of the mediators Slug, Zeb, and Twist, plus failure of the cells to invade and metastasize. In another study of NSCLC, NF- κ B inhibitors and siRNAs directed against NF- κ B p65 were used to implicate NF- κ B signaling in the regulation of response gene to complement 32 (RGC32)-induced EMT [99]. Because RGC32 drives EMT, migration, and invasion, the authors of this study argued that NF- κ B is also the master regulator of these RGC32-induced phenotypes. A study of cigarette smoke extract (CSE)-induced transformation of human bronchial epithelial cells (HBECS) also implicated NF- κ B in EMT and transformation [100]. Temporally, CSE activation of NF- κ B drove increased production of IL-6 and suppression of miR-200c. By blocking NF- κ B and systematically manipulating the IL-6, the authors of this study determined that NF- κ B was required and IL-6 was also necessary for maintenance of CSE-induced EMT, transformation, and malignant progression. Finally, in a study of mammary epithelial cells, serine cysteine protease inhibitor SCCA1 (Serpine B3) induced a prolonged non-lethal increase in unfolded protein response (UPR) that activate NF- κ B, thereby driving increased expression of IL-6, along

with EMT and transformation of the cells [101]. Importantly, reversal of SCCA1 halted this NF- κ B-mediated EMT and oncogenic transformation.

Snail

The zinc-finger transcription factor Snail, encoded by the *SNAIL* gene, is upregulated following exposure to inflammatory mediators, such as TGF- β and PGE2 [88, 102]. Snail is a direct transcriptional repressor of E-cadherin that plays a pivotal role in mediating EMT in several malignancies [37, 103, 104], and it has been shown to exert global effects on cancer cell gene expression profiles, including EMT, invasion, and angiogenesis programs [32, 105–107]. However, recent studies suggest that Snail may play a broader role in carcinogenesis [108–110]. For example, Snail is expressed in a stem-cell-like subpopulation within immortalized murine mammary epithelial cells and immortalized HBECs that are capable of malignant transformation [110–111]. Discovery of the link between Snail and “stemness” and demonstration that Snail drives both the progression and *initiation* of cancer has far reaching clinical implications. Identification of the mechanisms by which the inflammation-induced transcriptional repressor Snail contributes to lung cancer pathogenesis, specifically to stemness induction, malignant conversion, and micrometastatic dissemination, would be a significant step forward for researchers in the EMT and cancer research fields.

In the setting of established lung cancer, Snail is upregulated in human NSCLC tissues, is associated with poor prognosis, and promotes NSCLC tumor progression *in vivo* [107]. Furthermore, Snail overexpression in NSCLC cell lines is associated with differential gene expression related to diverse aspects of lung cancer progression, in addition to EMT [32, 107]. Investigators have also demonstrated a feed-forward mechanism whereby Snail (which is typically induced by TGF- β) activates TGF- β , its downstream signaling apparatus, and its corresponding malignant phenotypes [32]. Importantly, this Snail-centered feedback loop was also found to be operative in models of both established disease and disease onset. Other groups have now made similar observations, indicating that the inflammation-Snail feedback loop is widespread across cancer types and can occur as a late or early event. For example, expression of Snail by epidermal keratinocytes drives only partial EMT, but Snail promotes cutaneous inflammation and hyperplasia that is conducive to tumor formation and further activation of Snail [113]. The authors of this study identified IL-17/IL-6/Stat3 signaling initiated by activated macrophages, and they determined that this signaling pathway acts in concert with Snail overexpressing epithelial cells to create an inflammatory and hyperproliferative microenvironment conducive to tumor formation. Snail also forms a potent feed-forward loop with stemness-associated factors, such as miR-34a/b/c [114]. In a model of colorectal cancer (CRC), Snail directly induced ZNF281 transcription and repressed miR-34a/b/c, thereby alleviating ZNF281 mRNA from direct downregulation by miR-34. Ectopic expression of ZNF281 was associated with malignant phenotypes, including EMT, migration, invasion, stemness, and sphere formation, while downregulation

of ZNF281 attenuated these phenotypes *in vitro* and inhibited lung metastases *in vivo*. These results suggest the common nature of these inflammation-EMT feed-forward loops, and they identify ZNF281 as yet another component of the EMT-regulatory network with possible indications for clinical cancer management.

Many groups investigating known mediators of carcinogenesis are now reporting EMT as a new mechanism of action for these mediators. In lung cancer cells, for example, PAK1 is upregulated, tyrosine-phosphorylated, and translocated to the nucleus in response to irradiation. However, investigators recently determined that JAK2 kinase activity was essential for PAK1 protein stability and binding to Snail and that the radiation-induced JAK2-PAK1-Snail signaling pathway increased EMT [115]. Furthermore, JAK2 inhibitors mediated radiosensitization and EMT blockade in a xenograft mouse model, providing evidence that (a) PAK1 is a newly identified regulator of EMT and (b) JAK2 inhibitors may have a role as radiosensitizers for lung cancer treatment.

Still other groups have begun to study the epigenetic contribution to the Snail-EMT-cancer axis. In one such study, Krohn et al. surveyed the tumor heterogeneity inherent to SCLC and endeavored to link EMT to the phenotypic and functional changes associated with cells that float in clusters versus those that are more adherent [116]. In fact, the distinct SCLC cell populations did have pronounced differences in key EMT effectors, like Snail, Slug, and Zeb1, in DNA methylation patterns, and in malignant phenotypes, like migration, invasion, MMP production, and drug resistance. The authors' suggest that EMT signaling likely contributes to the heterogeneity that characterizes most solid tumors is increasingly credible, as is the suggestion that epigenetic regulation plays an important role in EMT-dependent cancer initiation and progression. In another study, for example, Millanes-Romero et al. described the regulation of heterochromatin transcription by Snail/LOXL2 during EMT [117]. Preventing the downregulation of the major satellite transcription that occurs concomitantly with the release of heterochromatin foci compromised the migratory and invasive behavior of cells that had undergone EMT. MicroRNA antagonism was also shown to regulate breast cancer EMT, stemness, and metastasis via TET-family-dependent chromatin remodeling. Specifically, Song et al. showed that miR-22 triggered EMT, invasiveness, and metastasis in a mouse xenograft model, demonstrating that miR-22 exerted its metastatic potential by silencing anti-metastatic miR-200 [118]. This miR-200 silencing occurred via the direct targeting of the ten eleven translocation (TET) family of methylcytosine dioxygenases, which thereby inhibited demethylation of the miR-200 promoter. Furthermore, the authors determined that miR-22 overexpression correlates with poor clinical outcome and silencing of the TET-miR-200 axis in patients; a strong indicator that miR-22 is a key epigenetic modifier and inducer of EMT and stemness.

Other types of post-translational regulatory events are being investigated, and they too may represent attractive clinical targets within the inflammation-EMT-cancer axis. For example, Shah et al. identified a role for ubiquitin 1 (UBQLN1) in EMT and tumorigenesis. UBQLN1 is a ubiquitin-like domain and ubiquitin-associated domain containing protein involved in protein transport to the proteasome [119]. The authors of this study demonstrated that the loss of UBQLN1 causes EMT

and is associated with cytoskeletal reorganization, migration, and invasion. Furthermore, ZEB1 was required for induction of EMT following loss of UBQLN1, and Zeb1 was able to repress expression of UBQLN1, suggesting reciprocal regulation of EMT by UBQLN1 and Zeb1. UBQLN1 is lost or underexpressed in a large percentage of human cell lines and primary samples, so this likely represents a new mechanism for the regulation of EMT. Similarly, using a luciferase-based genome-wide E3 ligase siRNA library screen to identify mediators of Snail ubiquitylation and degradation, Zheng et al. discovered that Snail degradation by FBXO11 is dependent on Ser-11 phosphorylation of Snail by protein kinase D1 (PKD1) [96]. FBXO11 blocked Snail-induced tumor initiation and metastasis in multiple breast cancer models, establishing that the PKD1-FBXO11-Snail axis is a mechanism of post-translational regulation of EMT and its malignant outcomes.

Together, these results emphasize the central role of the transcriptional repressors, particularly Snail, in the inflammation-EMT-cancer axis. They also reflect the shift that has occurred over the past decade from mere descriptions of EMT effectors to more comprehensive descriptions of their mechanisms of action. The shift from a focus on established cancer to a focus on describing the role of EMT in tumor initiation is also evident. Finally, researchers of Snail and other EMT mediators are beginning to investigate the post-translational regulation of the inflammation-EMT-cancer axis, with the hope that understanding this additional layer of regulation will provide new opportunities to target EMT for the purpose of cancer prevention or treatment.

Hypoxia

Hypoxia is a reduction in tissue oxygen tension, and it characterizes most TMEs. Hypoxic areas are commonly found in the central necrotic region of a solid tumor mass, and increased expression of hypoxia-related proteins is often found at the leading edge of a tumor [95, 120, 121]. A solid tumor invariably becomes hypoxic as uncontrolled proliferation causes the tumor to outgrow the pre-existing vasculature, thereby depleting its oxygen and nutrient supply. Moreover, new blood vessels the tumor develops are aberrant and have poor blood flow, compounding oxygen deprivation. Chronic inflammation also contributes to the local deficiency of oxygen due to the combination of reduced circulation at inflammatory sites and increased metabolic demand from infiltrating immune cells. In a feed-forward manner, hypoxia promotes chronic inflammation—thus, hypoxia itself—in the developing TME via activation of NF- κ B signaling by macrophages, neutrophils, and non-immune cells, a finding recently confirmed *in vivo* in the lungs of mice [122–124]. Hypoxia is also associated with increased resistance to conventional radiation and chemotherapy, and not surprisingly, it correlates with poor clinical prognosis in many cancer types, including breast, pancreatic, and lung cancer [120, 121]. Although hypoxia has traditionally been considered a late-stage consequence of malignant tumor growth, it is now widely appreciated to play a critical role in the development and progression of tumors as well [95, 120, 121, 124].

The central mediator of the canonical cellular response to reduced oxygen levels is the bHLH heterodimeric transcription factor hypoxia-inducible factor 1 (HIF-1), which consists of a constitutively expressed β subunit (also known as aryl hydrocarbon receptor nuclear translocator) and an oxygen-regulated α subunit. Under normoxic conditions, oxygen-dependent prolyl hydroxylase domain enzymes hydroxylate HIF-1 α on two conserved proline residues. The von Hippel–Lindau (VHL) E3 ubiquitin ligase complex recognizes the hydroxylated proline and targets HIF-1 α for ubiquitin-mediated proteasomal degradation. When oxygen is limited, hydroxylation of HIF-1 α is inhibited, resulting in stabilization and translocation of HIF-1 α into the nucleus, where it heterodimerizes with HIF-1 β to form active HIF-1 transcription factor. Following recruitment of numerous co-activators, the HIF complex binds to hypoxia-response elements (HREs) in target genes to regulate a number of pathways, including cell survival, proliferation, ECM remodeling, angiogenesis, and apoptosis, consequently promoting tumor invasion and metastasis [120, 121, 124].

More recently, the hypoxic microenvironment has emerged as an important factor in the induction of EMT. Since the early observation of HIF-induced EMT in models of renal fibrosis [125], considerable evidence has further implicated the HIF pathway in EMT and carcinogenesis. In this regard, HIF activation is associated with loss of E-cadherin and induction of mesenchymal gene expression. Interestingly, the transcriptional repressors of E-cadherin, namely Snail, Slug, Twist, Zeb1, and Sip1, can be regulated by hypoxia in human cancer. In VHL-deficient renal cell carcinoma, stable expression of the HIF-1 α and HIF-2 α isoforms is associated with increased expression of Snail, Zeb1, and Sip1, loss of E-cadherin, and increased invasion [126, 127]. Similarly, Twist can be directly regulated by both HIF-1 α and HIF-2 α [128, 129]. In NSCLC and breast cancer cell lines, hypoxia or overexpression of HIF-1 α reduced E-cadherin expression and increased cell migration, invasion, and metastasis, effects that were reversed by knockdown of Twist by siRNA [128]. Moreover, co-expression of HIF-1 α , Snail, and Twist in HNSCC patient primary tumors correlated with the highest probability of metastasis and poor prognosis. In accord with these findings, a study by Hung et al. reported that overexpression of HIF-1 α , Snail, or Twist correlated with poor overall survival in patients with NSCLC [130]. Furthermore, co-expression of any two or all these markers correlated with a significantly worse prognosis, demonstrating the value of HIF-1 α , Snail, and Twist to predict the overall and recurrence-free survival in patients with resectable NSCLC. Although hypoxic induction of Snail transcription has been reported in multiple cancer types, an HRE in the minimal promoter of its gene *SNAIL* was only recently identified [131]. In addition to HIF-1 α and HIF-2 α binding to the HRE in its promoter region, expression of *SNAIL* can be upregulated during hypoxia via other mechanisms, namely, Notch signaling. In a range of tumor cell lines, hypoxic activation of Notch signaling induced EMT and promoted cell migration, invasion, and survival, effects that were attributed to direct upregulation of Snail and Slug expression, as well as the lysyl oxidase (LOX)-dependent stabilization of Snail protein [132, 133].

In addition to E-cadherin downregulation, hypoxia can increase the metastatic potential of tumor cells via other mechanisms. In NSCLC cell lines, HIF-1 α activation of MET sensitized tumor cells to HGF stimulation, leading to ECM degradation, cell dissociation, and increased cell migration through the lung parenchyma. Activation of the WNT/ β -catenin signaling pathway through HIF-1 α can also induce prostate cancer cells to be more motile and invasive [134, 135]. HIF-1 α -dependent activation of TGF- β 1 signaling and upregulation of survivin also contributes to EMT and resistance to apoptosis, respectively, in human NSCLC cells [136, 137]. Recently, hypoxia-induced metastasis has been linked to activation of the c-MYC pathway, upregulation of membrane-type 4 MMP via Slug, and induction of LOX, the latter of which acts both extracellularly to stabilize collagen deposition and intracellularly to foster EMT thru stabilization of Snail [132, 138–140].

Along with its role as a transcription factor, which mediates the canonical hypoxia response via binding to HRE, HIF- α subunits can also regulate cellular functions through molecular interactions with other signaling pathways to induce EMT, invasion, and metastasis. Thus, it is evident that hypoxia plays a critical role in the TME to promote lung carcinogenesis, and it is increasingly clear that induction and regulation of the EMT program is one of its main malignancy effector arms.

Reactive Oxygen Species (ROS)

ROS are products of normal cellular metabolism that help to maintain cellular homeostasis. Under physiological conditions, there is nearly constant low-level generation of ROS that act as signaling and regulatory mediators in pathways involving metabolism, cell cycle, intracellular reduction, cellular redox, and apoptosis [141–145]. Excessive or sustained ROS production associated with infection or irritation results in oxidative stress that causes oxidative damage to cellular constituents, such as lipids, proteins, enzymes, and DNA. In the setting of chronic inflammation, persistent high-level production of ROS can cause considerable tissue damage and yield a number of pathologic processes conducive to lung carcinogenesis, including DNA damage, activation of proto-oncogenes, resistance to apoptosis, and activation of the EMT program [146].

Several intrinsic cellular mechanisms exist to address redox imbalance and prevent unregulated proliferation, sustained EMT, or the accumulation of DNA mutations, including cell cycle-specific growth arrest, initiation/termination of signal transduction pathways and gene transcription, and repair of damaged DNA. ROS-mediated epigenetic regulation of gene expression and ROS-induced oxidative stress are two other important mechanisms often underlying lung carcinogenesis. Oxidative stress causes damage to the DNA and disruption of the cell repair process that can lead to chromosomal abnormalities [147, 148]. Because cancer cells typically acquire permanent functional changes to their DNA that confer a selective growth or survival advantage, these chromosomal changes can then increase the rate at which mutations are present and lead to an elevated risk of cancer de-

velopment. Studies have definitively demonstrated the direct interaction of ROS with DNA that results in structural alterations, including DNA base pair deletions, small-scale insertions, base modifications, chromosomal changes/loss, and translocation of segments [149]. The adduct of hydroxyl radicals on DNA nucleobases is perhaps the most extensively studied type of oxidative DNA damage. The 8-hydroxy-2'-deoxyguanosine (8-OHdG) and/or its tautomeric 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) have proven to be important mutagenic adducts to DNA and potential biomarkers of carcinogenesis [150, 151]. In addition, ROS may initiate carcinogenesis by modifying DNA nucleobases, and causing certain changes in oncogenes and tumor suppressor genes [152, 153]. The tumor suppressor gene p53 is the most frequently altered gene that is commonly observed in squamous NSCLC and SCLC patients. P53 mutations are primarily G to T alterations produced by DNA adduct formation from carcinogens, such as the polycyclic hydrocarbons commonly present in the lungs of smokers [154, 155], that allow mutant p53 accumulation in the cytoplasm where it functions as an oncogene.

Apoptosis, including programmed cell death modulated by p53, is a normal physiological process that maintains the balance between cell division and cell death. Cancer cells utilize survival strategies to evade apoptosis and maintain autonomous proliferation. In the case of chronic inflammation-induced cancers, the rise of malignant cells may be prevented via apoptosis and phagocytosis by inflammatory cells [156, 157]. ROS can act as a potent intrinsic stimuli to activate apoptosis under stressful conditions [158–161], stimulating pro-apoptotic signaling molecules, including JNK and p38 [162]. However, studies suggest that ROS can also mediate pro-survival effects in a variety of cancer cells by activation of anti-apoptotic redox-sensitive pathways, such as NF- κ B and heat shock proteins [163]. Thus, dysregulated oxidant production appears to involve well-established mediators of pro-survival signaling, in addition to mediators of the EMT program.

However, the role of ROS in tumor progression is not just limited to macromolecule damage. ROS can also act as signaling molecules, modifying their molecular targets through the formation of cysteine oxidative adducts or disulfide bonds that play a critical role in redox signaling cascades [164]. In this way, ROS play an important role as second messengers that initiate pathways and cellular processes, including ROS homeostasis, proliferation, apoptosis, survival, mitochondrial oxidative stress, antioxidant gene regulation, and DNA damage response [165].

Finally, sustained ROS exposure may exert its pathological effect through ROS-mediated activation of selected transcription factors that promote cell proliferation and survival. For example, the nuclear factor erythroid-derived 2 (NRF2) is important for the transcriptional expression of enzymes involved in xenobiotic detoxification, antioxidant response, and proteome maintenance. In the setting of oxidative stress, NRF2 controls cell fate by upregulation of antioxidant response element-bearing genes. Expression of the NRF2-dependent proteins is critical for maintaining cellular redox homeostasis via clearance of toxins and carcinogens [166]. However, studies have also demonstrated the pro-tumorigenic role of the transcription factor NRF2. It is overexpressed in multiple human cancers and cell lines, providing cancer cells with a survival and growth advantage, and it is responsible for acquired chemoresistance [167].

ROS can also activate MAPK signaling, which results in the activation of the transcription factors, activation protein-1 (AP-1) and NF- κ B. AP-1 activation plays an important role in the initiation of important genes involved in inflammation, proliferation, and differentiation, along with the induction of transformation and carcinogenesis [168–170]. Studies indicate that cellular thiol redox status is important for regulating stress-activated signal transduction pathways, including JNK and p38 kinase [171]. Subsequent AP-1 activation leads to the induction of various pro-inflammatory mediators commonly observed in an inflammatory response [172, 173]. Similarly, ROS-induced NF- κ B mediates the expression of several genes involved in inflammation, growth, differentiation, and survival, as well genes in the EMT program [174, 175]. ROS activates NF- κ B and regulates the expression of IL-6, TNF- α , IL-1 β , IL-8, iNOS, E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1), for example, [174, 176, 177]. Thus, sustained production of ROS is an important inducer of the feed-forward loop that connects chronic inflammation to EMT through NF- κ B.

Cigarette-Related Compounds

Cigarettes are replete with compounds that are known inducers of chronic lung inflammation. Tobacco smoke plays a critical role in increasing the risk of lung cancer by generating ROS, which cause oxidative damage and increase the production of pro-inflammatory mediators in the lung. COPD and emphysema are co-morbid conditions often found in lung cancer patients [100, 143]. The inflammatory pathways that link COPD, emphysema, and lung cancer likely involve genetic and epigenetic modulations due to chronic tissue injury and abnormal tumor immunity in susceptible hosts. While it has long been recognized that chronic inflammation is complicit in the pathogenesis of COPD and lung cancer, there is now increasing recognition that the EMT signaling program may be the linchpin that connects inflammation to COPD and lung cancer development.

For example, one recent study found a robust genomic link between COPD, lung cancer, and hedgehog signaling, the latter of which is a major signaling node activated during tobacco-smoke-induced EMT [178–180]. In a separate study, tobacco smoke and nicotine were shown to induce EMT in bronchial epithelial cells in a TGF- β -, Wnt3a-, and β -catenin-dependent manner [181]. Overexpression of the urokinase plasminogen activator receptor (uPAR) has also been implicated in regulation of tobacco-smoke-induced EMT of HBECs [179]. More specifically, mice chronically exposed to smoke had increased levels of well-known mediators of EMT, including TGF- β . In addition, low E-cadherin levels were associated with poor prognosis and correlated with the number of pack years in patients with lung cancer [182]. Other distinctive morphological characteristics of EMT have been documented in the airways of patients with smoking-related COPD [179].

Strategies to eliminate cigarette smoking have led to the emergence of new tobacco-related products as alternatives for cigarette smoking or tools for smoking

cessation. The electronic cigarette (ECIG) is a battery-powered electronic nicotine delivery system (ENDS) designed to deliver nicotine without combusting tobacco. The cartridge is heated through an inhalation-activated mechanism to produce a vapor that is then inhaled through the mouth and into the lungs. The refillable cartridge consists of a base liquid that typically contains a humectant, such as propylene glycol or vegetable glycerin (or a mixture of these components), nicotine, and flavoring.

Currently, ECIGs are advertised as a safer alternative to traditional tobacco cigarettes (TCIGs) and as a smoking cessation tool due to (a) lack of combustion and (b) the fact that nicotine is widely considered the addictive component in tobacco with limited ability to initiate cancer. However, there are concerns regarding the safety of long-term nicotine use in former smokers and its concurrent use with smoking. Preclinical models suggest that nicotine is the “estrogen of lung cancer” and may augment lung carcinogenesis by promoting tumor progression and metastasis in already established precancerous lesions [183, 184]. Nicotine and nicotine-derived nitrosamine ketone (NNK) signal through nicotinic acetylcholine receptors (nAChRs) to activate various growth-promoting and anti-apoptotic signaling pathways, including the NF- κ B, PI3K, MAPK, AKT, and SRC pathways [185, 186]. The discovery of nAChRs on human lung epithelial cells and lung cancers led to the observation that nicotine promotes lung cancer cell proliferation, invasion, and angiogenesis [187–190]. Activation of these pathways by nicotine also induces EMT, promotes anchorage-independent growth, and confers apoptosis resistance of lung cancer cells [183, 188, 191]. Furthermore, some studies have shown that nicotine itself induces the growth and metastasis of tumors in mice, independent of other tobacco carcinogens [182, 192, 193]. Taken together, these studies support the hypothesis that nicotine enhances carcinogenesis. This ultimately raises the concern that ECIGs may promote carcinogenesis in former and current smokers with precancerous lesions. Minimally, it suggests that ECIGs should not be advertised as “harmless,” as is the case currently.

Two additional controversies surrounding ECIGs are the lack of quality control standards applied to their manufacture, and the paucity of data on their safety and long-term health effects. Studies analyzing the content of the ECIG cartridge and/or vapor revealed the presence of major tobacco-specific nitrosamines, volatile organic compounds, and metals, none of which were disclosed in the product description or labeling [194–196]. Because ECIG use requires a much higher vacuum than TCIG use (which generally results in deeper inhalation) [197], it is possible that these compounds and nanoparticles are inhaled directly and more deeply into the lung, perhaps gaining access to the bloodstream and other organs. Similarly, the effects of various ECIG refill liquids on pulmonary fibroblasts revealed varying degrees of cytotoxicity, even between samples from the same brand and flavor with identical product labeling [198]. The results of the few available studies examining the short-term impact of ECIG usage are conflicting. Vansickel et al. assessed the acute effects of short-term ECIG exposure on plasma nicotine, heart rate, and carbon monoxide and found no significant changes in these measures [199]. Yet in another study, ECIGs were reported to have immediate (following 5 min of use)

adverse physiologic effects similar to tobacco smoking, including increased respiratory resistance and decreased fractional exhaled nitric oxide (FeNO) [200]. Because there are no studies examining the long-term effects of inhaled propylene glycol in humans, it is unclear whether exposure to vaporized nicotine in propylene glycol is truly harmless [201–203]. Taken together, there is no adequate public awareness that some ECIG products contain carcinogens and other potentially harmful constituents. Additional studies are required to comprehensively evaluate the impact of ECIGs on public health, including their impact on the inflammation-EMT-cancer axis.

Contribution of EMT to Lung Cancer

EMT is a physiological process whereby epithelial cells, normally interacting with adjacent cells and a basement membrane, undergo a series of biological changes that lead to a more fibroblast- or mesenchymal-like phenotype, including loss of cell–cell contacts, dissolution of polarity, and increased motility. EMT is a critical mechanism for metastasis, thus it is most commonly viewed as a late event, occurring well after a tumor has developed [204]. The molecular program mediating EMT is complex [205], but loss of the adherens junction protein E-cadherin via upregulation of the Snail, bHLH, and Zeb families of transcription factors is considered the hallmark feature of EMT that leads to the well-known metastatic behavior of tumor cells. Relatively, recent studies revealed that upregulation of members of these E-cadherin transcriptional repressor families and activation of EMT also mediate a variety of important tumor-*initiating* and -*promoting* characteristics, including expansion of stem cell phenotypes, anchorage-independent growth, apoptosis resistance, and early micrometastatic seeding. In the sections that follow, we describe the contribution of inflammation-induced EMT to established lung cancer and lung cancer initiation, covering both seminal reports in the field, as well as the most significant recent advances.

Contribution to Established Lung Cancer

The importance of the interaction between a tumor and its microenvironment clearly applies to the role of EMT in the progression of established lung cancers. In a study of human NSCLC specimens co-stained for Snail and E-cadherin, we discovered a non-uniform pattern of E-cadherin downregulation in the presence of Snail upregulation, with more pronounced repression of E-cadherin at the invading edge of the tumor [107]. This phenomenon likely reflects interactions between the tumor and variable EMT effectors in the TME. As described previously, these EMT effectors are often inflammatory mediators. The mechanisms involved in the interplay between inflammation and EMT in lung cancer progression are diverse, as are the malignant phenotypes that result from these complex interactions.

Inflammation Induces EMT

A small subset of the many inflammatory mediators known to populate the lung TME were described earlier, along with a brief description of the experiments that demonstrated how the cytokines, growth factors, enzymes, and other signaling mediators induce EMT to promote the progression and metastasis of lung cancer. More recent studies have attempted to further elucidate how these EMT effectors and pathways interact with each other in the TME to promote the growth and invasion of established tumors. In one such study, Chen et al. investigated the interaction between two known EMT effectors, IL-6 and CCL2 [206]. Both IL-6 and CCL2 are found in the lung TME and both have previously been shown to induce EMT in lung cancer [206]. Using a lung cancer cell line and an HBEC line, Chen and colleagues demonstrated how the induction of EMT and increased invasiveness resulting from IL-6 exposure was significantly enhanced with the addition of CCL2. The combination of IL-6 and CCL2 cooperatively elicited STAT3 phosphorylation, which then upregulated the production of IL-6 and CCL2, constituting a positive feedback loop that supported sustained EMT. The synergism of multiple EMT effectors in the lung TME illustrates the importance of considering both individual factors and the context in which they are found when designing future investigations or planning potential therapeutic interventions.

EMT Induces Inflammation

The relationship between inflammation and EMT in tumor progression is complex. Numerous studies now suggest that one mechanism by which EMT promotes the progression and metastasis of established lung cancers is via the induction of potent inflammatory mediators and pathways. One such example is the link between EMT and the CXCR2 axis. The CXCR2 ligands, CXCL8, and CXCL5, have been shown to be elevated in human NSCLC and play a role in the development of inflammation, angiogenesis, and carcinogenesis [207–210]. For example, ectopic Snail overexpression in human lung cancer cell lines promoted increased tumor burden and metastases *in vivo* [107]. Snail overexpressing tumors had elevated levels of CXCL8 and CXCL5 compared to vector control tumors, and the increased tumor burden associated with Snail overexpression was completely abrogated with CXCR2 blockade. This suggests that one mechanism by which Snail overexpression and EMT leads to tumor progression and metastasis in lung cancer is through the inflammatory CXCR2 pathway.

Another example of the complex interplay between EMT and inflammation in the promotion of lung cancer metastasis is described in the study by Risolino et al. [211]. In this study, the authors found that Pbx-regulating protein-1 (PREP1), a transcription factor known to induce EMT, impacts tumor progression by significantly sensitizing cells to the pro-metastatic effects of TGF- β via the SMAD3 pathway. A concentration of TGF- β as low as 0.1 ng/ml was sufficient to induce EMT in a lung cancer cell line in the context of PREP1 overexpression, whereas a fivefold higher cytokine concentration was required to produce the same effects in the control cell

line. Therefore, by modulating the threshold of sensitivity of a cell to a cytokine, an inducer of EMT is able to manipulate an inflammatory pathway to enhance EMT and tumor invasiveness.

Diverse Malignant Phenotypes, Including Resistance to Therapies

Although a great deal of research focuses on the metastatic potential conferred by the induction of EMT, numerous studies have also shown that EMT contributes to more than enhanced invasiveness during tumor progression. For example, gene expression profiling of lung cancer cell lines in which EMT had been induced by Snail overexpression revealed differential gene expression and suggested that diverse aspects of lung cancer progression are influenced by EMT, including angiogenesis, invasion, cell cycle regulation, CSCs, and the inhibition of tumor suppression [107]. In addition to its impact on these malignant phenotypes, EMT has also been shown to contribute to the development of resistance to standard lung cancer therapies. In a study by Kim et al., for example, initial radiation exposure of lung cancer cell lines led to activation of a Janus kinase 2 (JAK2)-p21-activated Ser/Thr kinase 1 (PAK1)-Snail signaling pathway which induced EMT and subsequent radioresistance of the cancer cells [115]. Also, EMT has been shown to confer resistance to systemic chemotherapies. In one study, the inhibition of Zeb1 in docetaxel-resistant human lung adenocarcinoma cells significantly enhanced their chemosensitivity [212]. Of particular note, E-cadherin downregulation or a gene signature associated with a mesenchymal phenotype, rather than epithelial phenotype, has been associated specifically with resistance to EGFR tyrosine kinase inhibitors (TKIs) in NSCLC [213–216].

In another interesting new study by Izumchenko et al., the authors suggest a potential mechanism that links inflammation-mediated EMT and EGFR TKI resistance [34]. They demonstrate that during TGF- β -mediated EMT, inhibition of the miR-200 family results in upregulation of MIG6, a negative regulator of EGFR. This occurs concomitantly with a TGF- β -induced EMT-associated kinase switch of tumor cells to an AKT-activated EGFR-independent state that is refractory to EGFR TKIs. The study authors also found that in the analysis of PDX lung cancers carrying wild-type EGFR, the MIG6/miR-200 ratio was inversely correlated with response to erlotinib in vivo, suggesting a potential role for this ratio as a predictive biomarker. Future studies that reveal the mechanisms behind EMT-induced drug resistance will be critical for the development of effective treatment strategies for lung cancer.

Contribution of EMT to Lung Cancer Initiation

As previously stated, a substantial body of work now indicates that upregulation of E-cadherin transcriptional repressor family members and subsequent activation of EMT mediate tumor-initiation, in addition to the metastasis of advanced stage

cancers. At the time, this discovery represented such a significant break from the prevailing dogma that new models (discussed below) were proposed to account for the paradigm shift. The new data and models also spurred a series of high impact commentaries by experts in the field to make clear the considerable clinical implications of the discovery. In the sections that follow, we present the seminal reports and expert commentaries that first described the contribution of inflammation-induced EMT to tumor initiation, and we review recent findings that have advanced the field. Finally, we present a recent shift in thinking about the “mechanics” of cell dissemination by investigators studying the role of EMT in carcinogenesis.

The Stem Cell Connection

Tumors harbor CSCs—perhaps more appropriately called cancer-initiating cells (CICs)—that are capable of giving rise to new tumors with all the cellular and molecular heterogeneity characteristic of the original tumor [217–219]. The identities and origins of these CICs within adult tissues and the mechanisms by which they drive carcinogenesis are areas of intense investigation. As previously described, many components of the developing lung TME, including inflammatory mediators, hypoxia, ROS, and cigarette smoke/vapor exposure, induce EMT. Many of these same factors have also been associated with CICs and carcinogenesis, suggesting that the inflammatory TME drives expansion and possible malignant conversion of stem cells into CICs via induction of EMT. Of note, the preponderance of research related to CICs, in particular, inflammation-driven and EMT-mediated stemness, has come from the breast cancer research community, and thus far the dearth of similar studies pertaining to lung carcinogenesis is striking.

In the seminal publication linking EMT, stemness, and carcinogenesis, Mani et al. demonstrated that immortalized human mammary epithelial cells induced to undergo EMT also acquired expression of stem cell markers [220]. Differentiated mammary epithelial cells that had undergone EMT via TGF- β treatment or ectopic overexpression of Snail or Twist gave rise to CD44⁺ CD24[–] cells with tumor-initiating capacity. An observational study utilizing human breast cancer specimens quickly followed and indicated that CICs isolated from breast cancer display a distinct EMT signature [221]. Ladybird homeobox 1 (LBX1), which directs expression of Snail, Zeb1, and Zeb2, was also noted to expand the CD44⁺ CD24[–] CIC subpopulation and to morphologically transform mammary epithelial cells [222]. Finally, in the setting of head and neck cancer, Yang et al. provided convincing evidence that Twist directly regulates the stemness factor B cell-specific Moloney murine leukemia virus integration site 1 (Bmi1) via cooperative repression of E-cadherin and p16. Both Twist and Bmi1 were required for the observation of EMT and tumor-initiating capacity, and both were associated with reduced patient survival [223]. In other investigations of lung carcinogenesis, Snail overexpression in immortalized HBECs was observed to drive expansion of stemness, anchorage-independent growth in vitro, and tumor formation and metastatic behavior in vivo [217]. Thus, there is now abundant evidence for CIC induction by EMT in numerous solid tumors, including in lung cancer [217–219].

Tumor Initiation

In the preceding pages, we provided numerous descriptions of inflammation-induced transcriptional repressors, like Snail and Zeb1, driving EMT and stemness that ultimately facilitate tumor initiation. However, there is increasing evidence that other features in the developing TME induce CICs, albeit more indirectly. For example, a hypoxic microenvironment is increasingly implicated as a driver of the EMT-stemness-carcinogenesis continuum. For example, cancer-associated fibroblasts (CAFs) have been shown to induce EMT and stemness through pro-inflammatory signaling which elicits COX-2/Rac1b-mediated release of ROS that ultimately drive a migratory and aggressive phenotype of prostate carcinoma cells [224]. This ROS-mediated induction of EMT and stemness by hypoxia is dependent on both NF- κ B and HIF-1 signaling. Using repetitive cycles of hypoxia and reoxygenation, Louie et al. also identified a CIC-like subpopulation of metastatic breast cancer cells with enhanced EMT and stemness phenotypes, as well as increased tumorigenic potential both *in vitro* and *in vivo* [225].

Inflammation-induced dysregulation of oncogene and tumor suppressor gene expression has also been investigated as a critical mediator of the EMT-stemness-carcinogenesis continuum. In one of the first accounts, Kurrey et al. described Snail and Slug activation of EMT, inactivation of p53-mediated apoptosis, and de-repression of stemness-associated genes under conditions of radiation- and drug-induced stress [226]. The authors proposed that the resulting CICs were capable of escaping the unfavorable primary tumor niche, traveling to distant sites, and surviving/colonizing the metastatic niche. This study led to the characterization of Snail and Slug as critical determinants of ovarian cancer progression and resistance to therapy. Using pancreatic epithelial cells derived from p53-/- mice that were also cultured under stress conditions, Pinho et al. described similar EMT and stemness features mediated by the self-renewal factor Bmi1 [227]. Chang et al. extended this observation in breast cancer by demonstrating that p53 is a transcriptional activator of the miR-200c gene [228]. Similarly, in their initial investigation of the putative intestinal stem cell marker DCAMKL-1 [229], Houchen and colleagues reported expression in both normal intestinal stem cells and colorectal cancer cells, evidencing promotion of tumorigenesis via miRNA- and c-myc-dependent mechanisms [230]. In a subsequent investigation by the group, DCAMKL-1 staining was also observed in both human pancreatic intraepithelial neoplasia lesions and pancreatic adenocarcinomas [231]. Knockdown of DCAMKL-1 in human pancreatic cell lines resulted in reduced expression of Snail, Slug, and Twist, elevated expression of miR-200a, downregulation of the proto-oncogenes c-myc and KRAS, and inhibition of Notch-1 via miRNA-dependent mechanisms. These new connections between EMT-induced stemness and critical genetic alterations that are widespread in human cancers (e.g., p53, c-myc, and KRAS), and in the tissues of those at-risk for cancer development, suggest that the EMT-stemness-carcinogenesis continuum may be widely applicable across cancer types. We anticipate identification of novel and abundant targets for prevention and therapy as the relationships between inflammation, EMT, and stemness in lung cancer initiation are further explored.

MicroRNAs are also induced and regulated by chronic inflammation and numerous components of the developing TME. A body of literature now substantiates regulation of the EMT-stemness-carcinogenesis continuum by miRNA. In one of the earliest studies of pancreatic and colon cancers, Wellner et al. discovered that Zeb1 promotes tumorigenicity by repressing stemness-inhibiting miRNAs [232]. In follow-up investigations, they determined that the miR-200 family targets Notch pathway components, such as Jagged1, to mediate enhanced Notch activation of Zeb1 in two aggressive types of human solid tumors [233]. MiR-200 essentially counteracts classical EMT properties, such as cell motility, and suppresses translation of stem cells factors, including Bmi1. In a model of CRC, Snail directly induced ZNF281 transcription and repressed miR-34a/b/c, thereby alleviating ZNF281 mRNA from direct downregulation by miR-34 [114]. Ectopic expression of ZNF281 was associated with malignant phenotypes, including EMT, migration, invasion, stemness, and sphere formation, while downregulation of ZNF281 attenuated these phenotypes in vitro and inhibited lung metastases in vivo. Tellez et al. used tobacco carcinogen exposure of immortalized HBECs to induce EMT and stemness phenotypes [234]. The observed induction of EMT was driven initially by epigenetic silencing of miR-200 and miR-205 that included chromatin remodeling with subsequent promoter methylation. Similarly, Song et al. recently reported that miR-22 triggered EMT, invasiveness, and metastasis in a mouse xenograft model, demonstrating that miR-22 exerted its metastatic potential by silencing anti-metastatic miR-200 [118]. This miR-200 silencing occurred via the direct targeting of the TET family of methylcytosine dioxygenases, which thereby inhibited demethylation of the miR-200 promoter.

The precise identity of the lung CIC or the aberrant stemness signaling pathways responsible for lung carcinogenesis remains controversial. However, it is abundantly clear that pathways associated with EMT (e.g., PGE2 or TGF- β) and stem cell maintenance (e.g., Wnt, Notch, miR-200, or miR-34a), or potentially the signaling nodes connecting these two pathways, are ideal points for investigations and eventual clinical intervention in lung carcinogenesis.

Premalignant Dissemination and Micrometastatic Seeding

The originally proposed and still prevailing model of lung cancer progression, termed the “linear progression model,” places the focus on the fully malignant primary tumor and its size, and metastatic dissemination is conditional upon both [110, 235]. Conversely, the more recently posited “parallel progression model” proposes that metastases may also arise from the early dissemination of premalignant epithelial cells prior to their full malignant conversion or collective growth into a large primary tumor [110, 235]. As per the linear progression model, EMT only occurs in rare cells at the leading invasive edge of advanced cancers, facilitating the final step of tumor progression—metastasis. However, many groups have now demonstrated that EMT also drives malignant transformation and early dissemination of epithelial

malignancies, including in tobacco-related cancers [110, 111, 236]. Harold Varmus and others have shown that in both murine models of breast cancer, as well as in breast cancer patients, (a) metastatic seeding occurs independent of primary tumor size and (b) primary tumor size does not imply a more genetically advanced and disseminated cell [237, 238]. These same studies indicate that during a premalignant stage, specifically ductal carcinoma in situ, metastatic seeding had already occurred in bone marrow, a common metastatic site for breast cancer, at the same frequency as late-stage disease. At the time, these alternate models of tumor initiation and dissemination and the evidence supporting them represented paradigm shifts in terms of our understanding of the protracted process of epithelial cell conversion from normal to cancer and its dissemination to distant organs [110].

Consistent with the parallel progression model and the findings of the Varmus group, it has been suggested that EMT also promotes dissemination of lung epithelial cells prior to, or concomitant with, their malignant conversion. Importantly, the parallel progression model may represent a more accurate model of lung cancer progression, given the clinical observation that 50% of the patients with early-stage lung cancer undergoing surgery return with metastatic disease, an indication that micrometastatic disease below the detection limits of our current imaging modalities was already present. Unfortunately, irrefutable evidence for the existence of parallel progression *in vivo* and *in situ* is still limited. In recent years, John Condeelis et al. have made significant strides toward this objective [239–241], but the imaging windows and advanced single cell tracking modalities developed by this group for the study of breast cancer dissemination have not yet been extended to other organs, such as the lung. Moreover, the mechanisms underlying parallel progression remain ill-defined at present, both in the setting of lung cancer and all other solid tumor types. These mechanisms, plus the actual mechanical properties, underlying premalignant dissemination and micrometastatic seeding are currently the subject of intense investigation.

One way in which the EMT program may facilitate the dissemination of premalignant cells to distant sites is by mediating biochemical and biophysical alterations in the ECM that would normally restrict the movement of the cells. For example, LOX-mediated collagen crosslinking is associated with increased matrix stiffness, and it appears to play a critical role in tumor invasion and metastasis [242]. Early studies by Paszek et al. revealed that increasing ECM stiffness yielded a malignant phenotype associated with activated FAK and ERK signaling [243]. This LOX-mediated ECM stiffening promoted tumor progression *in vivo* partially via activated FAK signaling [244]. Treatment with an LOX inhibitor reduced focal adhesions and PI3K signaling, demonstrating that LOX modulates tumor progression through ECM stiffening to drive focal adhesion assembly. In a separate study, ECM stiffening was required to cooperate with TGF- β to induce EMT in human breast tumor cells [245]. Finally, in a recent study of lung adenocarcinoma, altering both ECM stiffness and the concentration of cell-adhesive ligands in the TME significantly influenced epithelial morphogenesis as indicated by differences in the (a) extent of lumen formation, (b) patterns of intrasphere apoptosis and prolifera-

tion, and (c) expression of epithelial polarity markers [246]. Together, these findings suggest that mechanical properties of the TME are intimately linked to EMT and likely impact each of the steps described by the parallel progression model of lung carcinogenesis.

Mediators of EMT also likely play a role in early dissemination or micrometastatic seeding by altering the mechanical properties of the epithelial or tumor cells directly. The tools for investigating the molecular properties of these cells are advancing rapidly [247], along with the ability to integrate these physical measures with the genomic characteristics and functional profiles associated with a cell. For example, a correlation between the presence of filopodia on a murine mammary carcinoma line and metastatic outgrowth was described [248], although the precise relationship to mechanical properties affected by the filopodia was not fully delineated. Another team determined that TGF- β -induced stemness and EMT were associated with higher tumor recurrence and distal lung metastasis risk, and they used several measures to correlate these to EMT-induced alterations in cell stiffness and adhesion forces [249]. Still another team used atomic force microscopy (AFM) to determine that tongue squamous cell carcinoma cells with higher metastatic potential show decreased elastic modulus compared to cells with lower metastatic potential [250]. Osborne et al. also demonstrated that TGF- β -induced EMT results in decreased stiffness and loss of the normal stiffening response to force applied on integrins [251]. Furthermore, they determined that suppression of two RhoA guanine nucleotide exchange factors (GEFs) via TGF-B/ALK5-enhanced proteasomal degradation mediated the altered cell mechanics, stiffness, and invasion.

Agents that Target EMT and Lung Cancer

At this time, there are no ongoing lung cancer clinical trials that directly target EMT or evaluate a patient's EMT status. Some of the challenges of targeting EMT as a therapeutic approach include: (a) EMT-inducing transcription factors that repress E-cadherin are technically difficult to target using small molecular weight inhibitors; (b) even cancer-associated EMT is plastic, and mesenchymal cells may revert back to an epithelial state after dissemination or metastasis; and (c) EMT may be manifested only in a subset of tumor cells, such as the CICs or cells at the host-tumor interface, making it difficult to monitor the cells of interest. Therefore, targeting EMT *indirectly*, by focusing on EMT effectors, may be the most fruitful strategy to pursue at present. For example, it has been suggested that an miRNA-based therapeutic meant to reintroduce miR-200, a master post-transcriptional regulator of EMT, may be a feasible and impactful approach [252]. Whether this approach or another, therapeutics that successfully impact the EMT program for the treatment of lung cancer will likely involve the inhibition of inflammation-induced EMT effectors that are abundant in the TME and potentially induce EMT and other critical malignant phenotypes.

Primary Therapy

A number of drugs targeting inflammatory mediators known to induce EMT are already in clinical trials for the treatment of lung cancer. Part of the potential therapeutic effect of these drugs may be due to their impingement on the EMT program. For example, phase III clinical trials involving a COX-2 inhibitor (celecoxib), as well as phase II clinical trials of a small molecule inhibitor of TGF- β (tasisulam sodium), have been conducted in patients with advanced NSCLC, although with minimal proven benefit [253, 254]. Agents targeting the IL-6R/JAK/STAT3 pathway have also been developed and are in early phase clinical studies in advanced lung cancers [255]. Numerous preclinical studies are also investigating novel methods of combating inflammation-mediated EMT and tumor progression. In a study by Kim et al., the active component in ginseng was found to significantly inhibit TGF- β -induced EMT, invasion, and anoikis resistance of lung cancer cell lines, possibly providing an eventual alternative to conventional anti-EMT strategies [256].

Combined Therapies

Evolutionarily critical signaling programs, such as EMT, are characterized by extensive cross talk and effector redundancy. Therefore, therapeutic strategies will likely be more effective if they combine multiple drugs to address more than one EMT effector at a time and/or more than one functional arm of tumor progression at a time. For example, clinical trials have combined COX-2 inhibitors with both systemic chemotherapies or targeted therapies, such as the EGFR TKIs [253, 257, 258]. Another reason to combine therapeutic strategies relates to drug resistance. As previously described, EMT is associated with resistance to therapies. In a study by Li et al., the authors found that metformin reversed EMT and decreased IL-6 signaling activation in TKI-resistant lung cancer cell lines [259]. In addition, metformin was able to overcome IL-6-induced TKI resistance in TKI-sensitive lung cancer cell lines. They then demonstrated that the combination of metformin with gefitinib (an EGFR TKI) was able to potentiate gefitinib-induced anti-tumor activity in mouse xenografts established with EGFR TKI-resistant lung cancer cells. As in this case, a two-pronged approach to targeting lung cancer will likely continue to be more efficacious clinically.

Concluding Remarks and Future Perspectives

In this chapter, we described the inflammation-EMT-cancer connection, along with the key intrinsic and extrinsic mediators of this important signaling axis. The cancer research community has come to recognize that the carcinogenesis process is governed by more than just the epithelial cell genome. The epithelial cell proteome

and epigenome are now thought to be equally important, along with the interactions of the epithelial cell with the developing TME. Thus, the adjacent histologically normal appearing epithelium, immune effector cells, inflammatory mediators, and stroma comprising the TME are critical and dynamic constituents that are now known to facilitate the balance between acute versus chronic inflammation, physiologic versus pathologic EMT, and normal versus malignant epithelium. The broad array of elements involved in the inflammation-EMT-cancer axis makes it inherently complex to investigate, but this complexity also means the axis is rich in potential targets that may be exploited for the prevention and treatment of lung cancer.

Other noteworthy paradigm shifts also occurred in recent years within the cancer research community. The description of EMT as an inducer of stemness and tumor initiation, instead of simply a driver of the metastasis of late-stage disease, was perhaps most significant. The initial description of the parallel progression model that provided an explanation for micrometastatic seeding and recurrence among those with early-stage disease was also significant. The studies of Varmus and Condeelis further informed our understanding of the parallel progression model, including the temporal and mechanical characteristics of epithelial cell dissemination. However, investigations of lung epithelial cell movement have lagged behind those in other organs. Technological advances are needed to perfect real-time single-cell imaging of epithelial cell trafficking to the lung. Methods to better identify and isolate epithelial cells that are heterogeneous in their dissemination potential are also needed. It is anticipated that studies of the mechanics of epithelial cell acquisition of cancer cell form, function, adhesion, motility, and deformability will make significant advances in the coming years. And a better description of the mechanical cues from the microenvironment that affects the regulation of EMT would likely lead to agents targeting this signal that prevents movement.

Dumont et al. first described the concept of sustained or memorized EMT in 2008, when they identified a role for DNA methylation-mediated gene silencing in the cancer-associated EMT process [260]. There is now renewed interest in epigenetic regulation of EMT plasticity and carcinogenesis [261–264]. Thus, more investigations of the functional interaction between EMT mediators, such as Snail, Slug, and Zeb1, and the modulators of chromatin configuration, are anticipated, because these may provide attractive targets for clinical intervention.

The importance of proper subcellular location to protein function, particularly the function of major transcription factors, such as EMT-promoting Snail and Slug, has recently been emphasized [265]. This compartmentalization allows the organism to maintain tight regulation of potent transcription factors, facilitating the controlled plasticity described in the early pages of this chapter. In the setting of cancer, however, this complex regulatory process is often subverted, leading to the mislocalization of the Snail/Slug proteins and often yielding pathologic EMT. Several agents were recently shown to target the transcription factor nuclear transport machinery with great specificity, and preliminary results using nuclear export inhibitors in a cell-based model suggest that they reverse EMT and induce apoptosis. Along with the results of other investigators [266], we anticipate that these findings will spur further exploration of the potential to target EMT at the level of nuclear transport.

Similarly, several key studies have now highlighted the role of splicing factors, such as the epithelial splicing regulatory proteins (ESRPs) and RNA-binding FOX protein 2 (RBfox2), in the post-transcriptional regulation of EMT and carcinogenesis [267–270]. Additional studies are anticipated linking these splicing factors to carcinogenesis.

Finally, the appropriate patient selection appears to be a prerequisite for the success of targeted therapies in lung cancer. The concepts of risk stratification, patient selection, targeted therapy, and rational combinatorial therapeutic agent selection have been critical for most of the clinical successes enjoyed in recent years. These same concepts are now being advocated for lung cancer prevention [271], including any prevention targets discovered within the candidate-rich inflammation-EMT-cancer axis.

Acknowledgments Research reported in this publication was supported in part by funding from the National Cancer Institute (#T32-CA009120-36 and #U01CA152751), Department of Veteran Affairs (#2101BX000359-05A1), and Tobacco-Related Disease Research Program (#18FT-0060 and #20KT-0055).

References

1. Barbieri SS, Weksler BB (2007) Tobacco smoke cooperates with interleukin-1beta to alter beta-catenin trafficking in vascular endothelium resulting in increased permeability and induction of cyclooxygenase-2 expression in vitro and in vivo. *FASEB J* 21(8):1831–1843
2. Rom O, Avezov K, Aizenbud D, Reznick AZ (2013) Cigarette smoking and inflammation revisited. *Respir Physiol Neurobiol* 187(1):5–10
3. Walser T, Cui X, Yanagawa J, Lee JM, Heinrich E, Lee G, et al. (2008) Smoking and lung cancer: the role of inflammation. *Proc Am Thorac Soc* 5(8):811–815
4. Heinrich EL, Walser TC, Krysan K, Licican EL, Grant JL, Rodriguez NL, Dubinett SM (2012) The inflammatory tumor microenvironment, epithelial mesenchymal transition and lung carcinogenesis. *Cancer Microenviron* 5(1):5–18
5. O’Callaghan DS, O’Donnell D, O’Connell F, O’Byrne KJ (2010) The role of inflammation in the pathogenesis of non-small cell lung cancer. *J Thorac Oncol* 5(12):2024–2036
6. Balkwill FR, Mantovani A (2012) Cancer-related inflammation: common themes and therapeutic opportunities. *Semin Cancer Biol* 22(1):33–40
7. Gomes M, Teixeira AL, Coelho A, Araujo A, Medeiros R (2014) The role of inflammation in lung cancer. *Adv Exp Med Biol* 816:1–23
8. Franks AL, Slansky JE (2012) Multiple associations between a broad spectrum of autoimmune diseases, chronic inflammatory diseases and cancer. *Anticancer Res* 32(4):1119–1136
9. Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. *Cell* 144(5):646–674
10. Pasche B, Wang M, Pennison M, Jimenez H (2014) Prevention and treatment of cancer with aspirin: where do we stand? *Semin Oncol* 41(3):397–401
11. Rothwell PM, Fowkes FG, Belch JF, Ogawa H, Warlow CP, Meade TW (2011) Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. *Lancet* 377(9759):31–41
12. Thorat MA, Cuzick J (2013) Role of aspirin in cancer prevention. *Curr Oncol Rep* 15(6):533–540
13. Thun MJ, Jacobs EJ, Patrono C (2012) The role of aspirin in cancer prevention. *Nat Rev Clin Oncol* 9(5):259–267
14. Bauer AK, Rondini EA (2009) Review paper: the role of inflammation in mouse pulmonary neoplasia. *Vet Pathol* 46(3):369–390

15. de Visser KE, Eichten A, Coussens LM (2006) Paradoxical roles of the immune system during cancer development. *Nat Rev Cancer* 6(1):24–37
16. Schafer M, Werner S (2008) Cancer as an overhealing wound: an old hypothesis revisited. *Nat Rev Mol Cell Biol* 9(8):628–638
17. Thiery JP (2003) Epithelial-mesenchymal transitions in development and pathologies. *Curr Opin Cell Biol* 15(6):740–746
18. Lee K, Nelson CM (2012) New insights into the regulation of epithelial-mesenchymal transition and tissue fibrosis. *Int Rev Cell Mol Biol* 294:171–221
19. Kalluri R, Weinberg RA (2009) The basics of epithelial-mesenchymal transition. *J Clin Invest* 119(6):1420–1428
20. Giese A, Loo MA, Tran N, Haskett D, Coons SW, Berens ME (1996) Dichotomy of astrocytoma migration and proliferation. *Int J Cancer* 67(2):275–282
21. Hatzikirou H, Basanta D, Simon M, Schaller K, Deutsch A (2012) Go or grow: the key to the emergence of invasion in tumour progression? *Math Med Biol* 29(1):49–65
22. Zeisberg M, Neilson EG (2009) Biomarkers for epithelial-mesenchymal transitions. *J Clin Invest* 119(6):1429–1437
23. Massague J (2008) TGFbeta in Cancer. *Cell* 134(2):215–230
24. Samet JM (2000) Does idiopathic pulmonary fibrosis increase lung cancer risk? *Am J Respir Crit Care Med* 161(1):1–2
25. Willis BC, Borok Z (2007) TGF-beta-induced EMT: mechanisms and implications for fibrotic lung disease. *Am J Physiol Lung Cell Mol Physiol* 293(3):L525–534
26. Roberts AB, Wakefield LM (2003) The two faces of transforming growth factor beta in carcinogenesis. *Proc Natl Acad Sci U S A* 100(15):8621–8623
27. Shintani Y, Maeda M, Chaika N, Johnson KR, Wheelock MJ (2008) Collagen I promotes epithelial-to-mesenchymal transition in lung cancer cells via transforming growth factor-beta signaling. *Am J Respir Cell Mol Biol* 38(1):95–104
28. Thuaud S, Valcourt U, Petersen M, Manfioletti G, Heldin CH, Moustakas A (2006) Transforming growth factor-beta employs HMGA2 to elicit epithelial-mesenchymal transition. *J Cell Biol* 174(2):175–183
29. Aoyama D, Hashimoto N, Sakamoto K, Kohnoh T, Kusunose M, Kimura M, et al. (2013) Involvement of TGFbeta-induced phosphorylation of the PTEN C-terminus on TGFbeta-induced acquisition of malignant phenotypes in lung cancer cells. *PLoS ONE* 8(11):e81133
30. Ohshio Y, Teramoto K, Hashimoto M, Kitamura S, Hanaoka J, Kontani K (2013) Inhibition of transforming growth factor-beta release from tumor cells reduces their motility associated with epithelial-mesenchymal transition. *Oncol Rep* 30(2):1000–1006
31. Abulaiti A, Shintani Y, Funaki S, Nakagiri T, Inoue M, Sawabata N, et al. (2013) Interaction between non-small-cell lung cancer cells and fibroblasts via enhancement of TGF-beta signaling by IL-6. *Lung Cancer* 82(2):204–213
32. Grant JL, Fishbein MC, Hong LS, Krysan K, Minna JD, Shay JW, et al. (2014) A novel molecular pathway for snail-dependent, SPARC-mediated invasion in non-small cell lung cancer pathogenesis. *Cancer Prev Res (Phila)* 7(1):150–160
33. Toh B, Wang X, Keeble J, Sim WJ, Khoo K, Wong WC, et al. (2011) Mesenchymal transition and dissemination of cancer cells is driven by myeloid-derived suppressor cells infiltrating the primary tumor. *PLoS Biol* 9(9):e1001162
34. Izumchenko E, Chang X, Michailidi C, Kagohara L, Ravi R, Paz K, et al. (2014) The TGFbeta-miR200-MIG6 pathway orchestrates the EMT-associated kinase switch that induces resistance to EGFR inhibitors. *Cancer Res* 74(14):3995–4005
35. Kitamura K, Seike M, Okano T, Matsuda K, Miyanaga A, Mizutani H, et al. (2014) MiR-134/487b/655 cluster regulates TGF-beta-induced epithelial-mesenchymal transition and drug resistance to gefitinib by targeting MAGI2 in lung adenocarcinoma cells. *Mol Cancer Ther* 13(2):444–453
36. Zarogoulidis P, Yarmus L, Darwiche K, Walter R, Huang H, Li Z, et al. (2013) Interleukin-6 cytokine: a multifunctional glycoprotein for cancer. *Immunome Res* 9(62):16535
37. Yadav A, Kumar B, Datta J, Teknos TN, Kumar P (2011) IL-6 promotes head and neck tumor metastasis by inducing epithelial-mesenchymal transition via the JAK-STAT3-SNAIL signaling pathway. *Mol Cancer Res* 9(12):1658–1667

38. Giannoni E, Bianchini F, Masieri L, Serni S, Torre E, Calorini L, Chiarugi P (2010) Reciprocal activation of prostate cancer cells and cancer-associated fibroblasts stimulates epithelial-mesenchymal transition and cancer stemness. *Cancer Res* 70(17):6945–6956
39. Bao B, Ali S, Ahmad A, Azmi AS, Li Y, Banerjee S, et al. (2012) Hypoxia-induced aggressiveness of pancreatic cancer cells is due to increased expression of VEGF, IL-6 and miR-21, which can be attenuated by CDF treatment. *PLoS ONE* 7(12):e50165
40. Bao B, Ahmad A, Kong D, Ali S, Azmi AS, Li Y, et al. (2012) Hypoxia induced aggressiveness of prostate cancer cells is linked with deregulated expression of VEGF, IL-6 and miRNAs that are attenuated by CDF. *PLoS ONE* 7(8):e43726
41. Dalwadi H, Krysan K, Heuze-Vourc'h N, Dohadwala M, Elashoff D, Sharma S, et al. (2005) Cyclooxygenase-2-dependent activation of signal transducer and activator of transcription 3 by interleukin-6 in non-small cell lung cancer. *Clin Cancer Res* 11(21):7674–7682
42. Yao Z, Fenoglio S, Gao DC, Camiolo M, Stiles B, Lindsted T, et al. (2010) TGF-beta IL-6 axis mediates selective and adaptive mechanisms of resistance to molecular targeted therapy in lung cancer. *Proc Natl Acad Sci U S A* 107(35):15535–15540
43. Korkaya H, Kim GI, Davis A, Malik F, Henry NL, Ithimakin S, et al. (2012) Activation of an IL6 inflammatory loop mediates trastuzumab resistance in HER2 + breast cancer by expanding the cancer stem cell population. *Mol Cell* 47(4):570–584
44. Giles KM, Kalinowski FC, Candy PA, Epis MR, Zhang PM, Redfern AD, et al. (2013) Axl mediates acquired resistance of head and neck cancer cells to the epidermal growth factor receptor inhibitor erlotinib. *Mol Cancer Ther* 12(11):2541–2558
45. Carstens JL, Lovisa S, Kalluri R (2014) Microenvironment-dependent cues trigger miRNA-regulated feedback loop to facilitate the EMT/MET switch. *J Clin Invest* 124(4):1458–1460
46. Rokavec M, Oner MG, Li H, Jackstadt R, Jiang L, Lodygin D, et al. (2014) IL-6R/STAT3/miR-34a feedback loop promotes EMT-mediated colorectal cancer invasion and metastasis. *J Clin Invest* 124(4):1853–1867
47. Aggarwal BB (2003) Signalling pathways of the TNF superfamily: a double-edged sword. *Nat Rev Immunol* 3(9):745–756
48. Kim V, Rogers TJ, Criner GJ (2008) New concepts in the pathobiology of chronic obstructive pulmonary disease. *Proc Am Thorac Soc* 5(4):478–485
49. Mukhopadhyay S, Hoidal JR, Mukherjee TK (2006) Role of TNFalpha in pulmonary pathophysiology. *Respir Res* 7:125
50. Wu Y, Deng J, Rychahou PG, Qiu S, Evers BM, Zhou BP (2009) Stabilization of snail by NF-kappaB is required for inflammation-induced cell migration and invasion. *Cancer Cell* 15(5):416–428
51. Kawata M, Koinuma D, Ogami T, Umezawa K, Iwata C, Watabe T, Miyazono K (2012) TGF-beta-induced epithelial-mesenchymal transition of A549 lung adenocarcinoma cells is enhanced by pro-inflammatory cytokines derived from RAW 264.7 macrophage cells. *J Biochem* 151(2):205–216
52. Li CW, Xia W, Huo L, Lim SO, Wu Y, Hsu JL, et al. (2012) Epithelial-mesenchymal transition induced by TNF-alpha requires NF-kappaB-mediated transcriptional upregulation of Twist1. *Cancer Res* 72(5):1290–1300
53. Shiozaki A, Bai XH, Shen-Tu G, Moodley S, Takeshita H, Fung SY, et al. (2012) Claudin 1 mediates TNFalpha-induced gene expression and cell migration in human lung carcinoma cells. *PLoS ONE* 7(5):e38049
54. Saito A, Suzuki HI, Horie M, Ohshima M, Morishita Y, Abiko Y, Nagase T (2013) An integrated expression profiling reveals target genes of TGF-beta and TNF-alpha possibly mediated by microRNAs in lung cancer cells. *PLoS ONE* 8(2):e56587
55. Apte RN, Voronov E (2002) Interleukin-1—a major pleiotropic cytokine in tumor-host interactions. *Semin Cancer Biol* 12(4):277–290
56. Colasante A, Mascetra N, Brunetti M, Lattanzio G, Diodoro M, Caltagirone S, et al. (1997) Transforming growth factor beta 1, interleukin-8 and interleukin-1, in non-small-cell lung tumors. *Am J Respir Crit Care Med* 156(3 Pt 1):968–973
57. Apte RN, Kreltin Y, Song X, Dotan S, Recih E, Elkabets M, et al. (2006) Effects of micro-environment- and malignant cell-derived interleukin-1 in carcinogenesis, tumour invasiveness and tumour-host interactions. *Eur J Cancer* 42(6):751–759

58. Giavazzi R, Garofalo A, Bani MR, Abbate M, Ghezzi P, Boraschi D, et al. (1990) Interleukin 1-induced augmentation of experimental metastases from a human melanoma in nude mice. *Cancer Res* 50(15):4771–4775
59. Tu S, Bhagat G, Cui G, Takaishi S, Kurt-Jones EA, Rickman B, et al. (2008) Overexpression of interleukin-1beta induces gastric inflammation and cancer and mobilizes myeloid-derived suppressor cells in mice. *Cancer Cell* 14(5):408–419
60. Krelin Y, Voronov E, Dotan S, Elkabets M, Reich E, Fogel M, et al. (2007) Interleukin-1beta-driven inflammation promotes the development and invasiveness of chemical carcinogen-induced tumors. *Cancer Res* 67(3):1062–1071
61. Yano S, Nokihara H, Yamamoto A, Goto H, Ogawa H, Kanematsu T, et al. (2003) Multifunctional interleukin-1beta promotes metastasis of human lung cancer cells in SCID mice via enhanced expression of adhesion-, invasion- and angiogenesis-related molecules. *Cancer Sci* 94(3):244–252
62. Heinrich EL, Charuworn B, Dohadwala M, Dubinett SM (2008) IL-1B dependent epithelial-mesenchymal transition in non-small cell lung cancer [abstract]. In: Proceedings of the Frontiers in Cancer Prevention Research Conference - November 16-18, Washington, DC : Cancer Prev Res 1(7 Suppl): Abstract 26
63. St John MA, Dohadwala M, Luo J, Wang G, Lee G, Shih H, et al. (2009) Proinflammatory mediators upregulate snail in head and neck squamous cell carcinoma. *Clin Cancer Res* 15(19):6018–6027
64. Dong GW, Do NY, Lim SC (2010) Relation between proinflammatory mediators and epithelial-mesenchymal transition in head and neck squamous cell carcinoma. *Exp Ther Med* 1(5):885–891
65. Dohadwala M, Wang G, Heinrich E, Luo J, Lau O, Shih H, et al. (2010) The role of ZEB1 in the inflammation-induced promotion of EMT in HNSCC. *Otolaryngol Head Neck Surg* 142(5):753–759
66. Kiefel H, Bondong S, Pfeifer M, Schirmer U, Erbe-Hoffmann N, Schafer H, et al. (2012) EMT-associated up-regulation of L1CAM provides insights into L1CAM-mediated integrin signalling and NF-kappaB activation. *Carcinogenesis* 33(10):1919–1929
67. Lee CH, Chang JS, Syu SH, Wong TS, Chan JY, Tang YC, et al. (2015) IL-1B promotes malignant transformation and tumor aggressiveness in oral cancer. *J Cell Physiol* 230(4): 875–884
68. Leibovich-Rivkin T, Liubomirski Y, Bernstein B, Meshel T, Ben-Baruch A (2013) Inflammatory factors of the tumor microenvironment induce plasticity in nontransformed breast epithelial cells: EMT, invasion, and collapse of normally organized breast textures. *Neoplasia* 15(12):1330–1346
69. Li Y, Wang L, Pappan L, Galliher-Beckley A, Shi J (2012) IL-1beta promotes stemness and invasiveness of colon cancer cells through Zeb1 activation. *Mol Cancer* 11:87
70. Birchmeier C, Birchmeier W, Gherardi E, Vande Woude GF (2003) Met, metastasis, motility and more. *Nat Rev Mol Cell Biol* 4(12):915–925
71. Trusolino L, Bertotti A, Comoglio PM MET signalling: principles and functions in development, organ regeneration and cancer. *Nat Rev Mol Cell Biol* 11(12):834–848
72. Birchmeier C, Gherardi E (1998) Developmental roles of HGF/SF and its receptor, the c-Met tyrosine kinase. *Trends Cell Biol* 8(10):404–410
73. Siegfried JM, Weissfeld LA, Luketich JD, Weyant RJ, Gubish CT, Landreneau RJ (1998) The clinical significance of hepatocyte growth factor for non-small cell lung cancer. *Ann Thorac Surg* 66(6):1915–1918
74. Siegfried JM, Luketich JD, Stabile LP, Christie N, Land SR (2004) Elevated hepatocyte growth factor level correlates with poor outcome in early-stage and late-stage adenocarcinoma of the lung. *Chest* 125(5 Suppl):116S–119S
75. Grotegut S, von Schweinitz D, Christofori G, Lehembre F (2006) Hepatocyte growth factor induces cell scattering through MAPK/Egr-1-mediated upregulation of snail. *EMBO J* 25(15):3534–3545
76. Li G, Schaidt H, Satyamoorthy K, Hanakawa Y, Hashimoto K, Herlyn M (2001) Down-regulation of E-cadherin and Desmoglein 1 by autocrine hepatocyte growth factor during melanoma development. *Oncogene* 20(56):8125–8135

77. Kominsky SL, Argani P, Korz D, Evron E, Raman V, Garrett E, et al. (2003) Loss of the tight junction protein claudin-7 correlates with histological grade in both ductal carcinoma in situ and invasive ductal carcinoma of the breast. *Oncogene* 22(13):2021–2033
78. Canadas I, Rojo F, Taus A, Arpi O, Arumi-Uria M, Pijuan L, et al. (2014) Targeting epithelial-to-mesenchymal transition with Met inhibitors reverts chemoresistance in small cell lung cancer. *Clin Cancer Res* 20(4):938–950
79. Yu G, Jing Y, Kou X, Ye F, Gao L, Fan Q, et al. (2013) Hepatic stellate cells secreted hepatocyte growth factor contributes to the chemoresistance of hepatocellular carcinoma. *PLoS ONE* 8(9):e73312
80. Ogunwobi OO, Puszyk W, Dong HJ, Liu C (2013) Epigenetic upregulation of HGF and c-Met drives metastasis in hepatocellular carcinoma. *PLoS ONE* 8(5):e63765
81. Dubois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, Van De Putte LB, Lipsky PE (1998) Cyclooxygenase in biology and disease. *FASEB J* 12(12):1063–1073
82. Lee JM, Yanagawa J, Peebles KA, Sharma S, Mao JT, Dubinett SM (2008) Inflammation in lung carcinogenesis: new targets for lung cancer chemoprevention and treatment. *Crit Rev Oncol Hematol* 66(3):208–217
83. Krysan K, Reckamp KL, Dalwadi H, Sharma S, Rozengurt E, Dohadwala M, Dubinett SM (2005) Prostaglandin E2 activates mitogen-activated protein kinase/Erk pathway signaling and cell proliferation in non-small cell lung cancer cells in an epidermal growth factor receptor-independent manner. *Cancer Res* 65(14):6275–6281
84. Hida T, Yatabe Y, Achiwa H, Muramatsu H, Kozaki K, Nakamura S, et al. (1998) Increased expression of cyclooxygenase 2 occurs frequently in human lung cancers, specifically in adenocarcinomas. *Cancer Res* 58(17):3761–3764
85. Krysan K, Dalwadi H, Sharma S, Pold M, Dubinett S (2004) Cyclooxygenase 2-dependent expression of survivin is critical for apoptosis resistance in non-small cell lung cancer. *Cancer Res* 64(18):6359–6362
86. Baratelli F, Lin Y, Zhu L, Yang SC, Heuze-Vourc'h N, Zeng G, et al. (2005) Prostaglandin E2 induces FOXP3 gene expression and T regulatory cell function in human CD4 + T cells. *J Immunol* 175(3):1483–1490
87. Dohadwala M, Batra RK, Luo J, Lin Y, Krysan K, Pold M, et al. (2002) 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* 277(52):50828–50833
88. Dohadwala M, Yang SC, Luo J, Sharma S, Batra RK, Huang M, et al. (2006) Cyclooxygenase-2-dependent regulation of E-cadherin: prostaglandin E(2) induces transcriptional repressors ZEB1 and snail in non-small cell lung cancer. *Cancer Res* 66(10):5338–5345
89. Tomlinson DC, Baxter EW, Loadman PM, Hull MA, Knowles MA (2012) FGFR1-induced epithelial to mesenchymal transition through MAPK/PLCgamma/COX-2-mediated mechanisms. *PLoS ONE* 7(6):e38972
90. Kirane A, Toombs JE, Ostapoff K, Carbon JG, Zaknoen S, Braunfeld J, et al. (2012) Apricoxib, a novel inhibitor of COX-2, markedly improves standard therapy response in molecularly defined models of pancreatic cancer. *Clin Cancer Res* 18(18):5031–5042
91. Adhim Z, Matsuoka T, Bito T, Shigemura K, Lee KM, Kawabata M, et al. (2011) In vitro and in vivo inhibitory effect of three Cox-2 inhibitors and epithelial-to-mesenchymal transition in human bladder cancer cell lines. *Br J Cancer* 105(3):393–402
92. Fujii R, Imanishi Y, Shibata K, Sakai N, Sakamoto K, Shigetomi S, et al. (2014) Restoration of E-cadherin expression by selective Cox-2 inhibition and the clinical relevance of the epithelial-to-mesenchymal transition in head and neck squamous cell carcinoma. *J Exp Clin Cancer Res* 33:40
93. St John MA, Wang G, Luo J, Dohadwala M, Hu D, Lin Y, et al. (2012) Apricoxib upregulates 15-PGDH and PGT in tobacco-related epithelial malignancies. *Br J Cancer* 107(4):707–712
94. Peebles KA, Lee JM, Mao JT, Hazra S, Reckamp KL, Krysan K, et al. (2007) Inflammation and lung carcinogenesis: applying findings in prevention and treatment. *Expert Rev Anticancer Ther* 7(10):1405–1421

95. Chapman HA (2011) Epithelial-mesenchymal interactions in pulmonary fibrosis. *Annu Rev Physiol* 73:413–435
96. Zheng H, Shen M, Zha YL, Li W, Wei Y, Blanco MA, et al. (2014) PKD1 phosphorylation-dependent degradation of SNAIL by SCF-FBXO11 regulates epithelial-mesenchymal transition and metastasis. *Cancer Cell* 26(3):358–373
97. Fuchs SY, Chen A, Xiong Y, Pan ZQ, Ronai Z (1999) HOS, a human homolog of Slimb, forms an SCF complex with Skp1 and Cullin1 and targets the phosphorylation-dependent degradation of IkappaB and beta-catenin. *Oncogene* 18(12):2039–2046
98. Kumar M, Allison DF, Baranova NN, Wamsley JJ, Katz AJ, Bekiranov S, et al. (2013) NF-kappaB regulates mesenchymal transition for the induction of non-small cell lung cancer initiating cells. *PLoS ONE* 8(7):e68597
99. Sun Q, Yao X, Ning Y, Zhang W, Zhou G, Dong Y (2013) Overexpression of response gene to complement 32 (RGC32) promotes cell invasion and induces epithelial-mesenchymal transition in lung cancer cells via the NF-kappaB signaling pathway. *Tumour Biol* 34(5):2995–3002
100. Zhao Y, Xu Y, Li Y, Xu W, Luo F, Wang B, et al. (2013) NF-kappaB-mediated inflammation leading to EMT via miR-200c is involved in cell transformation induced by cigarette smoke extract. *Toxicol Sci* 135(2):265–276
101. Sheshadri N, Catanzaro JM, Bott AJ, Sun Y, Ullman E, Chen EI, et al. (2014) SCCA1/SERPINE3 promotes oncogenesis and epithelial-mesenchymal transition via the unfolded protein response and IL6 signaling. *Cancer Res* 74(21): 6318–6329.
102. Horiguchi K, Shirakihara T, Nakano A, Imamura T, Miyazono K, Saitoh M (2009) Role of Ras signaling in the induction of snail by transforming growth factor-beta. *J Biol Chem* 284(1):245–253
103. Dhasarathy A, Phadke D, Mav D, Shah RR, Wade PA (2011) The transcription factors Snail and Slug activate the transforming growth factor-beta signaling pathway in breast cancer. *PLoS ONE* 6(10):e26514
104. Zheng P, Meng HM, Gao WZ, Chen L, Liu XH, Xiao ZQ, et al. (2011) Snail as a key regulator of PRL-3 gene in colorectal cancer. *Cancer Biol Ther* 12(8):742–749
105. Peinado H, Ballestar E, Esteller M, Cano A (2004) Snail mediates E-cadherin repression by the recruitment of the Sin3A/histone deacetylase 1 (HDAC1)/HDAC2 complex. *Mol. Cell. Biol.* 24(1):306–319
106. Peinado H, Quintanilla M, Cano A (2003) Transforming growth factor beta-1 induces snail transcription factor in epithelial cell lines: mechanisms for epithelial mesenchymal transitions. *J Biol Chem* 278(23):21113–21123
107. Yanagawa J, Walser TC, Zhu LX, Hong L, Fishbein MC, Mah V, et al. (2009) Snail promotes CXCR2 ligand-dependent tumor progression in non-small cell lung carcinoma. *Clin Cancer Res* 15(22):6820–6829
108. Yang Y, Li Y, Wang K, Wang Y, Yin W, Li L (2013) P38/NF-kappaB/snail pathway is involved in caffeic acid-induced inhibition of cancer stem cells-like properties and migratory capacity in malignant human keratinocyte. *PLoS ONE* 8(3):e58915
109. Ishii G, Hashimoto H, Atsumi N, Hoshino A, Ochiai A (2013) Morphophenotype of floating colonies derived from a single cancer cell has a critical impact on tumor-forming activity. *Pathol Int* 63(1):29–36
110. Sanchez-Garcia I (2009) The crossroads of oncogenesis and metastasis. *New England Journal of Medicine* 360(3):297–299
111. Mani SA, Guo W, Liao MJ, Eaton EN, Ayyanan A, Zhou AY, et al. (2008) The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell* 133(4):704–715
112. Walser TC, Yanagawa J, Garon E, Lee JM, Dubinett SM (2010) Tumor microenvironment. In: Stewart DJ (ed) *Lung cancer: prevention, management and emerging therapies, current clinical oncology*. Humana Press, New York, pp 27–69
113. Du F, Nakamura Y, Tan TL, Lee P, Lee R, Yu B, Jamora C (2010) Expression of snail in epidermal keratinocytes promotes cutaneous inflammation and hyperplasia conducive to tumor formation. *Cancer Res* 70(24):10080–10089

114. Hahn S, Jackstadt R, Siemens H, Hunten S, Hermeking H (2013) SNAIL and miR-34a feed-forward regulation of ZNF281/ZBP99 promotes epithelial-mesenchymal transition. *EMBO J* 32(23):3079–3095
115. Kim E, Youn H, Kwon T, Son B, Kang J, Yang HJ, et al. (2014) PAK1 Tyrosine phosphorylation is required to induce epithelial-mesenchymal transition and radioresistance in lung cancer cells. *Cancer Res* 74(19):5520–5531
116. Krohn A, Ahrens T, Yalcin A, Plones T, Wehrle J, Taromi S, et al. (2014) Tumor cell heterogeneity in Small Cell Lung Cancer (SCLC): phenotypical and functional differences associated with Epithelial-Mesenchymal Transition (EMT) and DNA methylation changes. *PLoS ONE* 9(6):e100249
117. Millanes-Romero A, Herranz N, Perrera V, Iturbide A, Loubat-Casanovas J, Gil J, et al. (2013) Regulation of heterochromatin transcription by Snail1/LOXL2 during epithelial-to-mesenchymal transition. *Mol Cell* 52(5):746–757
118. Song SJ, Poliseno L, Song MS, Ala U, Webster K, Ng C, et al. (2013) MicroRNA-antagonism regulates breast cancer stemness and metastasis via TET-family-dependent chromatin remodeling. *Cell* 154(2):311–324
119. Shah PP, Lockwood WW, Saurabh K, Kurlawala Z, Shannon SP, Waigel S, et al. (2015) Ubiquitin1 represses migration and epithelial-to-mesenchymal transition of human non-small cell lung cancer cells. *Oncogene* 34(13): 1709–1717
120. Semenza GL (2010) Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. *Oncogene* 29(5):625–634
121. Harris AL (2002) Hypoxia—a key regulatory factor in tumour growth. *Nat Rev Cancer* 2(1):38–47
122. Nizet V, Johnson RS (2009) Interdependence of hypoxic and innate immune responses. *Nat Rev Immunol* 9(9):609–617
123. Fitzpatrick SF, Tambuwala MM, Bruning U, Schaible B, Scholz CC, Byrne A, et al. (2011) An intact canonical NF-kappaB pathway is required for inflammatory gene expression in response to hypoxia. *J Immunol* 186(2):1091–1096
124. Kim WY, Perera S, Zhou B, Carretero J, Yeh JJ, Heathcote SA, et al. (2009) HIF2alpha cooperates with RAS to promote lung tumorigenesis in mice. *J Clin Invest* 119(8):2160–2170
125. Higgins DF, Kimura K, Bernhardt WM, Shrimanker N, Akai Y, Hohenstein B, et al. (2007) Hypoxia promotes fibrogenesis in vivo via HIF-1 stimulation of epithelial-to-mesenchymal transition. *J Clin Invest* 117(12):3810–3820
126. Esteban MA, Tran MG, Harten SK, Hill P, Castellanos MC, Chandra A, et al. (2006) Regulation of E-cadherin expression by VHL and hypoxia-inducible factor. *Cancer Res* 66(7):3567–3575
127. Krishnamachary B, Zagzag D, Nagasawa H, Rainey K, Okuyama H, Baek JH, Semenza GL (2006) Hypoxia-inducible factor-1-dependent repression of E-cadherin in von Hippel-Lindau tumor suppressor-null renal cell carcinoma mediated by TCF3, ZFH1A, and ZFH1B. *Cancer Res* 66(5):2725–2731
128. Yang MH, Wu MZ, Chiou SH, Chen PM, Chang SY, Liu CJ, et al. (2008) Direct regulation of TWIST by HIF-1alpha promotes metastasis. *Nat Cell Biol* 10(3):295–305
129. Gort EH, van Haaften G, Verlaan I, Groot AJ, Plasterk RH, Shvarts A, et al. (2008) The TWIST1 oncogene is a direct target of hypoxia-inducible factor-2alpha. *Oncogene* 27(11):1501–1510
130. Hung JJ, Yang MH, Hsu HS, Hsu WH, Liu JS, Wu KJ (2009) Prognostic significance of hypoxia-inducible factor-1alpha, TWIST1 and Snail expression in resectable non-small cell lung cancer. *Thorax* 64(12):1082–1089
131. Luo D, Wang J, Li J, Post M (2011) Mouse snail is a target gene for HIF. *Mol Cancer Res* 9(2):234–245
132. Sahlgren C, Gustafsson MV, Jin S, Poellinger L, Lendahl U (2008) Notch signaling mediates hypoxia-induced tumor cell migration and invasion. *Proc Natl Acad Sci U S A* 105(17):6392–6397

133. Chen J, Imanaka N, Griffin JD (2010) Hypoxia potentiates Notch signaling in breast cancer leading to decreased E-cadherin expression and increased cell migration and invasion. *Br J Cancer* 102(2):351–360
134. Pennacchietti S, Michieli P, Galluzzo M, Mazzone M, Giordano S, Comoglio PM (2003) Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene. *Cancer Cell* 3(4):347–361
135. Jiang YG, Luo Y, He DL, Li X, Zhang LL, Peng T, et al. (2007) Role of Wnt/beta-catenin signaling pathway in epithelial-mesenchymal transition of human prostate cancer induced by hypoxia-inducible factor-1alpha. *Int J Urol* 14(11):1034–1039
136. Zhou G, Dada LA, Wu M, Kelly A, Trejo H, Zhou Q, et al. (2009) Hypoxia-induced alveolar epithelial-mesenchymal transition requires mitochondrial ROS and hypoxia-inducible factor 1. *Am J Physiol Lung Cell Mol Physiol* 297(6):L1120–L1130
137. Chen Y, Li D, Liu H, Xu H, Zheng H, Qian F, et al. (2011) Notch-1 signaling facilitates survival expression in human non-small cell lung cancer cells. *Cancer Biol Ther* 11(1):14–21
138. Erler JT, Bennewith KL, Nicolau M, Dornhofer N, Kong C, Le QT, et al. (2006) Lysyl oxidase is essential for hypoxia-induced metastasis. *Nature* 440(7088):1222–1226
139. Huang CH, Yang WH, Chang SY, Tai SK, Tzeng CH, Kao JY, et al. (2009) Regulation of membrane-type 4 matrix metalloproteinase by SLUG contributes to hypoxia-mediated metastasis. *Neoplasia* 11(12):1371–1382
140. Yoo YG, Christensen J, Huang LE (2011) HIF-1alpha confers aggressive malignant traits on human tumor cells independent of its canonical transcriptional function. *Cancer Res* 71(4):1244–1252
141. Brookes PS, Levonen AL, Shiva S, Sarti P, Darley-Usmar VM (2002) Mitochondria: regulators of signal transduction by reactive oxygen and nitrogen species. *Free Radic Biol Med* 33(6):755–764
142. Chatterjee S, Fisher AB (2004) ROS to the rescue. *Am J Physiol Lung Cell Mol Physiol* 287(4):L704–705
143. Hancock JT, Desikan R, Neill SJ (2001) Role of reactive oxygen species in cell signalling pathways. *Biochem Soc Trans* 29(Pt 2):345–350
144. Sauer H, Wartenberg M, Hescheler J (2001) Reactive oxygen species as intracellular messengers during cell growth and differentiation. *Cell Physiol Biochem* 11(4):173–186
145. Thannickal VJ, Fanburg BL (2000) Reactive oxygen species in cell signaling. *Am J Physiol Lung Cell Mol Physiol* 279(6):L1005–L1028
146. Rosanna DP, Salvatore C (2012) Reactive oxygen species, inflammation, and lung diseases. *Curr Pharm Des* 18(26):3889–3900
147. Mates JM, Segura JA, Alonso FJ, Marquez J (2008) Intracellular redox status and oxidative stress: implications for cell proliferation, apoptosis, and carcinogenesis. *Arch Toxicol* 82(5):273–299
148. Paz-Elizur T, Sevilay Z, Leitner-Dagan Y, Elinger D, Roisman LC, Livneh Z (2008) DNA repair of oxidative DNA damage in human carcinogenesis: potential application for cancer risk assessment and prevention. *Cancer Lett* 266(1):60–72
149. Wiseman H, Halliwell B (1996) Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. *Biochem J* 313(Pt 1):17–29
150. Delaney S, Jarem DA, Volle CB, Yennie CJ (2012) Chemical and biological consequences of oxidatively damaged guanine in DNA. *Free Radic Res* 46(4):420–441
151. Kawanishi S, Hiraku Y, Oikawa S (2001) Mechanism of guanine-specific DNA damage by oxidative stress and its role in carcinogenesis and aging. *Mutat Res* 488(1):65–76
152. Jackson JH (1994) Potential molecular mechanisms of oxidant-induced carcinogenesis. *Environ Health Perspect* 102(Suppl 10):155–157
153. Malins DC, Polissar NL, Gunselman SJ (1996) Progression of human breast cancers to the metastatic state is linked to hydroxyl radical-induced DNA damage. *Proc Natl Acad Sci U S A* 93(6):2557–2563
154. Bennett WP, Colby TV, Travis WD, Borkowski A, Jones RT, Lane DP, et al. (1993) p53 protein accumulates frequently in early bronchial neoplasia. *Cancer Res* 53(20):4817–4822

155. Denissenko MF, Pao A, Tang M, Pfeifer GP (1996) Preferential formation of benzo[a]pyrene adducts at lung cancer mutational hotspots in P53. *Science* 274(5286):430–432
156. Lauber K, Blumenthal SG, Waibel M, Wesselborg S (2004) Clearance of apoptotic cells: getting rid of the corpses. *Mol Cell* 14(3):277–287
157. Maderna P, Godson C (2003) Phagocytosis of apoptotic cells and the resolution of inflammation. *Biochim Biophys Acta* 1639(3):141–151
158. Crawford DR, Abramova NE, Davies KJ (1998) Oxidative stress causes a general, calcium-dependent degradation of mitochondrial polynucleotides. *Free Radic Biol Med* 25(9):1106–1111
159. Kane DJ, Sarafian TA, Anton R, Hahn H, Gralla EB, Valentine JS, et al. (1993) Bcl-2 inhibition of neural death: decreased generation of reactive oxygen species. *Science* 262(5137):1274–1277
160. Kristal BS, Chen J, Yu BP (1994) Sensitivity of mitochondrial transcription to different free radical species. *Free Radic Biol Med* 16(3):323–329
161. Reed JC (1997) Cytochrome c: can't live with it—can't live without it. *Cell* 91(5):559–562
162. Benhar M, Dalyot I, Engelberg D, Levitzki A (2001) Enhanced ROS production in oncogenically transformed cells potentiates c-Jun N-terminal kinase and p38 mitogen-activated protein kinase activation and sensitization to genotoxic stress. *Mol Cell Biol* 21(20):6913–6926
163. Vaquero EC, Edderkaoui M, Pandolfi SJ, Gukovsky I, Gukovskaya AS (2004) Reactive oxygen species produced by NAD(P)H oxidase inhibit apoptosis in pancreatic cancer cells. *J Biol Chem* 279(33):34643–34654
164. Ishikawa K, Takenaga K, Akimoto M, Koshikawa N, Yamaguchi A, Imanishi H, et al. (2008) ROS-generating mitochondrial DNA mutations can regulate tumor cell metastasis. *Science* 320(5876):661–664
165. Ray PD, Huang BW, Tsuji Y (2012) Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. *Cell Signal* 24(5):981–990
166. Lau A, Villeneuve NF, Sun Z, Wong PK, Zhang DD (2008) Dual roles of Nrf2 in cancer. *Pharmacol Res* 58(5–6):262–270
167. DeNicola GM, Karreth FA, Humpton TJ, Gopinathan A, Wei C, Frese K, et al. (2011) Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. *Nature* 475(7354):106–109
168. Ding M, Li JJ, Leonard SS, Ye JP, Shi X, Colburn NH, et al. (1999) Vanadate-induced activation of activator protein-1: role of reactive oxygen species. *Carcinogenesis* 20(4):663–668
169. Ding M, Shi X, Lu Y, Huang C, Leonard S, Roberts J, et al. (2001) Induction of activator protein-1 through reactive oxygen species by crystalline silica in JB6 cells. *J Biol Chem* 276(12):9108–9114
170. Suzuki T, Murakami M, Onai N, Fukuda E, Hashimoto Y, Sonobe MH, et al. (1994) Analysis of AP-1 function in cellular transformation pathways. *J Virol* 68(6):3527–3535
171. Wilhelm D, Bender K, Knebel A, Angel P (1997) The level of intracellular glutathione is a key regulator for the induction of stress-activated signal transduction pathways including Jun N-terminal protein kinases and p38 kinase by alkylating agents. *Mol Cell Biol* 17(8):4792–4800
172. Devalia JL, Davies RJ (1993) Airway epithelial cells and mediators of inflammation. *Respir Med* 87(6):405–408
173. Rahman I, Gilmour PS, Jimenez LA, MacNee W (2002) Oxidative stress and TNF-alpha induce histone acetylation and NF-kappaB/AP-1 activation in alveolar epithelial cells: potential mechanism in gene transcription in lung inflammation. *Mol Cell Biochem* 234–235(1–2):239–248
174. Akira S, Kishimoto T (1997) NF-IL6 and NF-kappa B in cytokine gene regulation. *Adv Immunol* 65:1–46
175. Sethi G, Sung B, Aggarwal BB (2008) Nuclear factor-kappaB activation: from bench to bedside. *Exp Biol Med* (Maywood) 233(1):21–31

176. Brennan FM, Maini RN, Feldmann M (1995) Cytokine expression in chronic inflammatory disease. *Br Med Bull* 51(2):368–384
177. Ward PA (1996) Role of complement, chemokines, and regulatory cytokines in acute lung injury. *Ann N Y Acad Sci* 796:104–112
178. Bartis D, Mise N, Mahida RY, Eickelberg O, Thickett DR (2014) Epithelial-mesenchymal transition in lung development and disease: does it exist and is it important? *Thorax* 69(8):760–765
179. Bartis D, Thickett DR (2014) Authors' response: epithelial-mesenchymal transition (EMT) is a common molecular programme in epithelial cells which can be triggered by injury. *Thorax* 69(8):769
180. Young RP, Whittington CF, Hopkins RJ, Hay BA, Epton MJ, Black PN, Gamble GD (2010) Chromosome 4q31 locus in COPD is also associated with lung cancer. *Eur Respir J* 36(6):1375–1382
181. Zou W, Zou Y, Zhao Z, Li B, Ran P (2013) Nicotine-induced epithelial-mesenchymal transition via Wnt/beta-catenin signaling in human airway epithelial cells. *Am J Physiol Lung Cell Mol Physiol* 304(4):L199–209
182. Nagathihalli NS, Massion PP, Gonzalez AL, Lu P, Datta PK (2012) Smoking induces epithelial-to-mesenchymal transition in non-small cell lung cancer through HDAC-mediated downregulation of E-cadherin. *Mol Cancer Ther* 11(11):2362–2372
183. Davis R, Rizwani W, Banerjee S, Kovacs M, Haura E, Coppola D, Chellappan S (2009) Nicotine promotes tumor growth and metastasis in mouse models of lung cancer. *PLoS ONE* 4(10):e7524
184. Spindel ER (2009) Is nicotine the estrogen of lung cancer? *Am J Respir Crit Care Med* 179(12):1081–1082
185. Hecht SS (2003) Tobacco carcinogens, their biomarkers and tobacco-induced cancer. *Nat Rev Cancer* 3(10):733–744
186. Schuller HM, Orloff M (1998) Tobacco-specific carcinogenic nitrosamines. Ligands for nicotinic acetylcholine receptors in human lung cancer cells. *Biochem Pharmacol* 55(9):1377–1384
187. Catassi A, Servent D, Paleari L, Cesario A, Russo P (2008) Multiple roles of nicotine on cell proliferation and inhibition of apoptosis: implications on lung carcinogenesis. *Mutat Res* 659(3):221–231
188. Dasgupta P, Rizwani W, Pillai S, Kinkade R, Kovacs M, Rastogi S, et al. (2009) Nicotine induces cell proliferation, invasion and epithelial-mesenchymal transition in a variety of human cancer cell lines. *Int J Cancer* 124(1):36–45
189. Lam DC, Girard L, Ramirez R, Chau WS, Suen WS, Sheridan S, et al. (2007) Expression of nicotinic acetylcholine receptor subunit genes in non-small-cell lung cancer reveals differences between smokers and nonsmokers. *Cancer Res* 67(10):4638–4647
190. Tsurutani J, Castillo SS, Brognard J, Granville CA, Zhang C, Gills JJ, et al. (2005) Tobacco components stimulate Akt-dependent proliferation and NFkappaB-dependent survival in lung cancer cells. *Carcinogenesis* 26(7):1182–1195
191. Maneckjee R, Minna JD (1994) Opioids induce while nicotine suppresses apoptosis in human lung cancer cells. *Cell Growth Differ* 5(10):1033–1040
192. Heesch C, Jang JJ, Weis M, Pathak A, Kaji S, Hu RS, et al. (2001) Nicotine stimulates angiogenesis and promotes tumor growth and atherosclerosis. *Nat Med* 7(7):833–839
193. Jarzynka MJ, Guo P, Bar-Joseph I, Hu B, Cheng SY (2006) Estradiol and nicotine exposure enhances A549 bronchioloalveolar carcinoma xenograft growth in mice through the stimulation of angiogenesis. *Int J Oncol* 28(2):337–344
194. Westenberger BJ (2009) Evaluation of e-cigarettes: DPATR-FY-09-23. Prepared by U.S. Food and Drug Administration/Center for Drug Evaluation and Research. St Louis, MO
195. Williams M, Villarreal A, Bozhilov K, Lin S, Talbot P (2013) Metal and silicate particles including nanoparticles are present in electronic cigarette cartomizer fluid and aerosol. *PLoS ONE* 8(3):e57987

196. Goniewicz ML, Knysak J, Gawron M, Kosmider L, Sobczak A, Kurek J, et al. (2014) Levels of selected carcinogens and toxicants in vapour from electronic cigarettes. *Tob Control* 23(2):133–139
197. Cobb NK, Abrams DB (2011) E-cigarette or drug-delivery device? Regulating novel nicotine products. *N Engl J Med* 365(3):193–195
198. Laugesen M (2008) Safety report on the Ruyan® e-cigarette cartridge and inhaled aerosol. New Zealand Ltd. Christchurch, New Zealand
199. Vansickel AR, Eissenberg T (2013) Electronic cigarettes: effective nicotine delivery after acute administration. *Nicotine Tob Res* 15(1):267–270
200. Vardavas CI, Anagnostopoulos N, Kougias M, Evangelopoulou V, Connolly GN, Behrakis PK (2012) Short-term pulmonary effects of using an electronic cigarette: impact on respiratory flow resistance, impedance, and exhaled nitric oxide. *Chest* 141(6):1400–1406
201. Maunders H, Patwardhan S, Phillips J, Clack A, Richter A (2007) Human bronchial epithelial cell transcriptome: gene expression changes following acute exposure to whole cigarette smoke in vitro. *Am J Physiol Lung Cell Mol Physiol* 292(5):L1248–L1256
202. Riker CA, Lee K, Darville A, Hahn EJ (2012) E-cigarettes: promise or peril? *Nurs Clin North Am* 47(1):159–171
203. Trtchounian A, Williams M, Talbot P (2010) Conventional and electronic cigarettes (e-cigarettes) have different smoking characteristics. *Nicotine Tob Res* 12(9):905–912
204. Kalluri R, Weinberg RA (2009) The basics of epithelial-mesenchymal transition. *Journal of Clinical Investigation* 119(6):1420–1428
205. Nieto MA, Cano A (2012) The epithelial-mesenchymal transition under control: global programs to regulate epithelial plasticity. *Seminars in Cancer Biology* 22(5–6):361–368
206. Chen W, Gao Q, Han S, Pan F, Fan W (2014) The CCL2/CCR2 axis enhances IL-6-induced epithelial-mesenchymal transition by cooperatively activating STAT3-Twist signaling. *Tumour Biol* 36(2):973–981.
207. Arenberg DA, Keane MP, DiGiovine B, Kunkel SL, Morris SB, Xue YY, et al. (1998) Epithelial-neutrophil activating peptide (ENA-78) is an important angiogenic factor in non-small cell lung cancer. *J Clin Invest* 102(3):465–472
208. Arenberg DA, Kunkel SL, Polverini PJ, Glass M, Burdick MD, Strieter RM (1996) Inhibition of interleukin-8 reduces tumorigenesis of human non-small cell lung cancer in SCID mice. *J Clin Invest* 97(12):2792–2802
209. Keane MP, Belperio JA, Xue YY, Burdick MD, Strieter RM (2004) Depletion of CXCR2 inhibits tumor growth and angiogenesis in a murine model of lung cancer. *J Immunol* 172(5):2853–2860
210. Wislez M, Fujimoto N, Izzo JG, Hanna AE, Cody DD, Langley RR, et al. (2006) High expression of ligands for chemokine receptor CXCR2 in alveolar epithelial neoplasia induced by oncogenic kras. *Cancer Res* 66(8):4198–4207
211. Risolino M, Mandia N, Iavarone F, Dardaei L, Longobardi E, Fernandez S, et al. (2014) Transcription factor PREP1 induces EMT and metastasis by controlling the TGF-beta-SMAD3 pathway in non-small cell lung adenocarcinoma. *Proc Natl Acad Sci U S A* 111(36):E3775–3784
212. Ren J, Chen Y, Song H, Chen L, Wang R (2013) Inhibition of ZEB1 reverses EMT and chemoresistance in docetaxel-resistant human lung adenocarcinoma cell line. *J Cell Biochem* 114(6):1395–1403
213. Yauch RL, Januario T, Eberhard DA, Cavet G, Zhu W, Fu L, et al. (2005) Epithelial versus mesenchymal phenotype determines in vitro sensitivity and predicts clinical activity of erlotinib in lung cancer patients. *Clin Cancer Res* 11(24 Pt 1):8686–8698
214. Thomson S, Buck E, Petti F, Griffin G, Brown E, Ramnarine N, et al. (2005) Epithelial to mesenchymal transition is a determinant of sensitivity of non-small-cell lung carcinoma cell lines and xenografts to epidermal growth factor receptor inhibition. *Cancer Res* 65(20):9455–9462

215. Witta SE, Gemmill RM, Hirsch FR, Coldren CD, Hedman K, Ravdel L, et al. (2006) Restoring E-cadherin expression increases sensitivity to epidermal growth factor receptor inhibitors in lung cancer cell lines. *Cancer Res* 66(2):944–950
216. Byers LA, Diao L, Wang J, Saintigny P, Girard L, Peyton M, et al. (2013) An epithelial-mesenchymal transition gene signature predicts resistance to EGFR and PI3K inhibitors and identifies Axl as a therapeutic target for overcoming EGFR inhibitor resistance. *Clin Cancer Res* 19(1):279–290
217. Gomperts BN, Spira A, Massion PP, Walser TC, Wistuba II, Minna JD, Dubinett SM (2011) Evolving concepts in lung carcinogenesis. *Semin Respir Crit Care Med* 32(1):32–43
218. Sullivan JP, Minna JD, Shay JW (2010) Evidence for self-renewing lung cancer stem cells and their implications in tumor initiation, progression, and targeted therapy. *Cancer Metastasis Rev* 29(1):61–72
219. Singh A, Settleman J (2010) EMT, cancer stem cells and drug resistance: an emerging axis of evil in the war on cancer. *Oncogene* 29(34):4741–4751
220. Mani SA, Guo W, Liao M-J, Eaton EN, Ayyanan A, Zhou AY, et al. (2008) The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell* 133(4):704–715
221. Creighton CJ, Li X, Landis M, Dixon JM, Neumeister VM, Sjolund A, et al. (2009) Residual breast cancers after conventional therapy display mesenchymal as well as tumor-initiating features. *Proc Natl Acad Sci USA* 106(33):13820–13825
222. Yu M, Smolen GA, Zhang J, Wittner B, Schott BJ, Brachtel E, et al. (2009) A developmentally regulated inducer of EMT, LBX1, contributes to breast cancer progression. *Genes Dev* 23(15):1737–1742
223. Yang MH, Hsu DS, Wang HW, Wang HJ, Lan HY, Yang WH, et al. (2010) Bmi1 is essential in Twist1-induced epithelial-mesenchymal transition. *Nat Cell Biol* 12(10):982–992
224. Giannoni E, Bianchini F, Calorini L, Chiarugi P (2011) Cancer associated fibroblasts exploit reactive oxygen species through a proinflammatory signature leading to epithelial mesenchymal transition and stemness. *Antioxid Redox Signal* 14(12): 2361–2371
225. Louie E, Nik S, Chen JS, Schmidt M, Song B, Pacson C, et al. Identification of a stem-like cell population by exposing metastatic breast cancer cell lines to repetitive cycles of hypoxia and reoxygenation. *Breast Cancer Res* 12(6):R94
226. Kurrey NK, Jalgaonkar SP, Joglekar AV, Ghanate AD, Chaskar PD, Doiphode RY, Bapat SA (2009) Snail and slug mediate radioresistance and chemoresistance by antagonizing p53-mediated apoptosis and acquiring a stem-like phenotype in ovarian cancer cells. *Stem Cells* 27(9):2059–2068
227. Pinho AV, Rooman I, Real FX (2011) p53-dependent regulation of growth, epithelial-mesenchymal transition and stemness in normal pancreatic epithelial cells. *Cell Cycle* 10(8):1312–1321
228. Chang CJ, Chao CH, Xia W, Yang JY, Xiong Y, Li CW, et al. (2011) p53 regulates epithelial-mesenchymal transition and stem cell properties through modulating miRNAs. *Nat Cell Biol* 13(3):317–323
229. May R, Sureban SM, Hoang N, Riehl TE, Lightfoot SA, Ramanujam R, et al. (2009) Doublecortin and CaM kinase-like-1 and leucine-rich-repeat-containing G-protein-coupled receptor mark quiescent and cycling intestinal stem cells, respectively. *Stem Cells* 27(10):2571–2579
230. Sureban SM, May R, Ramalingam S, Subramaniam D, Natarajan G, Anant S, Houchen CW (2009) Selective blockade of DCAMKL-1 results in tumor growth arrest by a Let-7a microRNA-dependent mechanism. *Gastroenterology* 137(2):649–659, 659 e641–642
231. Sureban SM, May R, Lightfoot SA, Hoskins AB, Lerner M, Brackett DJ, et al. DCAMKL-1 regulates epithelial-mesenchymal transition in human pancreatic cells through a miR-200a-dependent mechanism. *Cancer Res* 71(6):2328–2338
232. Wellner U, Schubert J, Burk UC, Schmalhofer O, Zhu F, Sonntag A, et al. (2009) The EMT-activator ZEB1 promotes tumorigenicity by repressing stemness-inhibiting microRNAs. *Nat Cell Biol* 11(12):1487–1495
233. Brabletz S, Bajdak K, Meidhof S, Burk U, Niedermann G, Firat E, et al. (2011) The ZEB1/miR-200 feedback loop controls Notch signalling in cancer cells. *EMBO J* 30(4):770–782

234. Tellez CS, Juri DE, Do K, Bernauer AM, Thomas CL, Damiani LA, et al. (2011) EMT and stem cell-like properties associated with miR-205 and miR-200 epigenetic silencing are early manifestations during carcinogen-induced transformation of human lung epithelial cells. *Cancer Res* 71(8):3087–3097
235. Klein CA (2009) Parallel progression of primary tumours and metastases. *Nat Rev Cancer* 9(4):302–312
236. Rhim AD, Mirek ET, Aiello NM, Maitra A, Bailey JM, McAllister F, et al. (2012) EMT and dissemination precede pancreatic tumor formation. *Cell* 148(1–2):349–361
237. Husemann Y, Geigl JB, Schubert F, Musiani P, Meyer M, Burghart E, et al. (2008) Systemic spread is an early step in breast cancer. *Cancer cell* 13(1):58–68
238. Podsypanina K, Du YC, Jechlinger M, Beverly LJ, Hambardzumyan D, Varmus H (2008) Seeding and propagation of untransformed mouse mammary cells in the lung. *Science* 321(5897):1841–1844
239. Wyckoff JB, Jones JG, Condeelis JS, Segall JE (2000) A critical step in metastasis: in vivo analysis of intravasation at the primary tumor. *Cancer Res* 60(9):2504–2511
240. Wang W, Wyckoff JB, Frohlich VC, Oleynikov Y, Huttelmaier S, Zavadil J, et al. (2002) Single cell behavior in metastatic primary mammary tumors correlated with gene expression patterns revealed by molecular profiling. *Cancer Res* 62(21):6278–6288
241. Beaty BT, Wang Y, Bravo-Cordero JJ, Sharma VP, Miskolci V, Hodgson L, Condeelis J (2014) Talin regulates moesin-NHE-1 recruitment to invadopodia and promotes mammary tumor metastasis. *J Cell Biol* 205(5):737–751
242. Jung HY, Fattet L, Yang J (2015) Molecular pathways: linking tumor microenvironment to epithelial-mesenchymal transition in metastasis. *Clin Cancer Res* 21(5): 962–968
243. Paszek MJ, Zahir N, Johnson KR, Lakins JN, Rozenberg GI, Gefen A, et al. (2005) Tensional homeostasis and the malignant phenotype. *Cancer Cell* 8(3):241–254
244. Levental KR, Yu H, Kass L, Lakins JN, Egeblad M, Erler JT, et al. (2009) Matrix crosslinking forces tumor progression by enhancing integrin signaling. *Cell* 139(5):891–906
245. Leight JL, Wozniak MA, Chen S, Lynch ML, Chen CS (2012) Matrix rigidity regulates a switch between TGF-beta1-induced apoptosis and epithelial-mesenchymal transition. *Mol Biol Cell* 23(5):781–791
246. Gill BJ, Gibbons DL, Roudsari LC, Saik JE, Rizvi ZH, Roybal JD, et al. (2012) A synthetic matrix with independently tunable biochemistry and mechanical properties to study epithelial morphogenesis and EMT in a lung adenocarcinoma model. *Cancer Res* 72(22):6013–6023
247. Buckley ST, Davies AM, Ehrhardt C (2011) Atomic force microscopy and high-content analysis: two innovative technologies for dissecting the relationship between epithelial-mesenchymal transition-related morphological and structural alterations and cell mechanical properties. *Methods Mol Biol* 784:197–208
248. Shibue T, Brooks MW, Inan MF, Reinhardt F, Weinberg RA (2012) The outgrowth of micrometastases is enabled by the formation of filopodium-like protrusions. *Cancer Discov* 2(8):706–721
249. Wu TH, Chou YW, Chiu PH, Tang MJ, Hu CW, Yeh ML (2014) Validation of the effects of TGF-beta1 on tumor recurrence and prognosis through tumor retrieval and cell mechanical properties. *Cancer Cell Int* 14(1):20
250. Zhou Z, Zheng C, Li S, Zhou X, Liu Z, He Q, et al. (2013) AFM nanoindentation detection of the elastic modulus of tongue squamous carcinoma cells with different metastatic potentials. *Nanomedicine* 9(7):864–874
251. Osborne LD, Li GZ, How T, O'Brien ET, Blobe GC, Superfine R, Myhre K (2014) TGF-beta regulates LARG and GEF-H1 during EMT to affect stiffening response to force and cell invasion. *Mol Biol Cell* 25(22):3528–3540
252. Creighton CJ, Gibbons DL, Kurie JM (2013) The role of epithelial-mesenchymal transition programming in invasion and metastasis: a clinical perspective. *Cancer Manag Res* 5:187–195
253. Groen HJ, Sietsma H, Vincent A, Hochstenbag MM, van Putten JW, van den Berg A, et al. (2011) Randomized, placebo-controlled phase III study of docetaxel plus carboplatin with celecoxib and cyclooxygenase-2 expression as a biomarker for patients with advanced non-small-cell lung cancer: the NVALT-4 study. *J Clin Oncol* 29(32):4320–4326

254. Scagliotti GV, Ilaria R, Jr., Novello S, von Pawel J, Fischer JR, Ermisch S, et al. (2012) Tasisulam sodium (LY573636 sodium) as third-line treatment in patients with unresectable, metastatic non-small-cell lung cancer: a phase-II study. *J Thorac Oncol* 7(6):1053–1057
255. Harada D, Takigawa N, Kiura K (2014) The role of STAT3 in non-small cell lung cancer. *Cancers (Basel)* 6(2):708–722
256. Kim YJ, Choi WI, Jeon BN, Choi KC, Kim K, Kim TJ, et al. (2014) Stereospecific effects of ginsenoside 20-Rg3 inhibits TGF-beta1-induced epithelial-mesenchymal transition and suppresses lung cancer migration, invasion and anoikis resistance. *Toxicology* 322:23–33
257. Gadgeel SM, Ruckdeschel JC, Heath EI, Heilbrun LK, Venkatramanamoorthy R, Wozniak A (2007) 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* 2(4):299–305
258. O'Byrne KJ, Danson S, Dunlop D, Botwood N, Taguchi F, Carbone D, Ranson M (2007) Combination therapy with gefitinib and rofecoxib in patients with platinum-pretreated relapsed non small-cell lung cancer. *J Clin Oncol* 25(22):3266–3273
259. Li L, Han R, Xiao H, Lin C, Wang Y, Liu H, et al. (2014) Metformin sensitizes EGFR-TKI-resistant human lung cancer cells in vitro and in vivo through inhibition of IL-6 signaling and EMT reversal. *Clin Cancer Res* 20(10):2714–2726
260. Dumont N, Wilson MB, Crawford YG, Reynolds PA, Sigaroudinia M, Tlsty TD (2008) Sustained induction of epithelial to mesenchymal transition activates DNA methylation of genes silenced in basal-like breast cancers. *Proc Natl Acad Sci USA* 105(39):14867–14872
261. Kiesslich T, Pichler M, Neureiter D (2013) Epigenetic control of epithelial-mesenchymal-transition in human cancer. *Mol Clin Oncol* 1(1):3–11
262. Cieslik M, Hoang SA, Baranova N, Chodaparambil S, Kumar M, Allison DF, et al. (2013) Epigenetic coordination of signaling pathways during the epithelial-mesenchymal transition. *Epigenetics Chromatin* 6(1):28
263. Carmona FJ, Davalos V, Vidal E, Gomez A, Heyn H, Hashimoto Y, et al. (2014) A comprehensive DNA methylation profile of epithelial-to-mesenchymal transition. *Cancer Res* 74(19):5608–5619
264. Roll JD, Rivenbark AG, Sandhu R, Parker JS, Jones WD, Carey LA, et al. (2013) Dysregulation of the epigenome in triple-negative breast cancers: basal-like and claudin-low breast cancers express aberrant DNA hypermethylation. *Exp Mol Pathol* 95(3):276–287
265. Muqbil I, Wu J, Aboukameel A, Mohammad RM, Azmi AS (2014) Snail nuclear transport: the gateways regulating epithelial-to-mesenchymal transition? *Semin Cancer Biol* 27:39–45
266. Neggers JE, Vercruysse T, Jacquemyn M, Vanstreels E, Baloglu E, Shacham S, et al. (2015) Identifying drug-target selectivity of small-molecule CRM1/XPO1 inhibitors by CRISPR/Cas9 genome editing. *Chem Biol* 22(1): 107–116
267. Braeutigam C, Rago L, Rolke A, Waldmeier L, Christofori G, Winter J (2014) The RNA-binding protein Rbfox2: an essential regulator of EMT-driven alternative splicing and a mediator of cellular invasion. *Oncogene* 33(9):1082–1092
268. Warzecha CC, Jiang P, Amirikian K, Dittmar KA, Lu H, Shen S, et al. (2010) An ESRP-regulated splicing programme is abrogated during the epithelial-mesenchymal transition. *EMBO J* 29(19):3286–3300
269. Lu ZX, Huang Q, Park JW, Shen S, Lin L, Tokheim CJ, et al. (2015) Transcriptome-wide landscape of pre-mRNA alternative splicing associated with metastatic colonization. *13(2): 305–318*
270. Tavanez JP, Valcarcel J (2010) A splicing mastermind for EMT. *EMBO J* 29(19):3217–3218
271. Dubinett SM, Spira A (2013) Challenge and opportunity of targeted lung cancer chemoprevention. *J Clin Oncol* 31(33):4169–4171

Inflammation and Lung Cancer

Dubinett, S.M. (Ed.)

2015, X, 212 p. 5 illus., 3 illus. in color., Hardcover

ISBN: 978-1-4939-2723-4