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Inflammation Fuels Tumor Progress and Metastasis

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Abstract

Inflammation is a beneficial response that can remove pathogens, repair injured tissue and restore homeostasis to damaged tissues and organs. However, increasing evidence indicate that chronic inflammation plays a pivotal role in tumor development, as well as progression, metastasis, and resistance to chemotherapy. We will review the current knowledge regarding the contribution of inflammation to epithelial mesenchymal transition. We will also provide some perspectives on the relationship between ER-stress signals and metabolism, and the role of these processes in the development of inflammation.

Keywords

Epithelial-mesenchymal transition; Inflammation; Metastasis

A similarity between the chronic inflammatory processes associated with wound healing and tumor development was noticed by Rudolf Virchow more than 150 years ago, as both processes present with significant leukocyte infiltration [1]. An inflammatory niche, containing stromal fibroblasts, endothelial cells, infiltrated lymphocytes and secreted cytokines, chemokines and growth factors is not only essential for the normal wound healing process but also contributes significantly to the development and progression of cancer. Here, we discuss recent discoveries on the inter-relationships of inflammation, epithelial-mesenchymal transition (EMT), endoplasmic reticulum (ER) stress and metabolism, which collectively contribute to oncogenesis.

Tight Relationship between Chronic Inflammation and Cancer Development and Metastasis

Wound healing and tumor development share many similarities in regulatory processes, signaling pathways, and gene expression profiles. Both events involve cell survival,

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migration, and proliferation, and are controlled by numerous secretory factors, including growth factors, cytokines and chemokines, from the microenvironment of the wound or cancer. The similarities of these two events led Dvorak to postulate that cancer is a “wound that never heals” [2]. Indeed, daily irradiation under sun, chronically exposure to cigarette smoke or other environmental carcinogens in lung, and constant viral or bacterial infection in stomach, liver, and cervix produce chronic tissue injury and subsequent oncogenesis in these organs [3]. Many epidemiologic and clinical studies also show that approximately 25% of all solid tumors result from chronic inflammation [4], and that the consumption of anti-inflammatory agents (such as non-steroidal anti-inflammatory drugs) positively correlates with a reduced incidence of colorectal, breast, pancreatic, and gastric cancers [5]. During wound healing, the major function of inflammation is to destroy pathogens and infectious agents, to activate the immune system by eliciting innate and acquired immune responses, and to remodel injured tissues. This acute inflammation is a fast self-contained event; the signals and regulatory machinery that normally initiate and sustain the processes terminate when healing is complete. However, in cancer, inflammation becomes a persistent process, with a “failure to heal” lesions. Chronic inflammation and the subsequent persistent tissue injury, lead to changes in epithelial cytoarchitecture and surrounding stromal components, and enhance genetic mutations and epigenetic aberrations of epithelia cells. This alteration in tissue homeostasis causes a feed-forward chronic inflammatory response that never fades away, promoting tumor proliferation, angiogenesis, invasion, and metastasis through activation of surrounding stromal cells and recruitment of immune cells. Inflammation has been recognized as a “hallmark of cancer” [6, 7]. The fundamental mechanisms responsible for chronic inflammation in tumors remain elusive and many outstanding questions remain to be addressed; for instance, how does chronic inflammation enhance tumor cells resistant to apoptosis, invade to adjacent tissues, and metastasize to distant organs? Here we summarize recent findings from our laboratory and studies obtained by others that focus on the mechanisms and signaling pathways underlying inflammation-mediated metastasis.

Inflammation and EMT

(a) Overview of EMT

The concept of EMT was first proposed in 1967 by Elizabeth Hay from a seminal observation on chicken embryo development [8]. EMT is a phenotypic conversion during embryonic development with tissue remodeling and cell migration that shape the future organism; the same processes occurs during wound healing. Pathologically, a similar EMT process ensues for fibrotic diseases and during metastasis. During EMT, epithelial cells lose the expression of adherens and tight junction molecules that hold cells together; they change to a mesenchymal morphology and gain the ability to break basal membranes and invade to adjacent tissue or distant organs [9]. EMT is divided into three subtypes based on different biological conditions [10–12]. Type 1 EMT describes a constellation of events in which epithelial cells become motile mesenchymal cells during gastrulation, mesoderm formation, and neural crest delamination. These primary mesenchymal cells can revert, and form secondary epithelia in mesodermal and endodermal organs through mesenchymal-epithelial transition (MET). Type 2 EMT involves the program of generating tissue fibroblasts from epithelial or endothelial cells during wound healing and tissue remodeling. This type of

EMT will eventually lead to tissue fibrosis and organ malfunction if inflammation becomes chronic. Type 3 EMT describes how tumor cells undergo a phenotypic transition in order to detach, invade, and metastasize through the blood or lymphatic systems and form a metastatic lesion through MET at distant sites [13]. Despite this classification, the genetic elements and regulatory mechanisms among three subtypes of EMT are likely similar and well conserved. For example, E-cadherin is a cell-cell adhesion molecule that is required to form epithelial adherent junctions in a homotypic, calcium-dependent mechanism [14, 15]. The cytoplasmic tail of E-cadherin binds to α - and β -catenin to modulate cytoskeleton and gene expression during cell-cell adhesion and cell migration. Loss of E-cadherin disrupts the cell-cell adhesion and alters gene expression by increasing β -catenin nuclear localization [14, 15]. Loss of E-cadherin expression is an important hallmark of EMT and is often correlated with the tumor grade and poor patient survival. In contrast, N-cadherin, which is highly expressed in mesenchymal cells, fibroblasts, neural tissue, and cancer cells, is elevated during EMT. This cadherin switch, from E-cadherin to N-cadherin, is closely associated with invasiveness, motility and metastasis of tumor cells. Quite interesting, this switch of gene expression pattern is also noted in discoidin domain receptor (DDR) family and integrin members. DDR1 is highly expressed in epithelial cells whereas DDR2 is mainly expressed in mesenchymal cells. DDR1 expression is switched to DDR2 during EMT. In addition, integrins modulate the interaction of cells with the extracellular matrix (ECM), $\alpha 3 \beta 1$ integrin is a laminin-binding receptor whereas $\alpha 5 \beta 1$ integrin is a receptor for fibronectin. Expression of $\alpha 3$ is downregulated whereas $\alpha 5$ expression is elevated in EMT [16–18].

(b) Distinct but Complementary Roles of Snail and Twist in EMT

EMT is orchestrated by a network of EMT-transcription factors (EMT-TFs), including Snail/Slug, Twist1, ZEB1/ZEB2, E12/E47, Goosecoid, and SIP1 [19–21]. These factors act as a molecular switch to induce the EMT program by repressing a subset of adherens and tight junction molecules (such as cadherins, claudins, integrins, mucins, plakophilin, occludin and ZO1) and by activating expression of genes that can facilitate cell migration and invasion. Snail is zinc-finger transcription factor that plays an evolutionary conserved role in mesoderm formation from vertebrates to human [20]. First identified in *Drosophila melanogaster*, Snail represses expression of neuroectodermal genes such as *single-minded* and *shotgun*, which are essential for the formation of the mesoderm and neural crest [22]. In *Drosophila*, Snail inhibits *E-cadherin* expression, promotes cadherin endocytosis, and this is essential for the delamination and ingression of primary mesenchymal cells [23]. Snail is critical for gastrulation and normal development in mice [24]. Mice with homozygous knockout of Snail died at embryonic stage due to failure of mesoderm formation, which prevents gastrulation. Human Snail resides in chromosome 20q13, a region that is commonly amplified in breast cancer. The carboxyl-terminal region of Snail family members contains 4–6 C2H2-type zinc fingers, which specifically bind to DNA promoters containing an E-box sequence (CAGGTG). The N-terminus of all vertebrate Snail family members possesses a highly evolutionarily conserved SNAG (Snail/Gfi) domain, which is required for the transcriptional repressive activity of Snail (Figure 1). Snail directly recruits three key chromatin-modifying enzymes (LSD1, G9a and Suv39H1) through the SNAG domain to silence the E-cadherin promoter [25–27]. We also discovered that this Snail

repressor complex suppresses the expression of fructose-1,6-bisphosphatase (FBP1) and induces aerobic glycolysis and cancer stem cell (CSC)-like properties during tumor development and metastasis [28]. In addition, Snail was found to interact with the co-repressor complex SIN3A and HDAC1/2 to alter the chromatin structures for E-cadherin silencing [29].

Snail is a highly labile protein; we discovered that Snail is regulated by both protein stability (ubiquitination) and cellular location (phosphorylation) [30]. The repressive functions of Snail require residence in the nucleus; cytoplasmic Snail has a very short half-life and is targeted for ubiquitin-mediated proteasome degradation through GSK-3 β -mediated phosphorylation. We identified and characterized the kinase (GSK-3 β) [30], phosphatase (SCP1) [31], and ubiquitin E3 ligase (β -Trcp) [30] that govern Snail regulation. In addition, the p21-activated kinase 1 (PAK1) can also modulate the subcellular localization of Snail; Pak1 phosphorylates Snail at S246 and promotes the nuclear translocation of Snail in EMT [32]. In addition to β -Trcp (FBXW1), several E3 ligases, FBXL14 (Ppa), FBXL5, and FBXO11, have been shown to regulate the protein stability of Snail under different cellular contexts.

On the other hand, Twist belongs to the family of transcription factors that contain basic helix-loop-helix (bHLH) domain (Figure 1). Many studies indicate that Twist is required for EMT and the acquisition of CSC-like properties; cells with abundant Twist expression have the CD44⁺CD24⁻ phenotype and self-renewal ability [33–35]. Twist-induced EMT is well known to contribute to tumor cell invasion and metastasis by activating several signaling pathways such as Mir-10b and PDGFR [21, 36, 37]. It has also been reported that Twist plays an important role in the inhibition of oncogene-induced senescence and acquisition of chemotherapeutic resistance [38–42]. Twist is also subjected to post-translational modifications and degradation. Two residues, Thr125 and Ser127, at the C-terminal of the bHLH domain are phosphorylated by protein kinase A (PKA) [43]. PKB/AKT allows the Ser42 phosphorylated Twist to confer resistance to p53-mediated apoptosis in response to DNA damage, and promotes EMT and metastasis in breast cancer by enhancing TGF- β signaling [44, 45]. Three mitogen-activated protein kinases, p38, c-Jun N-terminal kinases (JNK), and extracellular signal-regulated kinases 1/2 were reported to phosphorylate Twist at serine 68 in vitro and prevent its protein degradation as well as promote breast cancer cell invasion [46]. Interleukin 6 (IL-6) activates casein kinase 2, which phosphorylates Twist at Ser 18 and Ser 20, leading to Twist stabilization and increased cancer cell motility [47]. Recent studies also show that the stability of Twist is negatively regulated by GSK-3 β -mediated phosphorylation on the WR motif (also known as the Twist box) of Twist protein [48].

The C-terminal bHLH domain of Twist recognizes the DNA sequence known as the E-box motif. Many mutations which cause Saethre-Chotzen syndrome are located within the bHLH domain demonstrating its functional importance [49]. The WR motif resides on the c-terminus of bHLH domain. Several molecules were reported to associate with the WR domain of Twist, and several point mutations in the WR domain are also linked to Saethre-Chotzen syndrome [50–52]. By using unbiased protein purification coupled with mass spectrometry analysis, we identified that Twist is di-acetylated at Lys73 and Lys76 [53].

This di-acetylation creates the “histone H4-mimic” GK-X-GK motif that is essentially for the interaction with the second bromodomain of BRD4 and the recruitment of BRD4/pTEFb/RNA-PolII complex to the promoter and superenhancer of Twist target genes; these events lead to the transcription activation of downstream signaling program.

Although Twist has been shown to bind to the E-cadherin promoter and repress its transcription in a manner similar to that of Snail, our studies indicate that Snail and Twist have distinct yet complementary functions in EMT regulation by functioning as a repressor and activator, respectively. Snail functions as a transcriptional repressor to suppress genes that prevent cell migration and growth (such as E-cadherin and FBP1); whereas Twist acts as a transcriptional activator to induce genes that favor cell migration and proliferation (such as Wnt5A). An analogy would be a moving car that has disabled brakes and an accelerating engine (Figure 1). Our results are supported by the findings seen during mesoderm formation in *Drosophila*, in which Snail functions as a transcriptional repressor to prevent ectoderm gene expression; whereas Twist serves as a transcriptional activator to induce mesodermal gene expression. Snail and Twist work synergistically, controlling distinct sets of genes, to coordinate EMT and mesoderm formation.

(c) EMT Occurs at the Tumor Invasive Front and is Driven by an Inflammatory Tumor Microenvironment

EMT is a reversible and dynamic event that is initiated and propelled by microenvironmental signals that cells received. In line with this contention, EMT is commonly observed at the tumor-stromal boundary of many invasive tumors [54, 55]. The tumor microenvironment evolves as tumor develops; this includes fibroblasts, endothelial cells and infiltrated immune cells (such as neutrophils, myeloid-derived suppressor cells (MDSC), as well as extracellular matrix (ECM) proteins, such as collagen, hyaluronic acid and fibronectin. Oncogenic signaling within tumors frequently drives the recruitment of normal fibroblasts (NAFs) and reprograms them into cancer associated fibroblasts (CAFs) [56]. CAFs are activated fibroblasts that share similarities with fibroblasts and are stimulated by inflammatory conditions or activated during wound healing [57]. In the tumor microenvironment, CAFs and the infiltrated immune cells secrete growth factors, such as fibroblast growth factor (FGF), epidermal growth factor (EGF), and hepatocyte growth factor (HGF), cytokines (IL-1 β , IL-6, and IL-8), and chemokines (CXCL12, CXCL14 and CCL7) that all together play an essential role in promoting tumor proliferation and facilitating metastasis [58]. A high content of inflammatory cells, particularly tumor-associated macrophages (TAM), are commonly observed at the invasive fronts of advanced carcinomas and correlate with increased metastasis [59]. TAMs are a key component of infiltrated immune cells in tumor stroma, they play an important role in chronic inflammation and have been viewed as an “obligate partner for tumor-cell migration, invasion and metastasis” [59]. TAMs modulate inflammation and adaptive immunity, increase tumor growth, enhance angiogenesis, and promote tissue remodeling and fibrosis. Patient samples from different solid tumors show a strong correlation of TAM content in tumor with a poor clinical outcome [60]. Intriguingly, TAMs often cluster around blood vessels and the tumor margin and produce a variety of proteases to facilitating ECM remodeling, basement membrane degradation, and tumor cell

invasion. In fact, metalloproteinase 9 (MMP9) and urokinase-type plasminogen activator (uPA) are produced mainly from TAMs.

CAFs constitute another significant component of the tumor stroma and mediate changes in extracellular matrix composition to one with increased amounts of collagens (desmoplastic response) [61, 62]. CAFs are activated fibroblasts that share similarities with fibroblasts and are stimulated by inflammatory conditions or activated during wound healing [57]. Hypoxia and ROS can also promote activation of CAFs. CAFs are phenotypically and functionally distinct from NAFs and can be identified based on the expression of several markers including α -SMA, FAP, FSP1, and PDGFR. TGF- β , PDGF, bFGF, IL-6, and LPA, which can induce desmoplastic reactions in tumors, also stimulate the differentiation and proliferation of NAFs to CAFs. Distinct from NAFs, CAFs significantly promote tumor growth in xenograft models when mixed with tumor cells. The tumor-promoting effects of CAFs are mainly mediated through a paracrine mechanism involving multiple secreted factors, including HGF, CTGF, EGF, IGF, NGF, bFGF, Wnt ligands, and MMP. In addition to these paracrine events, CAFs secrete many chemokines. For instance, CAFs secrete CXCL12, which binds and activates CXCR4 in tumor cells, to induce migration and proliferation of tumor cells [63]. In addition, CAFs can change their metabolic profile and provide metabolic intermediates necessary for the growth of tumor cells [64]. We showed recently that downregulation of isocitrate dehydrogenase 3 α (IDH3 α) plays a critical role in the metabolic reprogramming in CAFs [65]. Downregulation of IDH3 α decreases the effective level of α -KG by reducing the ratio of α -KG to fumarate and succinate; this event results in PHD2 inhibition and HIF1 α protein stabilization. The accumulation of HIF1 α , in turn, promotes glycolysis by increasing the uptake of glucose, upregulating expression of glycolytic enzymes under normoxic conditions, and inhibiting oxidative phosphorylation by up-regulating NDUFA4L2. CAF from tumor samples exhibit low levels of IDH3 α , and overexpression of IDH3 α prevents transformation of fibroblasts into CAFs. Together, these findings reveal IDH3 α is a critical metabolic switch in CAFs.

Myeloid-derived suppressor cells (MDSC) are also present in many cancer patients and animals with xenografted or naturally developed tumors [66, 67]. MDSCs, characterized by CD11b⁺ Gr-1⁺ in mice, are recruited and activated by several inflammatory factors, such as IL-1 β , IL-6, and VEGF [68]. MDSCs also produce pro-inflammatory factors to further amplify the inflammatory response in tumor stroma. MDSCs crosstalk with macrophages to impair the activation of CD4(+) and CD8(+) T cells [69]. In Tgfb β 2-deficient mice, MDSCs are recruited to the tumor invasive front through chemokine receptors CXCR2 and CXCR4, and thus enhance tumor cell invasion and dissemination [70]. A high level of MDSCs correlates with tumor grade, staging, metastatic tumor burden, and drug resistance in patients [71].

In addition to the stromal cells discussed above, lymphocytes, neutrophils, mast cells, T-regulatory cells and platelets, also contribute to the development of tumor microenvironment and, foster the growth, and facilitate the invasion and metastasis of tumor cells.

(d) EMT Generates Cancer Stem Cells

Epithelial-mesenchymal Transition (EMT) is a de-differentiation program that imparts tumor cells with the phenotypic and cellular plasticity required for metastasis, drug resistance, and disease recurrence. Morphologically, EMT confers tumor cells with fibroblast-like properties that result in reduced intercellular adhesion and increased motility. Functionally, EMT endows tumor cells with CSC-like traits and renders them resistant to therapeutics, with a proclivity for recurrence after treatment [33, 72–74]. Consistent with this notion, circulating tumor cells (CTCs), isolated from the blood of breast cancer patients, are enriched in EMT and CSC markers [75–83]. During development, part of the epithelial cells in ectoderm give rise to mesoderm through EMT, and these cells can further differentiate into different cell lineages and generate various organs, such as muscle, bone, and connective tissues. Through EMT, neural crest cells can also delaminate from neural crest and give rise to glial and neuronal cells, adrenal glandular tissues, pigment-containing cells of the epidermis and skeletal and connective tissues. These observations indicate that EMT is a cellular differentiate/de-differentiate program utilized during embryonic development and tissue remodeling and that tumor cells hijack this program for their own benefit for generating CSC-like properties during metastasis. Intriguingly, basal-like breast cancer (BLBC), which is a subtype of breast cancer that associates with distant metastases, disease recurrence and short survival times [84–88], is enriched with EMT characteristics and CSC-like traits. This subtype of breast cancer contains high percentage of CSC cells that possess CD44⁺/CD24[−] expression; these cells display E-cadherin downregulation and fibronectin and vimentin upregulation, and they also contain high levels of EMT-TF expression, such as Snail, Slug, Twist, and FOXC2. Mani et al showed that EMT can endow tumor cells with CSC-like traits [33, 89]. Expression of Snail or Twist in immortalized mammary epithelial cells increases tumorsphere-formation, a phenotypic characteristic associated with stem cells. In addition, TGFβ not only induces EMT but also increases the population of CSC-like cells (CD44⁺/CD24[−]) in breast cancer, suggesting that tumor microenvironment contributes greatly to promote metastasis by propelling EMT. Clinically, breast tumor samples from patients developed resistance to chemo- and endocrine therapies are enriched with cells containing CSC characteristics and EMT markers. Interestingly, a distinct population of highly invasive pancreatic stem cells display the expression of both CD133⁺ and CXCR4 at the invading edge for tumor [90]. The observations that EMT occurs at the tumor invasive front and that EMT provides tumor cells with “metastatic cancer stem cell” traits underscores the contention that EMT is critical for tumor progression and metastasis. In line with this contention, several developmental pathways (such as Wnt, Hh and Notch) that control self-renewal and differentiation of stem cells also involve in EMT induction. Therefore, EMT confers tumor cells with invasive power for metastatic dissemination at the primary tumor, and the CSC-like properties needed for colonization and proliferation of metastatic lesion.

Hypoxia and Inflammation

A hypoxic condition, in which the level of O₂ pressure is below 10 mmHg, is commonly found in many human solid tumors. Cells respond to hypoxia by stabilizing HIF1α that, in turn, heterodimerizes with HIF1β and induces the transcription of numerous target genes

involve in angiogenesis, glucose metabolism, survival and metastasis. For example, HIF1 α induces VEGF to promote angiogenesis. HIF1 α also increases CXCR4 expression in renal cell carcinoma to promote organ specific metastatic dissemination [91]. The crosstalk between hypoxia and EMT was identified recently, HIF1 α regulates the expression of several EMT-TFs, such as Snail, Slug, Twist, ZEB1 and SIP1, directly or indirectly, and thus promotes EMT induction and increases metastasis under hypoxia [92–96]. Interestingly, the level of HIF1 α correlates with Snail and Twist expression; co-expression of these proteins predicts the highest probability of metastasis and correlates with the worst prognosis in head and neck cancer [96]. Expression of uPA, its receptor (uPAR), MMP-2 and MMP-9 are also increased under hypoxia, and their expression promote invasion and metastasis both *in vitro* and *in vivo* [97]. In addition, expression of LOX, which can stabilize Snail and promote its activity [98, 99], is also increased under hypoxia. LOX promotes the remodeling of tumor microenvironment by facilitating the maturation of newly synthesized collagen fibrils, and thus increases invasion and metastasis through enhancing the focal adhesion kinase activity in tumor cells. The interaction of Snail with LOX highlights a synergistic effect on EMT induction under hypoxia. The cross-talk among pathways involved in inflammation, hypoxia and EMT *in vivo* is an interesting area that requires further investigated.

ER Stress Signals Contribute to Chronic inflammation in Tumor Development

The main purpose of an inflammatory response is to remove a foreign agent disturbing tissue homeostasis. In the normal physiological context, inflammation is terminated and the homeostatic state recovered after tissue repair or pathogen elimination. It is now widely accepted that inadequately resolved chronic inflammation promotes the development of cancer. Although a constant presence of antigens are found in some types of inflammation-associated cancers, such as hepatitis antigen in liver cancer and HPV antigen in cervical cancer, most tumors that have chronic inflammation with no noted antigens. The mechanisms by which these tumors maintain constant inflammation remains an enigma. Emerging evidence suggest that ER stress signaling represents a fundamental mechanism of chronic inflammation. All newly synthesized secretory and membrane-bound proteins, the molecules a cell uses to communicate with the outside world, are glycosylated, properly folded, and assembled in the lumen of endoplasmic reticulum (ER). Cells sense environmental stresses by increasing protein unfolding in ER, and develop three parallel branches of the sensing mechanism, collectively named as the “unfolded-protein response” (UPR), to cope with stress [100–103]. The first branch is the activation of transcription factor 6 (ATF6), which migrates to the Golgi apparatus upon activation. There, ATF6 undergoes intramembrane proteolytic cleavage, which liberates the soluble N-terminal basic-zipper-containing cytoplasmic domain of ATF6. This ATF6 fragment subsequently migrates to the nucleus to induce robust expression of heat-shock proteins needed to enhance the folding ability of the ER. The second branch requires PKR-like ER kinase (PERK) that undergoes oligomerization and autophosphorylation when cells under ER stress. Activated PERK phosphorylates the α -subunit of eukaryotic translation-initiating factor EIF2 α ; the phosphorylated EIF2 α causes a global translational repression to reduce the overload on ER, and concurrently increases transcription factor ATF4 for heat-shock protein production.

The ATF6 and PERK branches appear in metazoan cells later in evolution; however, IRE1/XBP1 represents the sole UPR-signaling branch conserved from yeast to metazoans including humans. IRE1 is an ER-bound kinase and a ribonuclease that promotes post-transcriptional maturation of XBP1 mRNA. When cells receive ER stresses, IRE1 triggers an unusual processing of the XBP1 mRNA, in which a 26 nucleotide intron-like region of mRNA is spliced out, resulting in a reading-frame shift of XBP1 mRNA. This truncated mRNA encodes a matured XBP1 (XBP1s) that contains an active transactivation domain at its C-terminus. Using a genome-wide promoter binding assay, XBP1 was found to regulate a broad array of genes involved in ER function and physiology, cell differentiation, and DNA repair [104].

UPR monitors cellular stresses by inducing transcription of genes (e.g., heat-shock proteins) that increase folding capacity, with a reduction in the sensitivity of cells to inflammation [105]. When stress is extensive and devastating, this process serves as a cytoprotective function by executing an apoptotic program for killing rogue cells without causing chaos in the organism. Thus, UPR functions as a checkpoint to mitigate stresses and ensure cellular homeostasis. If UPR is disabled due to the loss of XBP1, an extended and “unresolved ER stress” results, with a chronic inflammatory response and metabolic changes [106]. An unresolved ER stress leads to the activation of the upstream kinase IRE1 that recruits TRAF2 for activation of NF- κ B and JNK pathways and proinflammatory gene expression [106–110]. For example, when *C. elegans* larvae are infected by *P. aeruginosa*, it activates the immune response mediated by p38 MAPK family member 1 (PMK-1) as well as the IRE1/XBP1-mediated stress response [111, 112]. Activation of XBP1 inhibits the harmful effect of PMK-1 activation in response to immune attack and reduces the sensitivity of larvae to infection-mediated inflammation. Loss of XBP1 resulted in larval lethality due to hyperactivation of PMK-1, and conversely, the knockdown of PMK-1 rescued this lethality. In addition, the intestinal epithelial cells of XBP1^{-/-} mice show spontaneous enteritis exemplified by crypt abscesses and large amount of infiltrated immune cells. These mice are highly susceptible to dextran sulfate sodium-induced colitis than their wild-type counterparts [106, 109, 113]. The intestinal epithelium of XBP1^{-/-} mice possess an elevated inflammatory response indicated by increased secretion of cytokines and chemokines. In addition, cultured XBP1^{-/-} intestinal epithelial cells showed a sensitive inflammatory responses to TNF α stimulation. All of these pathological changes are similar to the alterations seen in human inflammatory bowel disease (IBD). Consistent with this notion, XBP1 mutations were associated with IBD, Crohn’s disease, and ulcerative colitis [102]. Together, these genetic and pathological observations indicate that XBP1 plays a conserved and critical role in reducing sensitivity to inflammation through its ability to remove stress and restore cellular homeostasis.

Similar to the phenomenon of IBD seen in XBP1^{-/-} mice, BLBC also harbors abundant infiltrating immune cells in comparison with other breast cancer subtypes [114–117]. In addition, BLBC cells have intrinsic NF- κ B activation [118–122]. Using multiple analyses, we identified that XBP1 expression is greatly reduced in cells from human BLBC tumor samples and mouse breast cancer tissues. Loss of XBP1 expression due to Snail-mediated repression is associated with NF- κ B activation and the enhanced CCL2 expression in BLBC cells. Based on these analyses, we speculate that loss of XBP1 results in “unresolved ER

stress”, which signals endogenous tissue injury and activates NF- κ B pathway for the induction of CCL2. This “inside out” signal subsequently recruits TAMs and lymphocytes to generate an inflammatory/wound microenvironment [123–125], which in turn provides additional cytokines and growth factors to further boost EMT and foster CSC-like traits in a vicious cycle when continuously unchecked.

Metabolism and Inflammation in Cancer Development

Unlike normal tissue, cellular proliferation in tumors is an uncontrolled process. When the size of a tumor reaches the oxygen and nutrient diffusion limit, tumor cells encounter not only a profound metabolic challenge, but also hypoxia and nutrient deprivation. To survive in this hostile environment, tumor cells reprogram their intrinsic metabolic machinery to cope with these challenges and thus modulate the inflammation and immune response of the body. Cancer-associated cachexia (CAC), characterized by progressive loss of skeletal muscle mass, anorexia, anemia, lipolysis, and insulin resistance, is the most important tumor-associated systemic syndrome that causes approximately 15%–20% of cancer death. Many lines of evidence indicate that the excess of cytokines released from tumors (IL-6, TNF α , IL-1, and IFN γ), trigger a systemic inflammation, leading to increased energy expenditure in patients. In advanced stages of cancer, IL-1 β is more strongly associated with the clinical features of cachexia such as general weakness, loss of appetite, weight loss, and sarcopenia than other cytokines [126]. Increased IL-6 in a murine colon cancer correlates with the development of cachexia, whereas treatment with monoclonal antibody to murine IL-6 suppressed it [127]. In experimental CAC models, administration of the cytokines listed above led to anorexia, weight loss, acute-phase protein response, protein and fat breakdown, and increased levels of cortisol and glucagon, as well as decreased the level of insulin. All these together result in insulin resistance, anemia, fever, and elevated energy expenditure [128]. Although the mechanistic interaction between systemic inflammation and tumor development in patients has not yet been fully elucidated, it seems possible that a set of cytokines has to work in concert to induce CAC and that a single factor might therefore be poorly predictive of CAC.

1,25(OH) $_2$ D is an active form of vitamin D, which regulates calcium metabolism and bone strength. In addition, vitamin D binds its vitamin D receptor (VDR) in the nucleus to modulate gene expression, this results in numerous cellular effects in differentiation, anti-proliferation, and anti-microbial, anti-inflammatory, and immunomodulatory functions in different tissues and organs [129–131]. Several metabolic active tissues, such as kidneys, bone, and intestine, contain highest VDR expression, whereas the vast majority of other human tissues show low to moderate level of VDR. The *Vdr* gene resides on chromosome 12q13.11; several nucleotide polymorphisms of *Vdr* gene have been reported in close correlation with the severity of chronic rheumatic diseases [132, 133]. Thus, the *Vdr* gene itself can affect some chronic diseases and 1,25(OH) $_2$ D-bound VDR can modulate transcription, leading to various cellular functions. Liver fibrosis, the result of excessive deposition of extracellular matrix components, is an irreversible wound-healing and tissue damaging/repair response that requires the activation of TGF β /SMAD pathway in hepatic stellate cells (HSCs). Intriguingly, the VDR pathway is an endogenous inhibitor of TGF β activation in the wound healing process [134]. VDR competes with SMAD3 at the

profibrotic target gene promoters in hepatic stellate cells and thus inhibits fibrosis. Mice with *Vdr*^{-/-} develop hepatic fibrosis spontaneously, suggesting that VDR is an endocrine antagonist to inhibit the wound-healing response in liver. In addition, calcipotriol, a VDR ligand, strongly suppresses inflammation and fibrosis in pancreatitis [135]. Interestingly, VDR is a known target of Snail and is repressed by Snail during EMT [136], suggesting that Snail-mediated VDR repression provides a feed-back activation of TGF β signaling and inflammatory response, facilitating fibrosis and EMT.

The tryptophan catabolic pathway plays an important role in tumor immunology. In 1998, Munn and Mellor showed that indoleamine 2,3-dioxygenase (IDO), a key enzyme that catalyzes the first step in tryptophan break down, is highly expressed in the placenta; this expression is required to prevent the mother's immune system to attack and reject the fetus [137]. The connection between tryptophan metabolism and immunosuppression is exciting, because it explains why expression of the father's genes, exposed in the fetus and normally marked for destruction by the mother's immune system, does not occur. IDO prevents the immune rejecting from happen and thus guarantees that women can nourish healthy fetuses to full-term. IDO has also been implicated in chronic viral infections and allergies, and in various autoimmune and inflammatory disorders in which immune control is disrupted [138]. Now, it appears that tumor cells hijack this metabolic pathway to evade the immune suppression. Early studies showed that many human tumors contain high level of IDO expression, and this often correlates with a poor clinical outcome. In addition, administration of an IDO inhibitor suppresses the growth of human lung cancer cells injected into mice [139, 140]. Recent investigations indicate that kynurenine, a metabolite from the breakdown of tryptophan, is responsible for immune suppression and the pro-survival functions elicited by tumors [141]. Kynurenine generated by the tumor cells interacts to the aryl hydrocarbon receptor (AHR) in immune cells, causing suppression of effector T cells (T_{eff}) and inhibition of regulatory B cells (B_{reg}) and dendritic cells, and thus shuts down the immune response. These studies indicate that disruption of immune escape could restore immune attack; this notion was recognized as a priority task for the National Cancer Institute for developing the IDO inhibitor and Clinical trials began in 2007.

Conclusions

In the past several years, we have experienced a wealth of data in delineating the essential roles of chronic inflammation in EMT, ER stress and metabolism. New ideas and concepts are emerged and suggest that these essential cellular processes are intricately linked to each other. However, tumor cells hijack these cellular mechanisms for their own benefits in proliferation, invasion, metastasis, and drug resistance. Despite all these efforts, we are only starting appreciate the complexity of tumors and our limited understanding of the molecular processes by which tumor cells deployed under chronic inflammation. Therefore, future investigations to reveal the contribution of chronic inflammation, ER stress and metabolism in tumors will lead to a comprehensive understanding of oncogenesis and will provide us with effective therapeutic strategies for treating metastatic disease.

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Figure 1. Schematic diagram of the signaling pathways associated with Snail and Twist induced EMT

An integrated and complex signaling network, including RTKs, TGF- β , Notch, Wnt, TNF- α , and BMPs signaling pathways, activate Snail and Twist, resulting in the EMT induction. Snail functions as a transcriptional repressor to suppress genes that prevent cell migration and growth (such as E-cadherin and FBP1); whereas Twist acts as a transcriptional activator to induce genes that favor cell migration and proliferation (Wnt5A) in an analogy of a moving car that has disabled brakes and an accelerating engine.