Immune Promotion of Epithelial-mesenchymal Transition and Generation of Breast Cancer Stem Cells

Jennifer M. Reiman1, Keith L. Knutson1, and Derek C. Radisky2

Abstract

Elements of the immune system act as intimate regulators of cancer progression, inhibiting early stages of tumor growth, through immunosurveillance while facilitating later stages of tumor progression. Recent findings have revealed that activated CD8 T cells can stimulate mammary epithelial tumor cells to undergo epithelial-mesenchymal transition (EMT) and to acquire the greatly increased tumorigenic capability and chemotherapeutic resistance of breast cancer stem cells (BCSC). These studies provide a window to understanding how BCSC arise and are maintained within tumors, and how to best target these processes for therapeutic benefit. Cancer Res; 70(8); 3005–8. ©2010 AACR.

Background

Epithelial-mesenchymal transition (EMT) is a fundamental biologic process by which epithelial cells detach from the surrounding tissue and acquire characteristics of mesenchymal cells, a unique motile, spindle-shaped cell with end-to-end polarity. Cells that have undergone EMT can migrate out of their epithelial layers to distant points and either remain mesenchymal or redifferentiate into epithelial cells by a process known as mesenchymal-epithelial transition (MET). EMT is best characterized for its role in embryonic development; induction of EMT is necessary for formation of the mesoderm and endoderm during gastrulation and for delamination of the neural crest. Following EMT, disseminated mesenchymal cells act as progenitors for many different tissue types. EMT occurring in epiblast cells at gastrulation, for example, gives rise to skeletal muscle, bone, and connective tissue, and EMT in neural crest epithelia gives rise to glial cells, neurons, melanocytes, cranial bone, and connective tissue (1). There is increasing evidence that EMT or EMT-related processes are involved in the underlying pathologies of several disease states including tumor initiation and progression (2). Investigations using culture models have shown that many of the components of the tumor microenvironment are capable of inducing EMT, including transforming growth factor-β (TGFβ), matrix metalloproteinases, and growth factors. Studies using animal models have shown that key transcription factors involved in developmental EMT, including Snail and Twist, are also activated in cancers. Artificial expression of Snail and Twist in epithelial tumor cells induces transition to a mesenchymal phenotype, which is accompanied by increased malignant properties such as invasion and metastasis (3). There is currently considerable debate about the role of EMT in tumor progression, and more specifically, whether EMT of tumor cells must necessarily result in a complete, irreversible transition to a mesenchymal state, or whether a partial or transient EMT may be more common (4).

Stem cells have long been investigated for their central role in organ development, but recent studies have shown that cells with stem and/or progenitor characteristics play critical roles as well in tumor formation and progression (5). Stem cells have been characterized as having low proliferative rates, existing as minority populations within tissues in defined niches, and having responses to extracellular stimuli that are distinct from those of the more differentiated cells within the organ (6). Stem cells exhibit self renewal and can divide symmetrically, to produce additional stem cells, or asymmetrically, to generate progenitor cells that can subsequently differentiate into the many different cell types within the organ. Although it was long suspected that some cancers may develop from transformed stem and/or progenitor cells, or that some cancer cells could acquire stem and/or progenitor characteristics such as limitless proliferation, it was not until 1994 that so-called tumor-initiating cells, also known as cancer stem cells (CSC), were first identified in human acute myeloid leukemia (7). Subsequent studies have identified CSCs in solid tumors, including breast, brain, colon, and pancreas (reviewed in ref. 8). Investigations of CSCs have taken on increased urgency with the discovery that they are resistant to conventional cancer treatments, suggesting that newer therapies should be directed specifically to the CSC subpopulations to improve outcomes. Identification of CSCs and the determination of their derivation and maintenance within tumors have become important areas of research. A significant advance toward these objectives was provided by a recent series of studies showing that induction of EMT in human or mouse...
mammary epithelial or epithelial tumor cells can result in the generation of cells with stem cell properties, such as self renewal and resistance to toxicants (9–11).

Research suggests that the immune system has a role in preventing cancer development. By targeting premalignant and malignant cells in a process called immunosurveillance, immune responses can eliminate cancer cells prior to tumors becoming clinically apparent or can attenuate tumor growth and progression. Investigations of immunosurveillance have established roles for both innate and adaptive immune effectors in preventing spontaneous or chemically induced tumors in mice (12). Immunocompromised mice have increased tumor formation, and tumors emerging in these mice are more immunogenic (i.e., rejected by immune competent mice) than those developing in fully immune competent mice, suggesting that emergent tumors are immunoedited or, in other terms, have a different phenotype because of their prior interactions with immune effectors (13). However, the immune system can also promote tumor development. For example, chronic inflammatory responses mediated by activated B cells and associated antibodies have been directly shown to be critical in the initiation of skin cancer in K14-HPV16 mice (14). Chronic inflammation is often associated with increased cancer risk in humans; patients with inflammatory bowel disease have an increased risk of colorectal cancer; Helicobacter pylori infection is associated with gastric adenocarcinomas and mucosa-associated lymphomas; and chronic hepatitis is associated with hepatocellular carcinoma (15). For patients with chronic inflammatory conditions, therefore, suppressing the immune response can reduce subsequent cancer development. Thus, an important debate has emerged that is attempting to reconcile the tumor-protective and the tumor-promoting roles of the immune system. One possible scenario is that the initial immune response against the tumor is modified by the tumor for promoting its own survival and progression. Another hypothesis takes into account the intrinsic genetic instability of tumor cells, and proposes that the immune system may select for or induce tumor cells that have acquired immune evasive properties. Which scenario is closer to the truth remains to be determined, but what is clear is that understanding tumor and immune system interactions may be an important goal for cancer prevention and treatment.

**Immuoediting Promotes Tumor Epithelial-mesenchymal Transition and Acquisition of Breast Cancer Stem Cell Properties**

In recent years, the study of tumor immunosurveillance has led to the concept of immunoediting, a model used to...
describe the dynamic interaction of tumors with the immune system. As put forth by Dr. Robert Schreiber and his colleagues, immunooediting encompasses three phases: elimination, the initial destruction of tumor cells; equilibrium, in which the tumor growth is suppressed; and escape, in which tumors evade immune destruction by becoming less immunogenic or more immune suppressive (12, 13). The fact that mutated or downregulated major histocompatibility molecules is an often observed feature of tumor cells suggests that immunooediting may be a general phenomenon (13). Previously we showed that immunooediting in a mouse model of breast cancer relapse generates tumors that have undergone EMT (16). When epithelial tumors from neu-transgenic mice that express the cell surface rat neu oncogene under control of the mammary epithelial cell-specific mouse mammary tumor virus (MMTV) promoter were transplanted into nontransgenic syngeneic mice, a T-cell–dependent rejection occurred, but the mice subsequently relapsed with tumors enriched in neu-negative variant tumor cells that had a mesenchymal phenotype (Fig. 1; refs. 16, 17). It seemed that an immunooediting process had either induced the epithelial tumor cells to activate an EMT, or selected for a subpopulation of tumor cells that had already undergone an EMT, to downregulate the expression of the epithelial-specific MMTV promoter and consequent loss of expression of the neu oncogene. These results represented the first direct demonstration that EMT may be a potential mechanism of immunooediting in cancer.

Subsequent investigations revealed that CD8 T cells were required for outgrowth of the neu-negative mesenchymal variants suggesting local induction of EMT rather than selection (11). Specifically, depletion of CD8 T cells prior to the injection of neu-positive tumor cells did not prevent the initial tumor rejection but these mice failed to relapse. Furthermore, CD8 T cells isolated from mice primed with neu-positive tumor cells were able to induce antigen loss when co-cultured with neu-positive tumor cells. Detailed subsequent characterization of tumor cells isolated from relapsed mice showed that these tumors had characteristics of breast cancer stem cells (BCSC), as indicated by the cell surface CD24−/loCD44+ marker profile (18), and enhanced mammosphere formation (18). These CD24−/lo CD44+ mesenchymal tumor cells also showed potent tumorigenicity, effectively forming tumors with 1,000 to 10,000 fold fewer cells than with the neu-positive epithelial cells. Additionally, mesenchymal tumor cells had elevated expression of drug transporters, DNA repair enzymes, and enhanced resistance to chemotherapy and radiation. One important characteristic of these BCSC-like tumor cells is that they gave rise in vivo to tumors with a heterogeneous tumor population consisting of both CD24+ and CD24− tumor cells. Histopathologic analysis of tumors formed by injection of neu-negative cells showed a predominant epithelial phenotype with the re-acquisition of neu expression, suggesting that the immune-induced EMT was fully reversible through the reverse process, MET. Thus, in contrast to their typically ascribed protective role, CD8 T cells were capable of inducing tumors to undergo EMT and to acquire BCSC properties and a more aggressive tumor phenotype.

Selecting Targeting of the Immune System for Therapeutic Benefit

How do CD8 T cells induce EMT? Previous studies have shown that exposure to TGFβ or transfection with EMT-inducing transcription factors results in the conversion of cultured human mammary epithelial cells to stem-like cells (9, 10). Immune cells are known to produce a diverse array of EMT mediators, including TGFβ and tumor necrosis factor alpha (TNFα). TGFβ is produced by CD4 T-regulatory cells and chronically stimulated effector CD8 T cells (19), and TNFα is produced by activated macrophages and pro-inflammatory T cells (20). Another possible mediator of EMT is interleukin-like EMT inducer (ILEI), which is expressed in lymphocytes associated with chronic inflammation (21). Local inflammatory responses are also associated with EMT. For example, increasing numbers of leukocytes (macrophages and T cells) infiltrating the kidney after acute unilateral ureteral obstruction in neonatal mice correlated with increased EMT (22). Chronic renal allograft dysfunction is also associated with elevated intraepithelial T cells present in renal tubules, which were frequently associated with mesenchymal marker positive cells, and coculture of human renal cortical epithelial cells with a human intraepithelial T-cell line for 72 hours led to increased mesenchymal marker expression as a consequence of T-cell–produced TGFβ (23). Overall, the evidence suggests that immune effectors can induce EMT following an acute or chronic inflammatory response. Likely, CD8 T cells trafficking into the tumor microenvironment can produce direct mediators of EMT, or alternatively, can produce other cytokines or chemokines (e.g., MCP-1), which can attract in other immune effectors (e.g., macrophages) that provide the stimuli.

The identification of cells within tumors that possess BCSC characteristics has resulted in increased efforts to identify treatments that selectively target this subpopulation of cells. It is likely that traditional treatments for breast cancer such as surgery, chemotherapy, and radiation, which target the bulk of the tumor, may leave behind the more malignant CSCs capable of reseeding tumors and giving rise to subsequent metastases (24). Although identification of therapies that selectively target BCSCs is an important goal, a parallel and perhaps equally efficacious approach would be to target the mechanisms of plasticity that generate and maintain the BCSC population in tumors. For example, because immunity is able to induce BCSCs, one approach would be to define and to target the specific immune effectors of this process. Although we have identified activated CD8 T cells as the critical effectors of EMT in mouse mammary tumor cells, it is not possible to generally target CD8 T cells given their important role in protection against infection. Further, as alluded to above, it is likely that additional immune components participate in tumor evolution in vivo. Skewing of the macrophage response within the tumor microenvironment from a M2 (tumor-promoting) to M1 (tumor-eradicating) phenotype may have the potential to reduce tumor invasion and metastases. Having a Th2 or regulatory T-cell (Treg)–polarized CD4 T-cell response may promote breast cancer metastases, suggesting that agents (e.g., vaccines) that shift from Th2/Treg to an antitumor
Th1 response may be useful (25). Another approach to inhibit EMT-associated tumor progression is to target the pathways involved in the induction of EMT that specifically lead to the acquisition of BCSC characteristics, as recently shown for inhibitors of EMT pathways mediated by TGFβ or Hedgehog pathways (26, 27). The immune system has long been viewed as a co-conspirator with developing tumors; more recent data have shown that it can also selectively target tumor cells at early stages of cancer progression. An important goal now is to identify how to reduce the tumor promotion abilities of the immune system while preserving or increasing its ability to eliminate tumor cells.

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