# The role of the immune system in early epithelial carcinogenesis: B-ware the double-edged sword

**Cancer is commonly described as a disease of genetic mutations. However, epidemiologic and clinical evidence points to the important but multifaceted role of the host. The immune system has something to say about cancer evolution through promotion of malignancy by inflammatory myeloid cells of the innate immune system. In a report in this issue of *Cancer Cell*, B cells are implicated as key players in the regulation of chronic inflammation that promotes early events in epithelial carcinogenesis. These are surprising observations, linking antibodies of the adaptive immune system to innate immune responses that drive epithelial carcinogenesis.**

The pathogenesis of cancer is not an all-or-none phenomenon, but rather a consequence of multiple events. Peyton Rous recognized this notion more than 70 years ago when he coined the term "cancer progression" to describe how "tumors went from bad to worse," in reference to experiments observing conversion of virus-induced benign papillomas to carcinomas (Shope and Hurst, 1933). Leslie Foulds extended the concept in his remarkable set of experiments characterizing mouse carcinogenesis, leading him to realize that sequential cellular alterations were required to generate cancer (Foulds, 1954).

Much has been learned from the study of skin carcinogenesis. The multistep story of cancer was put on firmer footing with the two-step "initiation-promotion" model of chemically induced skin carcinogenesis (Berenblum and Haran, 1955). In these ageless experiments, a single small dose of a mutagen, an initiator, irreversibly (genetically) altered the state of the cell. This phase is necessary but insufficient to produce tumors. Tumors are produced through promotion by repeated applications of inflammatory compounds to the receptive initiated site, producing inflammation, cell proliferation, and other effects that are reversible. An oversimplistic interpretation of two-step carcinogenesis is: (1) initiation by activating or disabling mutations in molecules that regulate the cellular circuits and capacitors controlling cell division, survival, and senescence, and (2) promotion by cellular and extracellular signals leading to immortalized cells that are resistant to growth-inhibitory signals and apoptosis. The molecular details of cancer pathogenesis are rapidly being unraveled at the cellular level, but what is missing in this deconstruction is how the physiologic responses of the host fit in.

When one moves from the reductionist cellular point of view to a more global view of carcinogenesis and cancer pathogenesis, the innate immune system emerges as an important player at the interface between the host, malignant transformation, and cancer progression. The immune system has been called a "double-edged sword," describing its ability on the one hand to fight infectious pathogens and on the other to produce autoimmunity. This metaphor applies to the immune system's relationship to cancer—the immune system can destroy tumors, and yet paradoxically also promotes and sustains

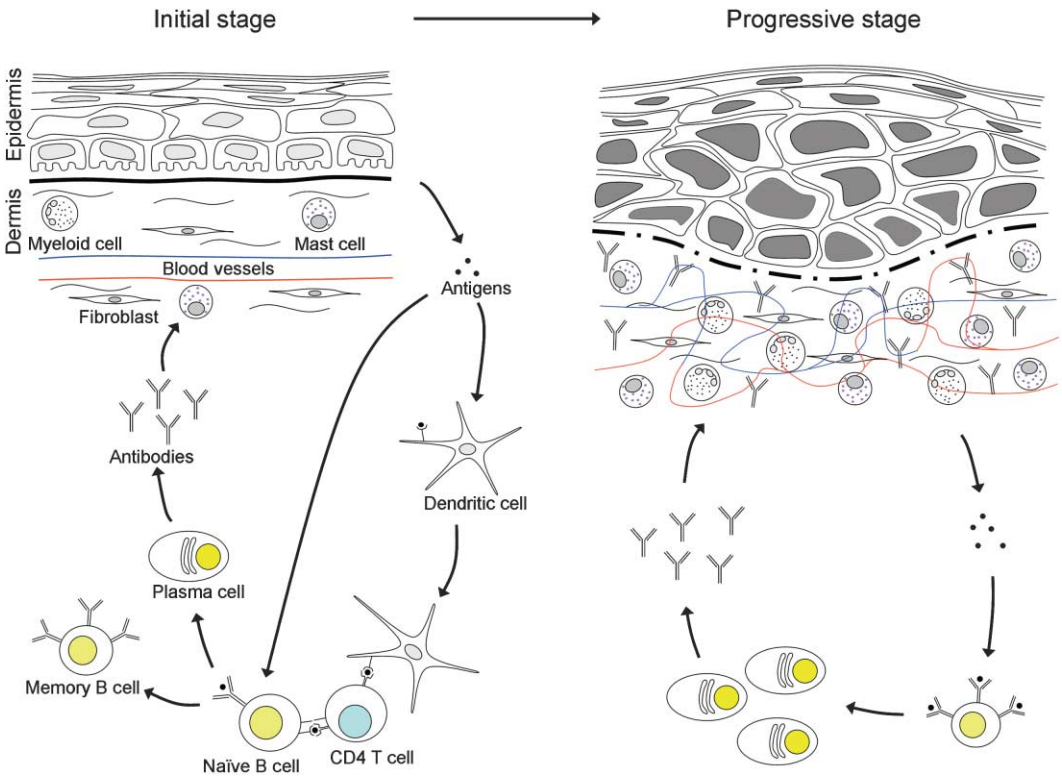


**Figure 1.** Model of antibody-mediated inflammation driving early skin carcinogenesis

Naive B cells are activated by skin-derived antigens (HPV16 E6, E7, and/or degraded products of keratinocytes and extracellular matrix?) in the presence of CD4 T cells, and differentiate into memory B cells and plasma cells. Antibodies produced by plasma cells stimulate resident myeloid cells (e.g., macrophages) in the dermis via activating Fc receptors to secrete VEGF, MMPs, and other inflammatory mediators, followed by further recruitment of innate immune cells. Chronic inflammation mediated by interaction of antibodies with innate immune cells facilitates cancer development.

**Table 1.** Macrophage-produced factors with potential to influence tumorigenesis

| Biologic effects | Factors and molecules |
|---|---|
| Growth and survival | Basic FGF, EGF, hepatocyte growth factor, PDGF, IL-6, TNF, polyamines, PGE2 |
| Angiogenesis | VEGF, MMP-9, IL-1, IL-8, urokinase-type plasminogen activator (uPA), CXCL1, CXCL8, HIF-1α, HIF-2α, PGE2 |
| Tissue invasion and metastases | Chemokines, PGE2, matrix metalloproteinases, uPA, plasmin |
| Mutations | Superoxide, peroxynitrite |
| Inhibition of T cell responses | IL-10, TGF-β, indoleamine-2,3-dioxygenase, PGE2, superoxide, peroxynitrite, arginase |

cancer. The rules for these choices are unclear.

A body of experimental and clinical evidence has emerged over the last decade showing that the immune system can prevent the development of incipient epithelial, mesenchymal, and lymphoid malignancies (Dunn et al., 2002). Mouse strains deficient in various arms of the immune system develop cancers at elevated rates compared to congenic immune-competent mice. The cancers that develop in immune-deficient mice are very immunogenic when transplanted back to immune competent mice, compared to the poor immunogenicity of spontaneous tumors arising directly in immune-competent mice. These observations suggest that tumors that arise in competent mice are shaped or edited by the immune system, evolving to escape further immune pressures. The details of immune surveillance have yet to be solved, but seem to involve lymphocytes, cytotoxic granules produced by lymphocytes, and signaling through type II interferon receptors. How adaptive immune cells (B cells and T cells with their remarkably specific receptors) and innate immune cells (natural killer and related cells, and myeloid lineage cells) contribute to cancer surveillance remains to be elucidated. We can say that the immune system serves as a tumor suppressor at the level of the host. Furthermore, immune therapies have become standard components of treatment for more advanced cancers, including cytokines, monoclonal antibodies, and T cells (crucial for the efficacy of allogeneic hematopoietic stem cell transplants). Immunity against cancer provides one edge of the blade that fights cancer.

The other edge of the blade is the role of the immune system in initiating and promoting cancer (reviewed by Balkwill et al., 2005). Clinical observations recorded through the centuries have pointed to the strong association of chronic inflammation and cancer. The great pathologist Rudolf Virchow recognized the association between inflammation and cancer and speculated that inflammation might play a role in tumor pathogenesis. Tumors arise in the context of stroma, which includes lymphocytes, myeloid cells (macrophages, dendritic cells, granulocytes, eosinophils, mast cells, and endothelial cells), fibroblasts, and connective tissue. Myeloid cells have drawn special interest because of their remarkable ability to produce a rich array of factors and small chemicals that can influence tumorigenesis both negatively and positively (Nathan and Sporn, 1991). Upon activation, macrophages express factors that promote growth and survival of tumors, angiogenesis, tissue invasion, and metastases (Table 1).

Because of the strong link between inflammation and cancer, most of the focus of researchers has been, appropriately, on cells and products of the innate immune system. Activated innate immune cells can interfere with T cell responses to cancer. Macrophages can: (1) inhibit antigen presentation by dendritic cells, directly suppress T cell responses, and indirectly downregulate immunity by inducing suppressor (regulatory) T cells through effects of IL-10 and TGF-β; (2) convert tryptophan to kynurenine through indoleamine-2,3-dioxygenase, disabling T cells; (3) deplete *L*-arginine by arginase leading to loss of CD3 ζ chain of the T cell receptor with impairment of signaling; (4) produce nitric oxide (NO) by inducible nitric oxide synthase (iNOS), creating nitrate adducts on tyrosine which block signaling from the IL-2 receptor; and (5) produce both superoxide and NO, which, under conditions of limited arginine availability, form the highly reactive oxidizing molecule peroxynitrite, instigating T cell apoptosis. Thus, ongoing inflammatory responses have the capacity to profoundly alter the ability of the host to mount adaptive immune responses locally within the inflammatory stroma of tumors.

Immunologists have long viewed the adaptive immune system, and particularly T cells, as central participants in immunity against cancer. This one-sided view has been repeatedly challenged through the years. Two relevant publications are worth mentioning from the many papers on this topic. A report from Daniel et al. examined the role of $CD4^+$ cells in epithelial tumors of transgenic mice expressing HPV16 early region genes (including E6 and E7) under a human keratin 14 promoter/enhancer (Daniel et al., 2003). HPV16 transgenic mice deficient in CD4 had a delay in tumor progression, albeit small, associated with decreased inflammation manifested as reduced infiltration of neutrophils and MMP-9 activity. These findings suggest a role for $CD4^+$ cells in tumor progression, an observation that makes sense from the perspective of $CD4^+$ T cells or NKT cells orchestrating inflammatory responses in their role as helper cells. In a second relevant study, Siegel et al. observed acceleration of tumor progression in transgenic mice expressing a mutant ras oncogene treated with a chemical tumor promoter following immunization with a mutated ras peptide (Siegel et al., 2000). Intriguingly, in the Siegel model, accelerated tumors were associated with the presence of antibodies against mutant ras protein.

Now, in this issue of *Cancer Cell*, de Visser and colleagues come along with the compelling evidence that soluble factors, probably antibodies, produced by B cells are the culprit in promoting de novo skin tumors in the HPV16 model (Figure 1) (de Visser et al., 2005). They demonstrate that HPV16 mice crossed to recombinase-deficient $RAG1^{-/-}$ mice, which have a complete absence of mature B cells and T cells, show a marked delay in early tumorigenesis associated with reduced inflammation. What is interesting is the finding that adoptive transfer of B cells or sera from wild-type transgenic mice restores inflammatory cell infiltrates, angiogenesis, epithelial hyperplasia, and tumor progression in premalignant lesions (Figure 1). The observations are novel with regard to linking soluble B cell products to inflammation in early steps in cancer development, addressing an old observation by tumor immunologists relating antibody responses (especially immune complexes with antigen, which are particularly proinflammatory) to accelerated growth of transplanted tumors. The results strongly point to antibodies in

establishing inflammation to promote earlier steps in cancer progression of skin previously "initiated" by the HPV16 transgenes. In contrast to the results of Daniel et al., $CD4^+$ cells were not required for this early tumor promotion. The discrepancy may relate to effects of different cells of the adaptive immune system at different stages of tumor development and progression. For Daniel et al., deficiency of CD4 cells correlated with a trend toward earlier lesions (hyperplasia versus dysplasia at 6 months) and a small but significant difference in incidence of squamous cell tumors at a later stage of tumor progression (at 12 months).

The observations of de Visser et al. raise a number of questions:

• Are antibodies mediating this tumor promotion, or might B cell-derived chemokines, cytokines, or other soluble molecules?

• If the active principle is antibody, what is the specificity? Cutaneous bacterial symbionts or pathogens, autoantigens (including the HPV transgene products), or products of mutations in transformed cells? Tantalizingly, de Visser et al. briefly relate unpublished observations that early development of tumors is not different in germ-free mice, suggesting that bacteria are not driving antibodies.

• If antibodies, what are the mechanisms for eliciting inflammation (Figure 1)? Signaling through activating Fc receptors on myeloid cells to initiate production of inflammatory molecules? Complement activation producing the anaphylatoxins C3a and C3b to drive inflammation through complement receptors on myeloid cells? Both?

• Do T cells play a role in the observations? Importantly, the authors could not rule out the complete absence of T cells in their adoptive transfer experiments.

If antibodies are found to be the mediators of the inflammatory response early on during carcinogenesis in this model, the answers to these questions will become relevant to developing strategies for prevention of cutaneous epithelial tumors and possibly other cancers.

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# Chromosomal instability in mouse metastatic pancreatic cancer—it's Kras and Tp53 after all

**A human pancreatic cancer progression model from intraepithelial neoplasia to ductal adenocarcinoma has been proposed. This process has been modeled in the mouse by activation of mutant *Kras* in pancreatic progenitor cells. In this issue of *Cancer Cell*, Hingorani et al. (2005) present a modification of their initial model by introducing a mutant Tp53. This combination of genetic alterations leads to rapid and increased frequency of neoplasia progression resulting in pancreatic cancers that manifest chromosomal instability in the presence of apparent intact telomeres. These findings introduce *Tp53*-mediated chromosomal instability as key event for carcinoma development in this mouse model.**

Pancreatic cancer likely reflects a model type of cancer displaying essentially all molecular and biological cancer hallmarks, such as genetic and epigenetic alterations, chromosomal instability, progression from preneoplastic lesions to an invasive and metastatic phenotype, and virtually complete resistance to any therapeutics tested so far. Along with this depressing clinical situation goes a considerable insight into the genetic and cellular events from the earliest preneoplastic lesions, found in as many as 50 percent of normal pancreata in the elderly population (Hruban et al., 2004), to late metastatic disease (Figures 1A and 1B). Due to significant progress in the development of genetically engineered murine models of human cancers (Van Dyke and Jacks, 2002), mouse models of pancreatic cancer have evolved that mimic the human disease genetically and morphologically in an astonishing way. By conditional activation of endogenously expressed oncogenic $Kras^{G12D}$ in the pancreas of mice, Hingorani, Tuveson, and colleagues were able to induce preneoplastic lesions that eventually progress to invasive and metastatic pancreatic adenocarcinoma (Hingorani et al., 2003 and Figure 1C). However, invasive and metastatic cancer developed at a considerable advanced age in mice, revealing a rather slow progression considering that PanIN-3 lesions can already be detected at the age of 4–6 months to the time of full-blown pancreatic cancer at around 12–15 months of age. By introducing tissue-specific deficiency of the Ink4a/Arf tumor suppressors, often mutated or silenced in