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Tumor Suppressor p16INK4A Regulates Polycomb-mediated DNA Hypermethylation in Human Mammary Epithelial Cells*

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Tumor Stroma and Regulation of Cancer Development

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Abstract

In the past 25 years, a majority of cancer studies have focused on examining functional consequences of activating and/or inactivating mutations in critical genes implicated in cell cycle control. These studies have taught us a great deal about the functions of oncogenes and tumor suppressor genes and the signaling pathways regulating cell proliferation and/or cell death. However, such studies have largely ignored the fact that cancers are heterogeneous cellular entities whose growth is dependent upon reciprocal interactions between genetically altered "initiated" cells and the dynamic microenvironment in which they live. This review highlights the aspects of cancer development that, like organogenesis during embryonic development and tissue repair in adult mammals, are regulated by interactions between epithelial cells, activated stromal cells, and soluble and insoluble components of the extracellular matrix.

ECM: extracellular matrix

Angiogenesis: growth of new blood vessels from preexisting vascular beds

Acute: a pathologic or physiologic event of severe and or short duration

Chronic: pathologic or physiologic condition that persists over a long period

Microenvironment: local area including all cellular and noncellular components

CANCER: A CHRONIC DISEASE INVOLVING MULTIPLE TISSUE COMPARTMENTS

What is cancer? Are cancers merely homogeneous masses of aneuploid cells with heightened migratory capabilities? If they were, their therapeutic elimination would have been possible years ago. Instead, cancers are heterogeneous multicellular entities containing cells of multiple lineages whose interactions with one another, the extracellular matrix (ECM), and soluble molecules in their vicinity are dynamic and favor cell proliferation, movement, differentiation, and ECM metabolism, while simultaneously restricting cell death, stationary polarized growth, and ECM stability. Thus, when referring to a "cancer cell" or a discrete property of "cancer," one should articulate which type of cell is being discussed, e.g., a mutated epithelial cell versus a vascular, inflammatory, or other activated stromal cell. Also important is the appreciation that angiogenesis, cell migration, and matrix and tissue remodeling are not unique properties of cancerous growths but instead are regulated programs normally utilized during development and in adult tissues responding to acute tissue stress. What then is unique about cancer as a chronic disease? Why is cancer disadvantageous to the organism harboring it, and what lessons can we learn from development or normal wound repair to aid in eradication of cancerous tissues? This review compares and contrasts the important regulatory programs and molecules implicated in maintaining cell and tissue homeostasis during development and following acute stress as compared with their roles in malignancy.

Cancer Cells and the Extracellular Matrix

All cancers contain a diverse population of cells, including those harboring genetic mutations typically referred to as "tumor" or "cancer" cells as well as other cell types that are activated and/or recruited to the local microenvironment, e.g., fibroblasts, innate and adaptive immune cells, and cells that line blood and lymphatic vessels. Reciprocal interactions between these responding "normal" cells, their mediators, structural components of the ECM, and genetically altered neoplastic cells regulate all aspects of tumorigenicity (1–3).

A functional link between inflammation and cancer has long been recognized. Innate immune cells, including granulocytes, dendritic cells, macrophages, natural killer cells, and mast cells, are prominent components of premalignant and malignant tissues (**Figures 1–3**) and contribute functionally to cancer development largely due to their release of potent soluble mediators that regulate cell proliferation, migration, angiogenesis, tissue remodeling, metabolism, and genomic integrity. Individuals suffering from chronic inflammatory disorders harbor a greatly increased risk for cancer development, owing primarily to the progrowth environment generated by activated inflammatory cells (**Figure 4**).

Fibroblasts are important cells in any context. They are primarily responsible for the synthesis, deposition, and remodeling of the ECM, as well as for the production of many soluble paracrine growth factors that regulate cell proliferation, morphology, survival, and death. Historically, fibroblasts were thought to be passive participants in neoplastic programming of tissues; however, recent data indicate that they exert an active role (2) and, in combination with inflammatory cells, can promote neoplastic programming of tissues (4).

Blood vessels are essential for maintaining tissue homeostasis and are activated during all tissue repair and growth processes as well as during cancer development. Tumor angiogenesis, the growth of new vessels from preexisting vascular beds (**Figure 5**), is regulated in part by local changes in the relative balance between soluble and insoluble molecules that elicit either pro- or antiangiogenic effects

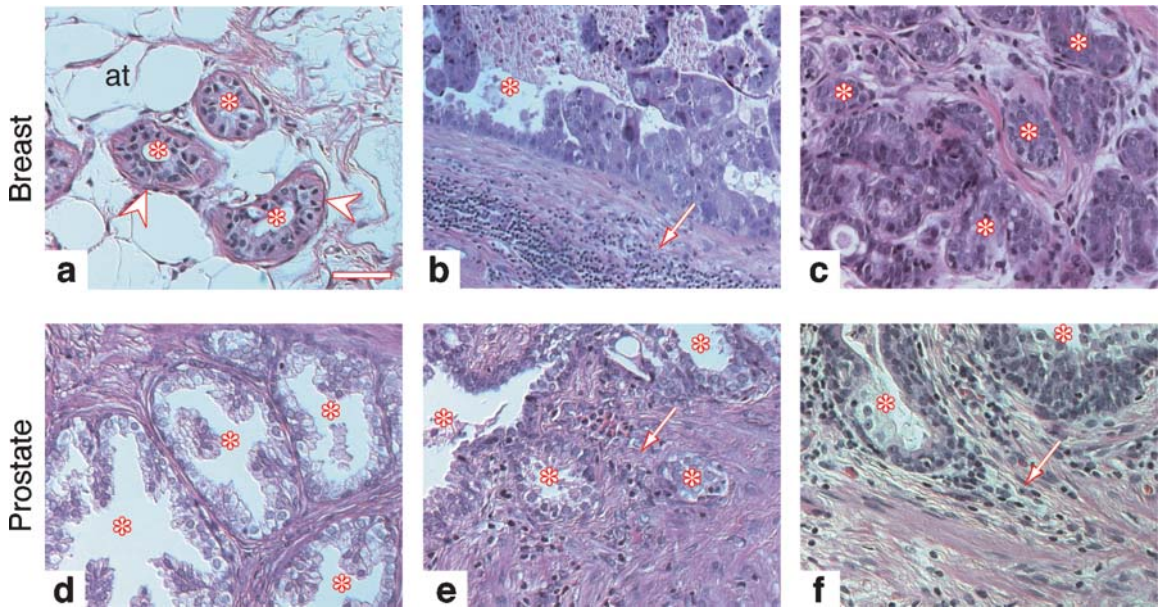

Figure 1

Stromal responses in breast and prostate carcinogenesis. (*a*) Normal mammary gland (*) from a nonpregnant woman of reproductive age showing a duct system, lined by epithelial cells and underlying myoepithelial cells (*arrowheads*). Ducts are surrounded by dense fibrous interlobular adipose tissue (at). (*b*) Ductal carcinoma in situ disorganization of ductal epithelium (*) stromal infiltration of inflammatory cells (*arrow*) in dense collagenous stroma are evident. (*c*) Mammary carcinoma in which ductal epithelial cells (*) form aberrant glandular structures and grow in cords without gland formation, surrounded by dense collagen bundles produced by activated fibroblasts. (*d*) Adult male prostate (benign nodular hyperplasia) with pseudostratified and columnar glandular epithelium with poorly stained foamy cytoplasm. (*e*) Prostatic intraepithelial neoplasia. Premalignant lesion consisting of closely packed irregular accini (*) lined by multiple layers of cuboidal epithelial cells adjacent to dense collagenous stroma and infiltrating inflammatory cells (*arrow*). (*f*) Invasive front of a prostatic adenocarcinoma in which rudiments of prostate gland are present (*) amid dense collagenous stroma infiltrated with diverse inflammatory cells (*arrow*). Bar: 50 μm (*a*, *d*); 100 μm (*b*, *c*, *e*, *f*).

on endothelial and perivascular cell proliferation, differentiation, migration, and/or tube formation.

The three-dimensional organization and architecture of the ECM or stroma surrounding the cells in any tissue are dynamic. The metabolism of ECM molecules—synthesis balanced by degradation—is an important aspect of tissue homeostasis and determines how cells respond to acute and chronic stress. Inappropriate synthesis or degradation of any ECM molecule can alter cell physiology and cause disease. Ultrastructural studies, immunohistochemistry, and biochemical analysis have each contributed to the appreciation that the stroma is altered at critical steps during the neoplastic process (5, 6). Early studies documented a change in the expression of proteins with acquired expression of α-smooth muscle actin, vimentin, smooth muscle myosin, calponin, tenascin, and desmin (5, 7). These proteins are often expressed as a response to wound healing or inflammation (8) and produce many new proteins that reflect a program of mesenchymal cell differentiation. During cancer development, these proteins and other ECM molecules embedded in the neoplastic tissue favor cell

Stroma: cells and connective tissue that provides contextual framework for an organ or tissue

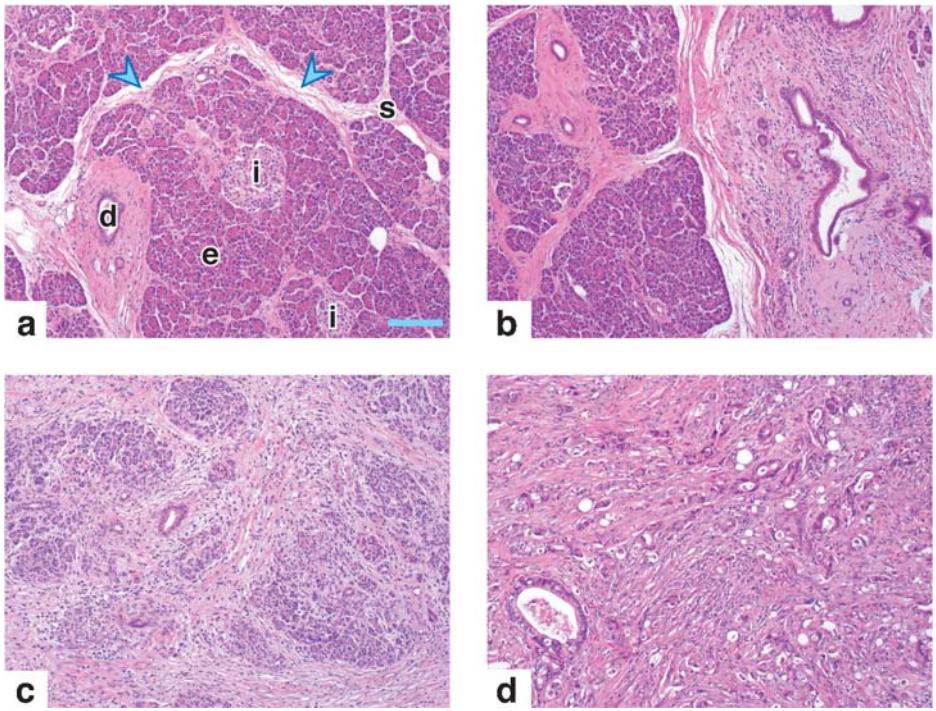

Figure 2

Inflammation and cancer in the human pancreas. H&E of human (*a*) normal pancreas, (*b*) early pancreatitis, (*c*) advanced pancreatitis, and (*d*) pancreatic ductal adenocarcinoma. The pancreas is a highly lobulated gland invested by a thin loose collagenous capsule (*arrowhead*) that extends as septa (s) between lobules. Exocrine acini (e) are densely packed, drain into ducts (d), and surround endocrine islets of Langerhans (i). Hereditary pancreatitis is characterized by a robust desmoplastic response thought to enhance neoplastic risk. Bar: 400 μm (*a–d*).

proliferation, inflammation, angiogenesis, and migration of cells into ectopic tissue compartments. Cancer development is like embryogenesis and adult wound repair in that it is also well-organized and follows highly regulated multistage events. All the cells and molecules comprising a tumor interact and engage in highly regulated reciprocal dialogues favoring malignancy.

THE ORIGINS OF CANCER

Initiation

The current predominant view of cancer is that of a disease involving irreversible genomic change—changes encompassing single mutations in specific genes, or alteration, amplification, or loss of large regions of the genome (9). Dominant gain-of-function and recessive loss-of-function alterations in critical gatekeeper genes, e.g., oncogenes and tumor suppressor genes, have been identified in virtually every form of human cancer and are believed to be at the root of the initiation of neoplastic growth. The sheer number of genes identified in the past 25 years harboring such alterations might suggest that random genetic mutations underlie cancer development. In reality, although a large diversity of mutated genes exist in cancer cells (9, 10), the overall neoplastic risk likely is influenced by a

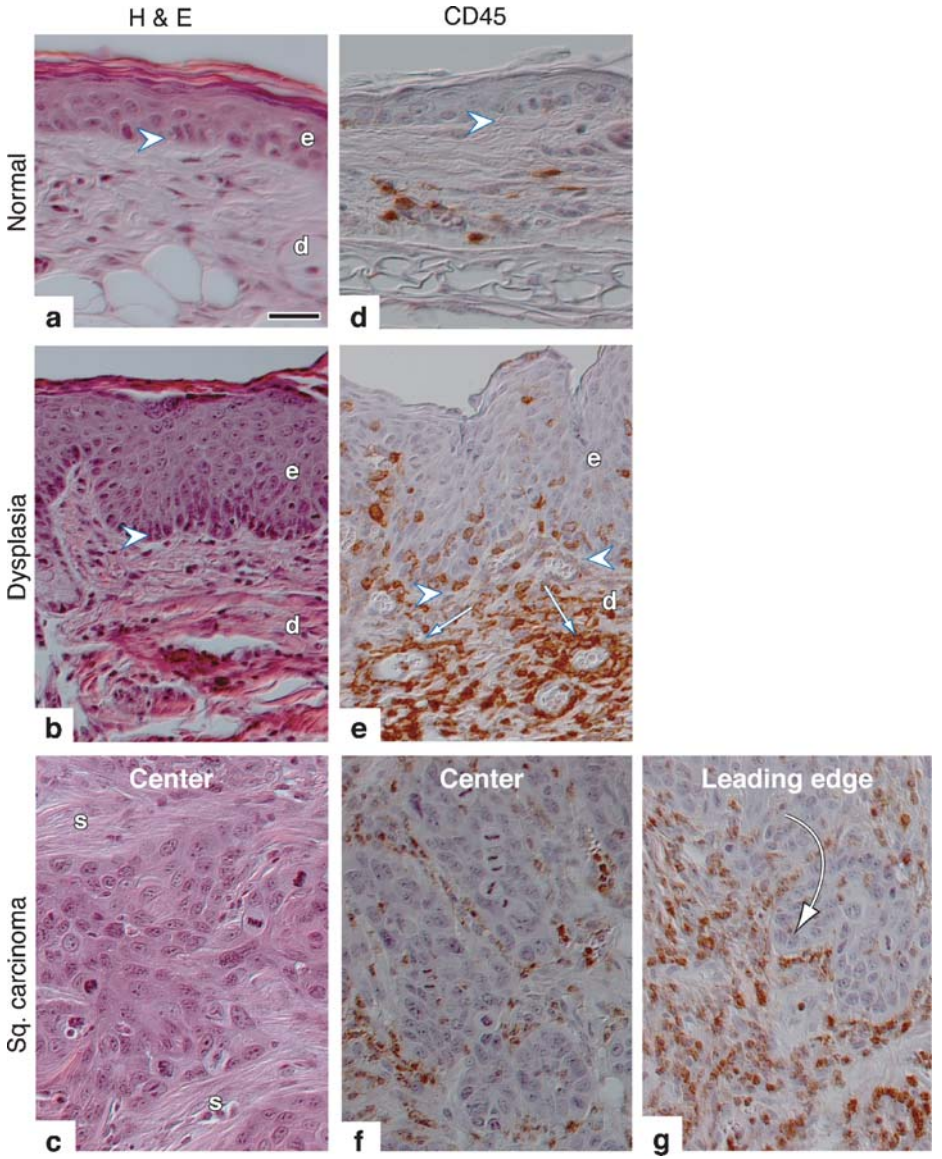

Figure 3

Malignant progression in K14-HPV16 (keratin 14-human papillomavirus type 16) transgenic mice. Skin from normal and transgenic animals stained with H&E (*a–c*) and immunostaining for CD45/leukocyte common antigen (*d–g*). (*a*) Normal ear skin demonstrating the normally thin, four-layered murine epidermis (e) composed of basal, spinous, granular, and corneum cells juxtaposed to the skin basement membrane (*arrowhead*) atop collagen-rich dermal matrix (d) containing few nucleated cells. (*b*) Dysplastic epidermis in which keratinocytes fail to terminally differentiate, basal and spinous layers are expanded, and cellularity in dermal compartment is increased. (*c*) Nests of fully malignant epithelial cells within a squamous cell carcinoma, growing amid dense stromal (s) matrix. (*d–g*) Immunostaining for CD45 (*brown staining*) in normal and neoplastic skin reveals an incremental increase in the presence of leukocytes most prominently infiltrating the dermal compartment in dysplastic skin around angiogenic blood vessels (*arrows*) (*e*) and at the leading edge (*curved arrow*) of an SCC (*g*) as compared with the tumor center (*f*). Bar: 50 μm (*a*, *d*), 100 μm (*b*, *c*, *e–g*).

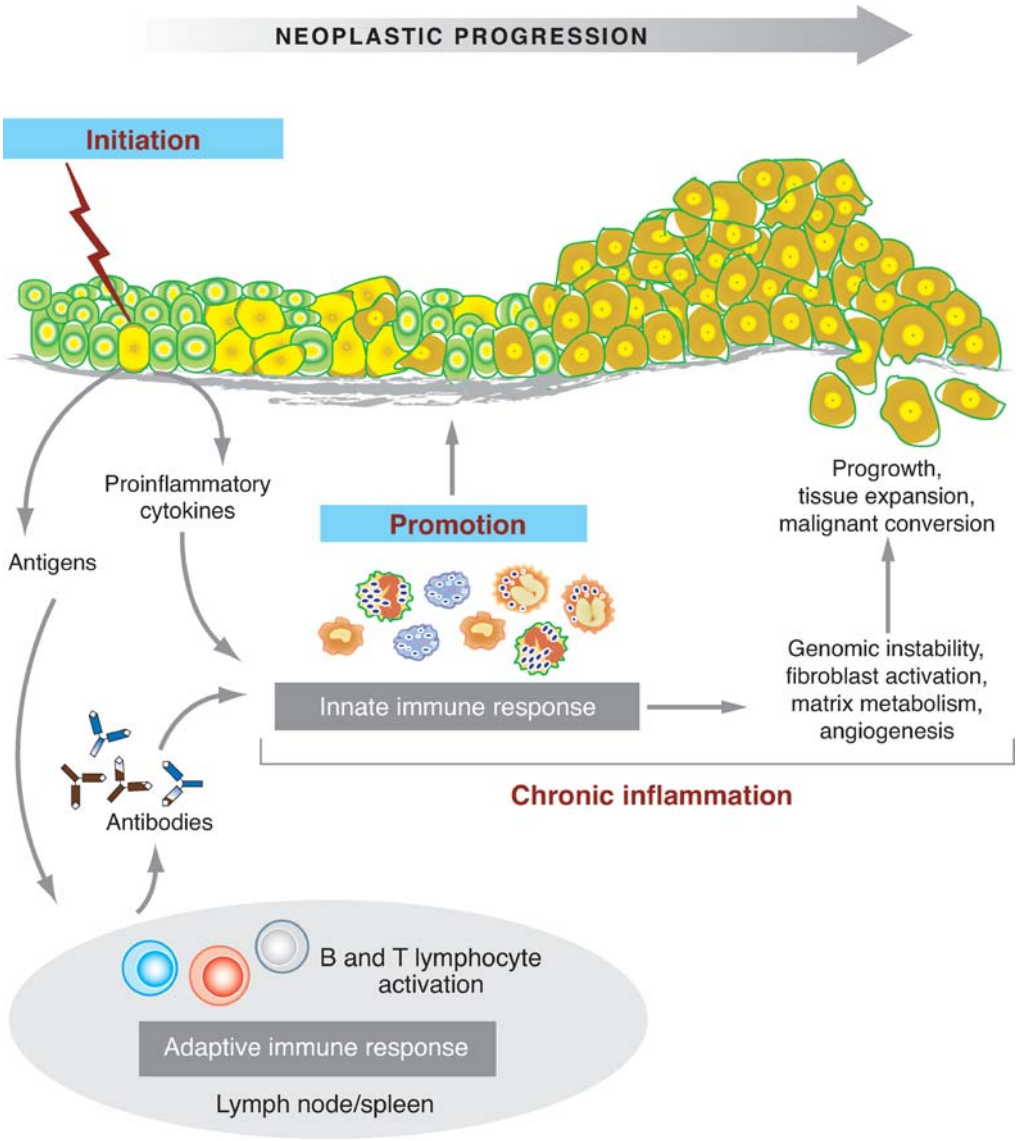

Figure 4

Model for role of innate and adaptive immune cells during inflammation-associated cancer development. Antigens present in early neoplastic tissues are transported to lymphoid organs by dendritic cells that activate adaptive immune responses, e.g., B and T lymphocytes, resulting in chronic activation of innate immune cells in neoplastic tissues. Activated innate immune cells promote tumor development via the modulation of gene expression programs in initiated neoplastic cells, culminating in altered cell cycle progression and enhanced survival. Inflammatory cells positively influence tissue remodeling and development of angiogenic vasculature by production of proangiogenic mediators and production of extracellular proteases. Tissues in which these pathways are chronically engaged exhibit enhanced risk of tumor development.

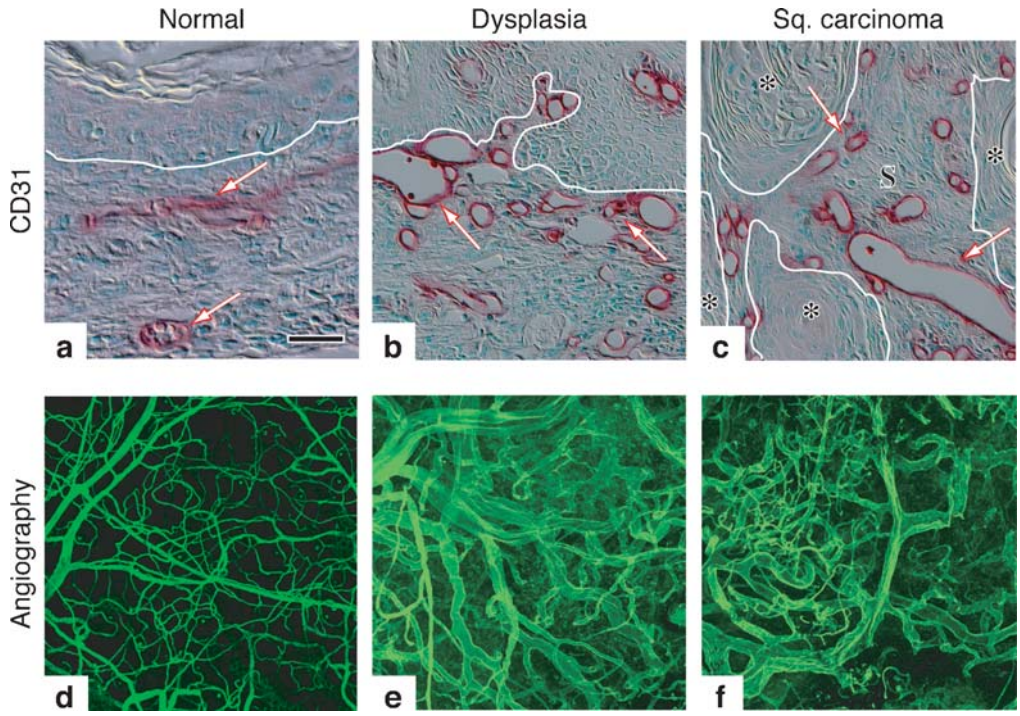

Figure 5

Angiogenesis during cancer development. (*a–c*) Immunoreactivity of CD31-positive endothelial cells in tissue sections reveals vascular (hematogenous and lymphatic) architecture in (*a*) normal mouse skin, (*b*) skin from HPV16 transgenic mice with dysplasia, and (*c*) in a squamous cell carcinoma. In normal skin, vessels are located deep in the dermal compartment (*arrows*). As neoplastic skin develops, vessels density and branching increases at sites where nascent vessels are juxtaposed to skin basement membrane (*arrows*). Stromal vascular structures in carcinomas are prominent (*arrows*) where their dilated nature is obvious. (*d–f*) Fluorescent angiography (220, 221) and whole-mount confocal microscopy of (*d*) normal mouse skin, (*e*) skin from HPV16 transgenic mice with dysplasia, and (*f*) a squamous cell carcinoma reveal three-dimensional organization and architecture of hematogenous vasculature. In premalignant and malignant tissue, angiogenic activation of blood vessels results in highly branched, dilated, and tortuous networks. The black line indicates skin basement membrane (*a*, *b*) and epithelial-stromal interfaces (*c*). Bar: 100 μm (*a–c*); 200 μm (*d–f*).

much lower number of critical physiological pathways that are either chronically enabled or disabled (11). Thus, genomic alterations affecting intrinsic cellular programs, e.g., cell cycle check-point control, programmed cell death, differentiation, metabolism, and cell adhesion, in combination with those affecting extrinsic programs, such as the immune response, matrix metabolism, tissue oxygenation, and vascular status, underlie human cancer development.

Inflammation as a Promoting Force in Cancer Development

Rous was the first to recognize that cancers develop from "subthreshold neoplastic states" (12, 13) caused by inherited mutations (14) or following somatic mutation of critical genes following viral or chemical carcinogen exposure (10, 15). "Initiation," as it is now known, is irreversible and persists in otherwise normal tissue indefinitely until nonspecific stimulation (now referred to as "promotion") occurs,

TGFβ: transforming growth factor β

NSAIDs: nonsteroidal anti-inflammatory drugs

COX-2: cycloygenase-2

typically following exposure of initiated cells to chemical irritants, such as phorbol esters, or from exposure to factors released at sites of chronic inflammation (16–22). In fact, Virchow hypothesized in 1863 (23) that cancer originates at sites of chronic inflammation, in part based on his hypothesis that some classes of irritants (promoters) enhance cell proliferation largely because of the tissue injury and ensuing inflammation that they cause. When tissues are wounded or exposed to a chemical irritant, damaged cells are removed by induction of cell death pathways, while cell proliferation is enhanced to facilitate tissue regeneration or wound healing, thus maintaining homeostasis. Proliferation and inflammation subside after the insulting agent is removed or the repair completed. By contrast, sustained proliferation of initiated cells in environments rich in inflammatory cells, growth/survival factors, activated stroma, and DNA damage–promoting agents potentiates and/or promotes neoplastic risk (17, 20, 22, 24).

Clinical and experimental studies linking inflammation and cancer. Clinical and experimental data indicate that innate immune cells, e.g., granulocytes (neutrophils, basophils, and eosinophils), dendritic cells, macrophages, natural killer cells, and mast cells, play a promoting role during cancer development (17). Many clinical studies have reported the abundance of innate immune cells, in particular mast cells and macrophages, in human tumor samples and correlated their presence with either angiogenesis or a clinical outcome (25–32).

How do inflammatory cells get coopted during neoplastic development? A plausible hypothesis is that many malignancies arise from areas of infection and inflammation simply as part of the normal host response (**Table 1**). Indeed, there is a growing body of evidence that many malignancies, e.g., gastric, cervical, and colon, are associated with bacterial or viral infections (24) or initiated by inherited mutations in genes encoding proteins that regulate tissue homeostasis (33, 34). In fact, more than 15% of malignancies worldwide are attributed to infections, corresponding to ~1.2 million cases per year (35). Population-based studies show that chronic inflammatory conditions predispose humans to certain cancers (**Table 1**) (20–22). Most notably, those patients with chronic *Helicobacter pylori* infection exhibit a 75% increased risk for gastric cancer, the second most common type of cancer globally (35, 36). In addition, experimental studies demonstrate that transforming growth factor β1 (TGFβ1)–deficient mice develop colon cancer and that this cancer is essentially eliminated by maintaining the mice in germ-free environments (37). Other clinical examples of the association between chronic inflammation and increased cancer risk are inflammatory bowel syndrome and colon cancer (20), chronic pancreatitis and pancreatic adenocarcinoma (19), and chronic hepatitis and hepatocellular carcinoma (20) (**Table 1**). Population-based studies examining the effect of various anti-inflammatory compounds, e.g., aspirin, nonsteroidal anti-inflammatory drugs (NSAIDs), and cycloygenase-2 (COX-2) inhibitors, have also concluded that chronic inflammation enhances cancer risk (38–41). These studies have revealed that long-term use of these compounds reduces colon cancer risk by ~50%, gastric and esophageal cancer risk by ~40%, and breast cancer by ~20% (24, 38, 41–46). However, other data show an increased risk of pancreatic cancer and non-Hodgkin's lymphoma among long-term aspirin users (47, 48). Thus, clinical data largely indicate a promoting role for inflammation in neoplastic progression and suggest that the elucidation of the mechanisms by which inflammatory cells participate in carcinogenesis may lead to the development of novel therapeutic agents against human cancer.

The availability of immune-competent mouse models of de novo carcinogenesis has facilitated mechanistic evaluation of the links between chronic inflammation, premalignant progression, tumor growth, and metastasis

Table 1 Chronic inflammatory conditions associated with neoplastic pathologies[a]

Pathologic condition	Associated neoplasm(s)	Etiologic agent
Asbestosis, silicosis	Mesothelioma, Lung carcinoma	Asbestos fibers, silica particles
Bronchitis	Lung carcinoma	Silica, asbestos, smoking (nitrosamines, peroxides)
Cystitis, bladder inflammation	Bladder carcinoma	Chronic indwelling, urinary catheters
Gingivitis, *lichen planus*	Oral squamous cell carcinoma	
Inflammatory bowel disease, Crohn's disease, chronic ulcerative colitis	Colorectal carcinoma	
Lichen sclerosis	Vulvar squamous cell carcinoma	
Chronic pancreatitis, hereditary pancreatitis	Pancreatic carcinoma	Alcholism, mutation in trypsinogen gene
Reflux esophagitis, Barrett's esophagus	Esophageal carcinoma	Gastric acids
Sialadenitis	Salivary gland carcinoma	
Sjögren syndrome, Hashimoto's thyroiditis	MALT[b] lymphoma	
Prostatitis	Prostate carcinoma	
Cancers associated with infectious agents		
Opisthorchis, *cholangitis*	Cholangiosarcoma, colon carcinoma	Liver flukes (*Opisthorchis viverrini)*, bile acids
Chronic cholecystitis	Gall bladder cancer	Bacteria, gall bladder stones
Gastritis/ulcers	Gastric adenocarcinoma, MALT	*Helicobacter pylori*
Hepatitis	Hepatocellular carcinoma	Hepatitis B and/or C virus
Mononucleosis	B-cell non-Hodgkin's lymphoma, Burkitts lymphoma	Epstein-Barr virus
AIDS	Non-Hodgkin's lymphoma, squamous cell carcinoma, Kaposi's sarcoma	Human immunodeficiency virus, human herpesvirus type 8
Osteomyelitis	Carcinoma in draining sinuses	Bacterial infection
Pelvic inflammatory disease, chronic cervicitis	Ovarian carcinoma, cervical/anal carcinoma	Gonnorrhea, chlamydia, human papillomavirus
Chronic cystitis	Bladder, liver, rectal carcinoma, follicular lymphoma of the spleen	Schistosomiasis

[a]Modified from References 17–20, 22, and 23.

[b]MALT, mucosa-associated lymphoid tissue.

formation (17, 23, 24, 49–53). Utilizing a transgenic mouse model of multistage epithelial carcinogenesis in which the early region genes of the human papillomavirus type 16 (HPV16) were expressed under control of the human keratin 14 (K14) promotor (K14-HPV16) mice (54, 55), researchers reported that despite transgene-driven oncogene expression in skin keratinocytes, absence of mast cells or inability to recruit innate immune cells to premalignant skin is sufficient to attenuate neoplastic progression (**Figures 3** and **4**) (50, 56, 57). Other studies have reported similar tumor-promoting roles for innate immune cells in the modulation of oncogene expression (58, 59). For example, secretion of the proinflammatory chemokine CXCL-8 by xenografted tumor cells is required for RasV12-dependent tumor-associated inflammation, onset of tumor vascularization, and tumor growth (59). Antibody-mediated depletion of granulocytes attenuates

HPV16: human papillomavirus type 16

K14: keratin 14

TNF: tumor necrosis factor

angiogenesis of RasV12-expressing tumors, suggesting that the recruitment of granulocytes by neoplastic cells facilitates tumor outgrowth (59). Several groups have reported that immature myeloid suppressor $GR1^+CD11b^+$ cells accumulate in the peripheral blood of cancer patients (60, 61) as well as in tumors and lymphoid organs of tumor-bearing animals (62, 63). Myeloid suppressor cells were initially identified as cells that indirectly enhance tumorigenesis by suppressing tumor-specific adaptive immune responses (61, 62, 64). However, myeloid suppressor cells also directly promote tumor growth by contributing to tumor-associated angiogenesis (63).

Macrophage infiltration is a key process during wound healing and similarly, tumor-associated macrophage (TAM) recruitment in human and animal models of cancer development are now known to be determinants of neoplastic progression (65). TAMs have been documented in precursor lesions of cutaneous melanoma, squamous and cervical carcinoma, and mammary adenocarcinoma (49, 58, 66, 67). Precursor lesions containing high numbers of TAMs may subvert normal macrophage-associated developmental processes and aid in the invasion of neoplastic cells into surrounding stroma (67) or in the direct remodeling of stroma, rendering it suitable for appropriate angiogenic and/or lymphangiogenic responses (68, 69). The biological effect of TAMs, however, depends on the local levels of chemokines, such as MCP-1, and the number of macrophages in the area (70). Studies using MCP-1-expressing tumor cells indicate that low concentrations of MCP-1 elicit modest macrophage recruitment and enhance angiogenesis and tumor growth in melanoma xenograft models (70). By contrast, high levels of MCP-1 induce more extensive macrophage infiltration, resulting in robust angiogenic responses and enhanced tumor growth but eventually tumor regression as well (70). Other studies suggest that fully malignant neoplastic cells can divert antitumor macrophage responses and suppress differentiation of mature tumor-antigen-presenting dendritic cells, thereby evading host adaptive immune response (67). Taken together, these data indicate that antitumor leukocyte responses depend upon the robustness of infiltration and relative inhibition of their antitumor activity. The data support the concept that oncogene expression in initiated cells is not sufficient for full neoplastic progression and underscore the role of inflammation as an important component of the host response involved in promoting cancer development.

Inflammation-derived mediators. What inflammatory cell–derived mediators enhance cancer risk and influence tumor development? The inflammatory component of a developing neoplasm includes a diverse population of leukocytes (**Figure 3**), all of which are variably loaded with an assorted array of chemokines, cytokines, cytotoxic mediators including reactive oxygen species, serine-, cysteine- and metallo-proteases, membrane-perforating agents, and soluble mediators of cell killing and cell proliferation, such as tumor necrosis factor (TNF), interleukins, and interferons (49–52, 63, 71–73). Individually, all of these molecules are known mediators of inflammation and evoke innate immune cell recruitment and/or activation, tissue remodeling, and angiogenesis. Together they create a microenvironment favoring cell proliferation, genomic instability, and expansion of cell populations into ectopic tissue microenvironments, i.e., malignant conversion and cancer development.

Leukocytes and other phagocytic cells can induce DNA damage in proliferating cells through their generation of reactive oxygen and nitrogen species, which normally are produced by these cells to fight infection and can react to form peroxynitrite, a mutagenic agent (74). Repeated tissue damage and regeneration of tissue in the presence of such highly reactive nitrogen and oxygen species damages DNA in proliferating cells, and can thus cause permanent genomic alterations, e.g., point

mutations, deletions, or rearrangements that promote neoplastic growth.

Several studies have provided molecular-level insight into which intracellular signaling pathways are coopted in initiated cells at risk for cancer development. The proinflammatory transcription factor nuclear factor κB (NFκB), a mediator of cell survival, proliferation, and growth arrest, has been identified as an important molecule linking chronic inflammation and cancer (51, 52, 75). Specific deletion of IKKβ—a key inhibitor of NFκB—in myeloid cells decreased carcinoma growth in a mouse model of colitis-associated cancer through reduced production of tumor-promoting paracrine factors (51). In addition, examination of a mouse model of inflammation-associated hepatocellular carcinogenesis similarly implicated activation of hepatocyte NFκB via production of inflammatory cell–derived TNF (52). These two mouse models reveal that the NFκB pathway acts in two ways to promote tumors: (*a*) by preventing death of cells with malignant potential and (*b*) by stimulating production of proinflammatory cytokines in cells of myeloid and lymphoid origin in tumor masses. These proinflammatory cytokines then signal to initiated and/or otherwise damaged epithelial cells and promote their proliferation and overall cell survival; thus, inflammatory cells in these contexts modulate gene expression and proliferation of neoplastic cells by paracrine regulation of NFκB.

In addition to influencing proliferation and survival of neoplastic cells, tumor-infiltrating inflammatory cells also regulate cancer development by affecting angiogenesis. A reduction in the extent of infiltration of inflammatory cells in mouse carcinogenesis models correlates with attenuated angiogenesis and reduced tumor growth (50, 57, 59). One mechanism by which inflammatory cells regulate angiogenesis is the production of proangiogenic mediators such as vascular endothelial growth factor (VEGF)-A (32, 76).

Tumor-infiltrating leukocytes also indirectly contribute to tumor development by producing extracellular proteases (49, 63, 71, 77–79). Matrix metalloproteinases (MMPs) are a large family of proteolytic enzymes that play key roles in cancer progression (80). MMPs regulate tumor development by remodeling ECM components as well as non-ECM substrates such as cytokines, growth factors, and cell-cell and cell-matrix adhesion molecules, thus contributing to angiogenesis, inflammation, and proliferation (80, 81).

NFκB: nuclear factor κB

VEGF: vascular endothelial growth factor

MMPs: matrix metalloproteinases

Several mechanistic studies have reported that inflammatory cell–derived MMPs functionally contribute to neoplastic progression (49, 63, 71, 81). For example, tumor incidence and growth in the K14-HPV16 mouse model of de novo epithelial carcinogenesis are reduced in the absence of MMP-9 (49). Neoplastic development is partially restored by reconstitution of MMP-9-deficient/K14-HPV16 mice by adoptive transfer of wild-type bone marrow–derived cells, indicating that inflammatory cells functionally contribute to de novo carcinogenesis, at least in part, by their deposition of MMP-9 into the neoplastic microenvironment (49, 82). Moreover, induction of cervical carcinoma in K14-HPV16 mice is markedly reduced when mice are systemically treated with an amino-bisphosphonate that acts on MMP-9-expressing macrophages (53). These data indicate that ECM remodeling and/or increased bioavailability of growth factors that are normally sequestered within the ECM are regulated by inflammatory cells present in the tumor microenvironment. Thus, inflammatory cells create an environment permissive for tumor growth.

Immune cells can also suppress cancer development. The immune system plays dual roles in tumor development and progression (83). In addition to the above studies indicating that immune cells can promote tumor development, other studies have reported that adaptive immune cells, e.g., B and T lymphocytes, may inhibit later stages of cancer development by affecting growth and/or dissemination of primary tumors. Studies supporting

this concept, also known as the immune-surveillance theory, have shown that infiltration of tumors by subsets of T lymphocytes can be beneficial and retard tumor growth (84–88). Based on the idea that a tumor can be a recognizable target for the adaptive immune system, several groups have attempted to activate adaptive immune cells to elicit antitumor immune responses (89). This topic is beyond the scope of the present article, but we refer readers to various excellent reviews (83, 90–94).

Alternatively, other groups have examined the role of adaptive immune cells during the early stages of cancer development, e.g., during premalignancy, and found that failure to appropriately activate/educate adaptive immune cells results in failed recruitment of innate immune cells into local neoplastic microenvironments (56, 57). As a consequence, tissue remodeling and angiogenic programs necessary for progression to the malignant states remain quiescent, resulting in attenuated tumor development (**Figure 4**). Taken together, these studies indicate that each stage of cancer development is regulated uniquely and that whereas activation of adaptive immune cells at the tumor stage may be beneficial for minimizing or eradicating malignant cells, activation during earlier stages elicits opposing effects.

Targeting inflammation and chemoprevention. It is now accepted that chronic inflammation fosters tumor development through diverse molecules and pathways. However, it is still unclear which pathways are key for activating inflammation in at-risk tissues and which are involved in maintaining chronic inflammatory states often associated with developing neoplasms. Furthermore, depending on the (pre)malignant states of the epithelial cells and/or level of inflammatory response, the contribution of immune cells to malignancy can be suppressive or enhancing. Thus, although undoubtedly complex, the identification of the major mediators and pathways responsible for triggering inflammatory cell infiltration into damaged tissue or their accumulation in premalignant tissues may provide therapeutic opportunities for the prevention and treatment of cancer. The efficacy of NSAIDS and COX-2 inhibitors (24, 41) in chemoprevention argues for anti-inflammatory therapy at the earliest stages of neoplastic progression. Alternatively, should future anticancer strategies focus on regulating NFκB activation, TNF-bioavailability, or metalloproteinase activity? In answering this question, it is important to point out that all organs are endowed with unique cell death and damage-response pathways that naturally invoke acute activation of innate immune cells. In skin, for example, keratinocyte cell death is by terminal differentiation (95). Inhibiting NFκB in keratinocytes promotes squamous cell carcinogenesis by reducing growth arrest and terminal differentiation of initiated keratinocytes (96) that proliferate in microenvironments in which growth factors, matrix remodeling enzymes, and reactive oxygen species produced by infiltrating inflammatory cells contribute to angiogenesis and keratinocyte DNA damage (17). By contrast, blockade of TNF attenuates skin tumor formation (97). The therapeutic regulation of TNF, however, must also be carefully considered, as this factor too possesses opposing activities that are cell type- and environment-dependent (98). Phase I clinical trials of TNF antagonists are currently underway in patients with advanced cancer; these experiments may help us understand the complexities of these responses (97, 98). Similarly, expression and activity of MMPs vary by organ and in response to damage (80), and although elimination or attenuation of MMP activity clearly evokes a survival advantage in immune-competent mouse models of cancer development, the efficacy of metalloprotease inhibitors in human clinical trials has been disappointing at best (99). So, what lessons can we learn from these failures? We must give special consideration to understanding the stage of tumor progression at which cytostatic agents targeting inflammatory

mediators are likely to work alone, and when a combination with standard debulking or cytotoxic therapy may be advantageous. Mouse models that more closely mimic human cancers are rapidly becoming available and must be applied in a way that also recapitulates the current therapeutic approach to the corresponding human disease.

Fibroblasts in Cancer: Phenotypes In Vivo and In Vitro

As the predominant cell in stroma, the fibroblast is responsible for the elaboration of most connective tissue components in the ECM, including collagens and structural proteoglycans, as well as various classes of proteolytic enzymes, their inhibitors, and various growth factors (8). Each organ has specialized requirements, and hence fibroblasts from different organs demonstrate organ-specific variations in the classes of biologically active molecules that they express (100). Furthermore, in response to varying physiologic signals, be they normal or pathologic, stromal fibroblasts change their phenotype and function (100).

Pathologists were the first to observe that fibroblasts in tumors were unique and underwent dynamic changes accompanying tumor progression (101, 102). In tumors, fibroblasts have been referred to as myofibroblasts, peritumoral fibroblasts, reactive stromal cells, and carcinoma-associated fibroblasts (CAFs). They typically exhibit a higher proliferative index, as compared with fibroblasts in normal tissues, often express α-smooth muscle actin, and are commonly surrounded by dense accumulations of fibrillar collagens (8, 103). This phenotype—common to several types of human cancer, e.g., breast, prostate (**Figure 1**), pancreatic (**Figure 2**), colon, and lung—is termed desmoplasia and is associated with the recruitment of inflammatory cells and activation of angiogenic programs.

CAFs isolated from malignant tissues exhibit altered phenotypes, most notably the enhanced production of collagens, hyaluronate, and epithelial growth factors (104, 105), disorganized patterns of growth, and enhanced proliferation (5, 106). Such phenotypes aid in tumor progression. Intriguingly, some of these phenotypes have also been detected in fibroblasts taken from distal, nontumorigenic sites in patients with cancer. Schor and coworkers (107, 108) found altered invasive properties of dermal fibroblasts from patients with cancer or with hereditary predispositions to cancer. Schor and colleagues (108) postulated that fibroblast abnormalities may influence the development of epithelial tumors and that hereditary defects may affect stromal-epithelial interactions and promote tumor formation. These important studies established that CAFs display altered phenotypes and raised the question of their possible functional significance in tumorigenesis.

CAFs: carcinoma-associated fibroblasts

Desmoplasia: hyperplasia of fibroblasts and disproportionate formation of fibrous connective tissue, mainly collagens, especially in the stroma of a carcinoma

Fibroblasts as a promoting force in cancer development. Tissue recombination experiments using fibroblasts and epithelial cells isolated from disparate sites have been instructive in analyzing the role of stromal fibroblasts in carcinogenesis (109). Early studies by Chung and coworkers (110–112) measured stromal effects on tumor progression by analyzing recombinant grafts containing tumorigenic epithelial cells with murine fibroblasts that were either normal, immortalized, transformed by viral or chemical carcinogens, or tumor associated. Depending on the characteristics of the epithelial tumor cell, fibroblasts exerted either a positive or negative effect on tumor development and progression (110–112). Taken together, these studies demonstrated that tumorigenic epithelial cells within recombinant grafts respond to fibroblast-derived signals and that the response depends on the types of gene mutated in adjacent epithelial cells.

In an alternative approach, using human tissues, Tlsty and coworkers (109) examined the effect of fibroblasts on nontumorigenic epithelial cells. These studies determined that CAFs send signals that either initiate abnormal epithelial growth or enhance the

progression of nontumorigenic cells to tumorigenic states. The combination of normal human prostatic epithelial cells with CAFs (obtained from prostatic adenocarcinoma) demonstrated an interaction that limited growth potential of the epithelial cells while reinstating their ability to form ductal structures resembling prostatic intraepithelial neoplasia. However, when CAFs were grafted with immortalized human prostatic epithelial cells, the resulting interaction resulted in tumors that often exceeded 5 grams (wet weight), surpassing the weight of control grafts by 500 fold (109). Remarkably, isolation of pure human epithelial cell populations from these tumors and subsequent grafting into animals demonstrated that the epithelial cells were then able to form tumors (i.e., contributing activity from CAFs was no longer necessary), as their transformation was accompanied by nonrandom chromosomal changes (109). Histological examination of these tumors demonstrated their malignant nature, e.g., enhanced cell proliferation, decreased cell death, angiogenesis, and genomic instability. Therefore, through a transient interaction, oncogenic signals from CAFs can stimulate nontumorigenic cells toward a malignant state, thus establishing an active role for fibroblasts in tumorigenic processes.

The stroma can acquire oncogenic activity by a variety of processes. The previous studies obtained fibroblasts from existing tumors and demonstrated their ability to stimulate oncogenesis. Can stroma develop oncogenic signals in the absence of a tumor? If stromal cells could acquire the properties that stimulate tumor initiation and progression through independent means, it would provide considerable insights into the risk factors for tumorigenicity. Recent experiments suggest that this is a possible mechanism of tumor generation and have begun to identify the processes involved.

Exposure to carcinogens. Decades ago, using skin (113) and bladder (114, 115) tissues, investigators observed enhanced tumor formation when carcinogen-treated stroma was heterotypically grafted with untreated epithelial cells. More recently, the effects of carcinogen treatment on stromal cells was examined in murine mammary tissues (116). Here, irradiation of epithelial cell–free mammary stroma (cleared fat pads) facilitated tumor progression of transformed epithelial cells that were subsequently introduced into treated stroma. In the irradiated stroma, mammary epithelial cells developed tumors faster and more often and reached a greater size than the same cells transplanted into unirradiated stroma. These data indicate that carcinogens can affect neoplastic processes not only by inducing genetic changes in epithelial cells but also by altering stromal cells such that they stimulate tumor progression.

Direct induction of fibroblasts by tumorigenic cells. Although the origin of fibroblasts in tumor stroma has been extensively debated, in vitro studies by Ronnov-Jessen and coworkers (117) provided evidence that the conversion of fibroblasts, but not of vascular smooth muscle cells or pericytes, constitutes the major source of myofibroblasts in mammary tissues. Isolation of each of these cell types and subsequent coculture with mammary tumor cells demonstrated that the majority of phenotypic and biochemical changes associated with desmoplasia were present in cocultures with fibroblasts and to a much lesser degree in cocultures with vascular smooth muscle cells. The finding that normal fibroblasts, placed in association with tumor cells, readily convert to α-smooth muscle actin–expressing cells suggests that factors elaborated from tumor cells modulate reprogramming. For example, TGFβ, often produced by tumor cells and present in a latent form in the ECM, induces α-smooth muscle actin and collagen production in cultured fibroblasts (118) and is a potential mediator of desmoplastic responses in tumors.

Wound healing. As discussed above, stroma at sites of wound healing and tumor growth share many characteristics (101, 119). Indeed, experimental evidence indicates that wounding can exert a tumor-promoting effect, and TGFβ has been postulated as a mediator of this effect (120). In Rous sarcoma virus–infected chickens, wounding leads to tumor formation (121). Subcutaneous injection of TGFβ substitutes for the wounding event, demonstrating the role of this molecule in altering the tissue microenvironment. The increased incidence of tumor formation at the sites of scar tissue (122) and in areas of chronic damage is also consistent with the idea that stromal changes accompanying wounding enhance tumorigenicity. The inflammatory response is present in both wound healing and carcinogenesis and may provide a common mechanistic link between the two.

Aging or induction of senescence. Age is a well-known risk factor for tumorigenesis, yet the mechanism underlying risk is uncertain, although accumulated genetic damage is likely involved. However, fibroblasts may play an important role as well. Increased collagen production and altered expression of MMPs are part of a gene expression program activated when fibroblasts enter senescence and when tissues age (123). This expression program can be activated either by prolonged growth of fibroblasts in culture, by aberrant expression of oncogenes (124), or merely by aging of the organism. Age-related alterations in fibroblasts may generate a microenvironment conducive to tumorigenicity (2, 125). Notably, chemically transformed rat hepatocytes demonstrate a greater tumorigenic potential when transplanted into older animals as compared with younger animals (126). Future studies will determine if aging fibroblasts contribute to alterations of stromal-epithelial interactions that modulate carcinogenesis.

Hormone imbalance. Extensive epidemiological data implicate hormonal imbalance in the generation of some forms of cancer (127–129), and experimental manipulation of hormone levels in animals verifies this observation (130). In the Noble rat model, exposure to hormones dramatically altered stromal-epithelial interactions and generated adenocarcinomas (131). Critical regulators, such as TGFβ, insulin-like growth factor-1, and VEGF, are altered upon administration of oncogenic doses of sex hormones, indicating that such alterations in these paracrine regulators of stromal-epithelial interactions can function as etiologic agents (131, 132).

Congenital or acquired mutations that alter stromal-epithelial signals. Fibroblasts containing inherited mutations that predispose to various cancers harbor abnormal phenotypes (2). If these mutations encode functional proteins involved in stromal-epithelial interactions or their regulation, these mutations may result in altering aspects of tissue specificity in the resulting tumor spectra. Such a situation has been postulated for juvenile polyposis of the colon (133, 134). In these polyps, deletions on chromosomes 10 and 18 have been detected in stromal cells, but not in epithelial cells, suggesting that inherited mutations in stromal cells may predispose one to carcinogenic conditions.

In addition to inherited mutations, acquired mutations in fibroblasts may contribute to carcinogenesis. New molecular tools have provided the means with which to examine tumor components individually. The combination of tissue microdissection (using laser capture microscopy) and the polymerase chain reaction has provided evidence that fibroblasts can acquire mutations independently from those in epithelial cells (135). Examination of fibroblast and epithelial cells from patients with ductal carcinoma in situ or infiltrating ductal carcinoma of the breast uncovered a group of mutations that can occur either in one cell type or both types. The high frequency of fibroblasts with acquired mutations whose proximity in the tissue is distal to carcinomatous tissue, in contrast to their

absence in normal mammary tissue, suggests that in some cases, stromal alterations may precede mutations in epithelial cells that are at risk for neoplastic progression.

Targeting fibroblasts for chemoprevention. Taken together, fibroblast alterations have tremendous potential to influence oncogenic processes. These alterations may be easily detectable prior to disease formation (136) or may arise in a concomitant time frame. Equally remarkable is the realization that the initial stimuli only need be applied in a transient manner to trigger formation of the lesion. In the examples described above, the need for altered fibroblasts to contribute oncogenic signals for the generation of a tumorigenic lesion was transient, i.e., the interaction of human CAF with epithelial cells (109), the single administration of a carcinogen administered to the stroma (116), or the rapid expression of MMPs (137). In each example, the transformed cell ultimately becomes independent of the fibroblast signals through alterations in its phenotype. In addition, fibroblast alterations may provide a gene expression signature that helps to predict the clinical course of disease (100). Further investigation of these processes will provide opportunities for the identification of novel targets for prevention and therapy. Because the fibroblast population does not seem to exhibit the extreme genomic changes that are so rampant in malignant epithelial cells, they may be a tractable target for therapy.

Tumor-Associated Angiogenesis

When any tissue expands or a primary tumor develops, growing beyond ~2–4 mm^3, influx of oxygen and nutrients and efflux of waste products must be ensured (138). To meet the metabolic needs of a rapidly growing tumor mass, development of a new blood vasculature is required and accomplished by activation of preexisting vascular beds, e.g., angiogenesis (138). During angiogenesis, a well-orchestrated series of events occurs, encompassing endothelial cell proliferation as well as directional migration of endothelial cells through remodeled basement membrane and perivascular stroma toward angiogenic stimuli (expanding populations of neoplastic cells) (139–141). Once endothelial cells display a proliferative and migratory phenotype, recruitment of perivascular support cells enables stabilization of nascent vessels, functional lumen formation, and appropriate blood flow. Activation of proangiogenic molecular and cellular programs in neoplasms is regulated at many levels and controlled by a diverse assortment of positively and negatively acting soluble and insoluble mediators whose balanced equilibrium is kept tightly in check under homeostatic conditions (142). However, under conditions of tissue stress, such as those that occur during the onset of incipient neoplasia, this balance is rapidly upset, favoring the proangiogenic program (139, 141, 143, 144). Whereas these cellular and molecular programs are common to physiological angiogenesis and appropriately turn on and off, tumor-associated angiogenic vasculature (**Figure 5**) is distinctly tortuous, chaotic in organization, and inherently unstable and leaky (145, 146). Thus, manipulating the stability of the tumor vasculature may constitute a viable anticancer therapeutic strategy (147), one that seeks to inhibit proliferation or migration of vascular endothelial cells.

Extracellular proteases and angiogenesis. MMPs are a family of at least 25 highly homologous, secreted or plasma membrane–associated zinc-binding proteinases (80, 148). MMPs can be produced by virtually all cell types, regulate many developmental processes, and participate in a variety of pathological conditions, including cancer, autoimmune diseases, and periodontitis (148). MMPs have been implicated as mediators of tumor angiogenesis at several discrete stages. This conclusion is based on MMP activity toward effector substrates that regulate angiogenesis by positive and negative mechanisms as well

as the observation that natural and synthetic inhibitors of MMP activity diminish tumor angiogenesis (80).

Previously, MMPs were thought to facilitate neoplastic progression by merely degrading ECM structural components, thereby allowing a cleared path for migrating neoplastic or endothelial cells. Indeed, cleavage of collagen type I is required for endothelial cell invasion of the ECM and vessel formation (149). Several proangiogenic polypeptide growth factors, most notably VEGF, basic fibroblast growth factor, and TNF, are highly expressed in developing tumors, as they are during physiologic wound healing; however, their bioavailability is limited, as they are either sequestered to ECM molecules or tethered to cells via membrane-spanning or anchorage domains (150). MMPs (and other extracellular proteases) regulate release of these factors, rendering them available for interaction with cognate receptors on vascular cells and thus activating the development of tumor-associated vasculature (80, 150). For example, MMP-9 targets the proangiogenic growth factor VEGF (77) by an indirect mechanism that targets an ECM VEGF-sequestering molecule. Both MMP-2 and MMP-9 activate latent TGFβ residing in the matrix (151), leading to the differential regulation of the proliferation of endothelial and perivascular cells and stabilization of nascent vessels (147, 152). In addition, several MMPs activate components of the plasma clotting system, such as fibrinogen and plasminogen, also implicated as regulators of angiogenesis (153–156). MMPs also modulate immune responses by processing (i.e., activating as well as inactivating) chemokines, a property important for resolution of acute inflammation and linked to progression of premalignant tissues (78, 157).

Whereas remodeling of some soluble or insoluble matrix molecules confers a proangiogeneic phenotype, remodeling of others confers antiangiogenic properties (158). Embedded within some ECM molecules are bioactive cryptic protein fragments released by proteolytic cleavage (80, 159). The first example of the release of a bioactive ECM fragment was the isolation of angiostatin from the urine of mice with Lewis lung cell carcinoma (160). Angiostatin, a plasminogen cleavage product containing kringle regions 1–4, inhibits endothelial cell proliferation, is believed responsible for maintaining Lewis lung cell metastases in a dormant state, and has demonstrated efficacy in limiting tumor burden in transgenic mice predisposed to pancreatic islet cell carcinogenesis (161). Several MMPs, including MMP-2, -7, -9, and -12, can generate angiostatin (159, 162). Another ECM fragment possessing antiangiogenic properties is endostatin, a 20-kDa NC1 fragment of type XVIII collagen (153). Endostatin is produced by cleavage of collagen type XVIII by MMP-3, -7, -9, -12, -13, and -20 (163) and acts by retarding endothelial cell proliferation (153, 160). Restin, by contrast, is a 22-kDa NC1fragment of type XV collagen that inhibits migration, but not proliferation, of endothelial cells in vitro and suppresses tumor-induced angiogenesis in renal xenograft carcinoma models (164). All three chains of type IV collagen (α1, α2, and α3) are potent inhibitors of angiogenesis and tumor growth (159, 165–168). The 24-kDa NC1 fragment of the α1 chain of type IV collagen, also termed arrestin, inhibits growth of human tumor xenografts in nude mice by significantly inhibiting growth factor–mediated angiogenesis (165). Furthermore, the antiangiogenic activity of arrestin is mediated by binding to endothelial α1β1 integrins (165). Likewise, canstatin, the 24-kDa NC1 fragment of the α2 chain of type IV collagen, suppresses growth of human tumor xenografts in nude mice by inhibiting angiogenesis (166). In vitro studies indicate that canstatin specifically inhibits proliferation, migration, and tube formation of endothelial cells (166). Lastly, the 24-kDa NC1 fragment of the α3 chain of type IV collagen, termed tumstatin, acts as an angiogenesis inhibitor, blocking both endothelial cell proliferation and functional lumen formation

(167–170). Studies using transgenic mouse models also indicate that tumstatin is generated by MMP-9 and suppresses angiogenesis via αvβ3 integrin interactions (171). Taken together, these studies indicate that protease-generated cleavage products of the ECM, basement membrane proteins, and other soluble molecules act as suppressors or activators of pathological angiogenesis in tissue-dependent and stage-dependent manners and implicate MMPs as important mediators of tumor-associated angiogenesis through both protumor and antitumor mechanisms.

Extracellular Matrix Molecules and Cancer Progression

The function of normal organs is determined by reciprocal communication between cells in an epithelial layer and their surrounding stroma (172), and the same organizational principles apply to cancer. The progression from a hyperproliferative state to malignancy can be characterized by increasingly abnormal communications between all cell types comprising the tumor mass (**Figures 1–3**) (2, 173). Thus, if disruptive stresses in the tumor microenvironment contribute to cancer development, therapeutic strategies aimed at restoring normal communication between different cell types within a tumor may be effective in combating neoplastic disease.

The three-dimensional ECM surrounding cells contains a mixture of fibrillar proteins, glycoproteins, proteoglycans, cytokines, and growth factors (174). The ECM provides both the structural support and contextual information for cells to determine the correct response to a given set of stimuli (175–177). Composition of ECM varies considerably both between and within different tissues, and ECM changes temporally as an adaptation to varying signals during normal developmental processes and during pathological processes such as those that occur in cancer. Epithelial cells can initiate incorrect stromal signaling, resulting in stromal cell production of growth factors that, in turn, stimulate inappropriate proliferation of neighboring epithelial cells (101). Alternatively, an aberrant matrix component produced by stromal cells in response to a local stress can be perceived by neighboring epithelial cells as a signal to proliferate or to enter a new developmental pathway (2, 3, 101, 178).

ECM supports cell adhesion and transmits signals through cell-surface adhesion receptors (179). The ECM of most tissues contains various types of fibrillar and nonfibrillar collagens, noncollagenous glycoproteins, and proteoglycans (180). By contrast, basement membranes are specialized forms of ECM, involved in separating tissue compartments and providing a scaffold on which some cell types, epithelial cells for example, adhere and exhibit polarized and/or differentiated growth (159). ECM remodeling may be essential for maintaining tissue integrity and involves a tightly regulated balance between ECM synthesis and ECM remodeling (159, 180, 181). During wound healing, pericellular ECM molecules in the immediate area of tissue damage are remodeled predominantly by MMPs secreted by epithelial cells, activated fibroblasts, or inflammatory cells (159, 180). In turn, fibroblasts and vascular cells synthesize the appropriate amounts of ECM components (type I collagen, fibronectin, etc.) important for tissue repair (159, 180). By contrast, in fibrotic environments (i.e., liver cirrhosis, renal and lung fibrosis, and scleroderma), the balance between ECM synthesis, accumulation, and degradation is shifted, which favors synthesis and accumulation, resulting in fibrosis, a phenotype that also can be caused by increased synthesis of ECM components independent of the degradative enzymes that remodel ECM (182, 183). A shift in favor of ECM remodeling is seen in degenerative pathologies such as arthritis (184) and tumor development (80, 150, 185). However, it is also clear that some fibrotic and inflammatory conditions increase cancer risk (17) and that some familial cancer syndromes result from gene defects that produce stromal changes before epithelial changes ever occur

(173, 186, 187). For example, a study by Moinfar and colleagues (135) examined genetic alterations in tumor-associated stroma from several independent cases of mammary carcinoma and found chromosomal rearrangements not present in the malignant carcinoma cells. These results indicate that characteristic mutations affecting stromal cells may have contributed to the formation of the epithelial tumors. Under normal homeostatic conditions, these misinterpretations are corrected by either cell cycle arrest or induction of cell death/differentiation programs; however, if the abnormal signal persists, cellular behavior can become increasingly abnormal, creating an evolving, interdependent, heterogeneous tissue (also known as a tumor) defined by its ability to grow and its unresponsiveness to normal physiological controls.

Matrix metabolism and collagen. Solid tumor growth cannot be sustained unless the tumor efficiently coopts fibroblasts, inflammatory cells, and vascular cells to help reset the balance between ECM remodeling (degradation) and ECM synthesis (181). In soft tissues, type I collagen accounts for 80%–90% of all collagenous proteins (188). It belongs to a family of 21 ECM proteins (188) that play a dominant role in maintaining the structural integrity of organs and tissues (189) as well as a more specialized role in regulating cell polarity, migration, survival, and phenotype. Proteins in this family also provide diffusible signaling molecules following breakdown (174). Within this family of molecules, types I, II, III, V, and XI collagens are included in the group of fibril-forming collagens on the basis of their structural and functional features (189).

Under normal homeostatic conditions, fibrillar collagens have a relatively slow metabolic turnover; however, under conditions of tissue remodeling, interstitial collagen metabolism is increased. Recent studies on human prostate, ovarian, lung, breast, and skin malignancies have shown a relationship between increased synthesis of type I collagen, increased collagen content in neoplastic tissues, and increased serum levels of type I collagen degradation products (190–195). These retrospective studies examining interstitial collagen status, in combination with in vitro studies demonstrating a functional relationship between collagenase-specific cleavage of type I collagen and growth factor–induced angiogenesis (149), suggest that type I collagen remodeling is an integral component of neoplastic stroma and may represent a potential target for anticancer therapeutic targeting.

When developing neoplasms undergo malignant conversion and acquire the capacity to invade normal tissue, increased architectural disorder at the invasive front of the neoplastic mass is characteristic (**Figures 1–3**) (185). Increased production of matrix remodeling enzymes and synthesis of many matrix components, most notably type I collagen, occur at this site (50, 185, 196, 197). Multiple cell types contribute to the maintenance of appropriate ECM composition at the invasive front, similar to that observed during wound healing. Activated fibroblasts migrate to premalignant tissue, in which they synthesize new matrix components, e.g., collagen type I and fibronectin, in response to a variety of factors including TFGβ, platelet-derived growth factor, interleukin-1α, interleukin-1β, and mast cell tryptase (17).

ECM composition is dynamic during development wound healing and in neoplastic states, and the architecture and form that ECM molecules assume are of critical importance for appropriate cellular responses. The architecture of tumor-associated ECM is fundamentally different from that of preexisting stroma (**Figures 1–3**) (198). Whereas preexisting collagen fibers in dermal stroma are readily identified by their parallel orientation with respect to the epithelium (188), newly synthesized peritumoral stroma (i.e., type I collagen) is characterized by its loosely woven morphology and nonplanar orientation (181). Increased collagen synthesis in the periphery of benign tumors results in tight

encapsulation of malignant cells that can limit neoplastic cell expansion (181). However, invading and metastasizing cancers acquire a capacity to orchestrate stromal responses in favor of malignant progression (i.e., invasion and metastasis) (181), suggesting that initial stromal responses may limit neoplastic propensity but are subsequently redirected to enhance neoplastic progression.

In ocular melanoma, for example, type I collagen is deposited as curved sheets around and in between tumor cell nests in which vascular structures are present (198–201). When type I collagen fibrils assume this arc-loop network, an efficient fluid-conducting meshwork is formed, which is closely apposed to hematogenous vasculature (202). Because vasculature in developing neoplasms is inherently leaky (203), these meshworks may efficiently allow for drainage of plasma and erythrocytes in some tumors while contributing to high interstitial pressure characteristics in others (204).

TARGETING THE TUMOR MICROENVIRONMENT: CLINICAL IMPLICATIONS

An important challenge remains to investigate novel anticancer therapies aimed at targeting both malignant tumor cells and the contexts in which they live. This review has examined facets of inflammation, angiogenesis, and fibroblast and extracellular matrix biology associated with neoplastic programming of tissues. As stated above, the efficacy of NSAIDS and COX-2 inhibitors (24, 41) in chemoprevention argues for the use of anti-inflammatory therapy at the earliest stages of neoplastic progression. Functional roles for extracellular proteases in early neoplastic progression and invasion, malignant conversion, and some metastatic events have clearly been demonstrated and prompted the first clinical testing of noncytotoxic biologic therapeutics. Unfortunately, results with metalloprotease inhibitors thus far have not been encouraging (99). The requirement for efficient vascularization of tumors and discovery of potent pro- and antiangiogenic molecules have produced a diverse array of angiogenesis inhibitors currently undergoing clinical evaluation (141, 147, 205–207). Some of these have demonstrated efficacy and have been approved for limited use in patients (208, 208a).

Fibrotic breast disease can also predispose one to breast cancer (122, 191, 195), and environmentally induced fibrotic disorders can increase incidence of lung and skin cancer (185, 209–212), underscoring the importance of developing therapeutic modalities that target tumor microenvironments. Therefore, future therapeutic strategies may consist of approaches targeting collagen synthesis with pharmacologic inhibitors such as Halofuginone. Halofuginone, which inhibits type I collagen synthesis by blocking TGFβ-mediated phosphorylation and activation of Smad3, has demonstrated efficacy in reducing fibrosis in scleroderma and liver disease (213–215). This inhibitor was recently approved as a therapeutic for scleroderma (215a) and is currently in clinical trials for treatment of various types of solid tumors (215b). Because remodeling of collagen fibrils is also involved in the alignment and formation of endothelial tubular structures (149, 216), inhibition of collagen synthesis may limit tumor-associated angiogenesis (217, 218). Furthermore, collagen type I is an important binding protein for a wide variety of mitogenic and morphogenic growth factors (219); therefore, disturbing the balance of regulated collagen metabolism may alter the bioavailability of these factors and subsequently inhibit neoplastic programming. The body of literature supporting these approaches suggests that we are at the dawn of a new era in cancer therapeutics. Thus, we are optimistic about the development of efficacious noncytotoxic chemopreventatives that would block neoplastic programming of tissues before life-threatening primary and/or metastatic diseases emerge.

SUMMARY POINTS

1. Malignant tumors are composed of several dynamic component parts.
2. As an oncogenic agent, stromal cells can provoke tumorigenicity in adjacent cells in the absence of preexisting neoplastic cells.
3. Chronic inflammatory disorders enhance overall risk for cancer development.
4. Biological processes can contribute opposite effects at different stages of carcinogenesis.
5. Stromal targets for therapy have advantages over epithelial targets.
6. Antiangiogenic therapy may stabilize tumor vasculature and enhance delivery of drugs to developing neoplasms.
7. Biologic therapies may inadvertently alter tissue programming and therefore should be fully explored in preclinical animal models of human disease pathogenesis.

FUTURE DIRECTIONS/UNRESOLVED ISSUES

1. Stromal signaling pathways that operate at each distinct stage of carcinogenesis and in each cell type should be identified and targeted.
2. Critical epithelial–stromal signaling pathways as therapeutic targets during the earliest stages of premalignant development should be identified.
3. The mechanisms by which oncogenic signals from stroma facilitate generation of neoplastic epithelial cells should be elucidated.

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