Multifocal epithelial tumors and field cancerization: stroma as a primary determinant

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It is increasingly evident that cancer results from altered organ homeostasis rather than from deregulated control of single cells or groups of cells. This applies especially to epithelial cancer, the most common form of human solid tumors and a major cause of cancer lethality. In the vast majority of cases, in situ epithelial cancer lesions do not progress into malignancy, even if they harbor many of the genetic changes found in invasive and metastatic tumors. While changes in tumor stroma are frequently viewed as secondary to changes in the epithelium, recent evidence indicates that they can play a primary role in both cancer progression and initiation. These processes may explain the phenomenon of field cancerization, i.e., the occurrence of multifocal and recurrent epithelial tumors that are preceded by and associated with widespread changes of surrounding tissue or organ “fields.”

Introduction
The vast majority of epithelial cancers are limited to in situ lesions that, for internal organs like breast, prostate, or lung, can remain undetected for the whole life of an individual (1, 2). The reason why only a minor fraction of these lesions progress to malignancy is not understood. In fact, many if not most of the genetic changes found in invasive and metastatic tumors are already present in premalignant lesions, raising the question of whether such changes are a primary cause or merely permissive for later cancer-spreading events. A related issue raised by deep sequencing analysis of tumors is the question of whether any of the identified driver mutations actually initiate the carcinogenic process (3). An extreme view is that none of these mutations are by themselves a driver of cancer development and that it is the ecological cellular environment that restrains or unleashes tumor growth (2, 4). Changes in tumor stroma are most frequently viewed as secondary to changes in the epithelium; however, recent evidence indicates that they may play a primary role. Such a possibility would help explain, not only dormancy of most epithelial cancers, but also field cancerization, a condition of major clinical significance defined as broader tissue and organ changes beyond localized areas of tumor development that result in multifocal and recurrent tumors (refs. 5, 6, and Figure 1).

In this Review, I will start with an overview of the clinical problem, followed by a discussion of underlying changes in epithelial and stromal tissues. I will focus on new insights into early stromal events that precede and determine the development of epithelial cancer. A defining primary role of the stroma may be of substantial conceptual and practical value for the development of new approaches to treat and prevent epithelial cancer.

The clinical problem
An important but overlooked fact is the multifocality of cancer, with a surprisingly high frequency of multiple lesions of primary origin (with estimates ranging between 3% and 25%) of same or different histological types, with concomitant or subsequent occurrence (synchronous versus metachronous lesions), and with occurrences at proximal versus distant organ sites (7–9). An obvious difficulty is distinguishing between truly independent primary lesions and separate lesions that are the result of distant spread with single initiating events. As a result, published frequencies of multiple primary (MP) cancers depend on operational definitions adopted by various cancer registries, like those of the Surveillance, Epidemiology and End Results Program (SEER) (http://seer.cancer.gov/) and the International Association of Cancer Registries (IACR) (http://www.iacr.com.fr/). Typically, multifocal malignant lesions originating at same body sites, including the entire lung, are considered for epidemiological purposes as single primary cancers. However, significant differences exist in the adopted criteria, including the counting of contralateral malignant breast lesions as MP cancers according to SEER, but not IACR, rules. A number of epidemiological studies have also reported on the incidence of multiple primary tumors within the same or neighboring organs, with potentially important insights (10–17). Notably, premalignant lesions are usually excluded from cancer statistics so that real frequencies of MP lesions are likely to be significantly underestimated, a conclusion supported by the staggering numbers of premalignant and malignant lesions that are discovered by autopsy studies of individuals with other causes of death (30%–40% of cases) (18–21).

Squamous cell carcinoma of the head and neck (HNSCC) is the sixth major cause of cancer death and a problem of major clinical significance (22). The concept of field cancerization was first developed in a landmark study of these tumors, in which a link was established between the common multifocality and recurrences of HNSCC and histological abnormalities, not visible to the naked eye, in surrounding epithelial and stromal tissues (6). There have been substantial advances in surgical treatment of HNSCC, in combination with radiotherapy and targeted approaches, such as EGFR inhibitors. However, these improvements have not led to any significant decrease in locoregional recurrences, secondary tumors, or early distant metastases, and the overall five-year survival rate has improved only marginally (22). Cutaneous field cancerization with multifocal and recurrent skin squamous cell carcinomas (SCCs) is also a common occurrence in organ transplant recipient patients treated with calcineurin inhibitors, representing a major cause of death (23).

Adenocarcinomas and SCCs of the lung are also frequently multifocal (24). While adenocarcinomas tend to be peripherally
Potential determinants of multifocal and recurrent epithelial cancer and field cancerization. Aging and environmental insults, such as UV irradiation or smoke, can target both epithelial and stromal compartments of organs, leading to stable genetic and epigenetic changes. Cross-talk between these two compartments can induce further pro-oncogenic alterations, such as secretion of growth factors and proteases, alterations in the extracellular matrix, and recruitment of inflammatory cells. These spreading alterations in both the epithelium and stroma are a phenomenon known as field cancerization.

Located and difficult to find at early stages, lung SCCs preferentially develop in the central and pericentral airways, allowing for the observation of early tissue alterations. The use of autofluorescence bronchoscopy imaging coupled with targeted biopsies and clinical follow-up studies has failed to provide convincing evidence of a sequential progression from mild/severe dysplastic lesions to carcinoma in situ (CIS) and invasive SCC of the lung (25, 26). Importantly, while malignant progression of dysplastic lesions is an infrequent event, they are often associated with development of lung SCC at other sites. In fact, high-grade dysplasia and CIS are an indication of elevated risk of cancer in the entire lung epithelium (24, 25).

Multifocal development of breast cancer is also frequent, and it is not clear whether these lesions are monoclonally derived (13, 27). Recurrences can also appear around the site of surgical resection and at more distal sites, including the contralateral breast (27). A major disease management decision facing patients with early-stage breast cancer (stage 0–II) is between conservative limited excisions (lumpectomy) and drastic mastectomy. The risk of early-stage breast cancer (stage 0–II) is between conservative limited excisions (lumpectomy) and drastic mastectomy. The risk of local or relatively distant recurrences after lumpectomy is significant (5%–22%; ref. 28), and minimal residual disease is a generally accepted explanation. Alternatively, recent experimental evidence discussed below suggests that de novo cancer cell development triggered by primary stromal alterations may take place.

In prostate cancer, multifocal lesions occur frequently, with >90% of patients diagnosed with the disease carrying two or more cancerous foci (29). There is great heterogeneity of these lesions and different rates of progression, each evolving independently of the other. This poses major clinical problems in terms of diagnosis, prognosis, and treatment (30). Systematic multiple biopsies of the prostate (“saturation biopsies”) are necessary to minimize chances that microscopic but highly aggressive lesions remain undetected (false-negative diagnosis). On the other hand, accurate prognostic and treatment decisions are hampered by the inability to predict behavior of apparently indolent lesions, with the resulting problem of overtreatment of patients (31).

In addition to the cancer types mentioned above, field cancerization plays an important role in the development of esophageal (32), gastric (33), colon (34), bladder (35), and cervical cancer (36). The association of this process with pancreatic and ovarian cancer is less appreciated but probably equally important (5). Field effects have also been implicated in nonepithelial cancer types, such as melanoma (37) and brain tumors (38). Further, bone marrow field effects have been invoked to explain the development of secondary myelodysplastic syndrome and/or acute leukemia in patients initially diagnosed with severe aplastic anemia (39, 40).

Epithelial precursor changes

Substantial attention has been devoted to genetic and/or epigenetic changes in epithelial cells as primary culprits in field cancerization leading to epithelial cancer. Such changes can occur in the absence of any histological abnormalities and at a significant distance (hundreds/thousands of cells) from tumors. They have been interpreted in the context of the multistep model of carcinogenesis, as established by classical experimental studies and clinical analysis of specific cancer types with well-defined sequential steps, like carcinoma of the colon (41).

According to this view, cancer fields result from the clonal expansion and spreading of epithelial cells with genetic alterations that have a role in cancer initiation and evolution (41). Brash and colleagues originally found that normal human skin, especially sun-exposed areas in aging individuals, contain a significant number of epidermal cells with pro-oncogenic p53 mutations (42). These cells are present as clusters that can increase in size over time. Cell populations with p53 mutations in apparently normal tissues have also been found in a number of other organs, including oral (43, 44), bronchial (45, 46), bladder (35), and esophageal (32, 47) epithelium. In concord with p53, its cousin p63 is also involved in epithelial stem cell potential and/or cell-cell adhesion (48–50). The functional implications of loss of normal p53 and/or altered p53/p63 balance seem obvious; however, the possibility that p53 mutations can be a marker of expanding clones of cells, rather than an initial or obligate cancer-triggering event, should also be considered. Consistent with this possibility is the finding that, in patients with primary oral SCCs, the presence of cells with p53 mutations in the normal epithelium is not associated with increased risk of secondary tumors (51). Additionally, discordant p53 mutations can be found in multiple cancer lesions in the same patients (52). Besides p53 mutations, other genetic events, specifically loss of heterozygosity (LOH) of common chromosomal regions, have been demonstrated in multiple lesions of the same patients and intervening normal epithelium in a number of organs, including breast (27), bronchial (53), and oral mucosa (54). LOH may contribute to clonal expansion and/or subsequent cancer development but also reflects genetic alterations that can randomly occur in a significant fraction of somatic cells, as recently reported for copy number variations in dermal fibroblasts from adults (55).

Another of the distinguishing features of cancer fields are the frequent epigenetic alterations that occur in the apparently normal cancer-surrounding epithelium (56–58). Increased DNA methylation at the promoter region of known or putative tumor-suppressing genes can lead to downregulation of their expression and function (59, 60), which may contribute to subsequent tumor development. A similar mechanism of epigenetic silencing
has been found for microRNAs with putative tumor-suppressive function in gastric cancer fields (61, 62). A link has been established between Helicobacter pylori infection and epigenetic silencing of tumor-suppressing genes and miRNAs in apparently normal gastric mucosa (58). A possibly primary role of chronic inflammation and associated production of highly reactive oxygen species (ROS) in field cancerization will be considered further below. In responding epithelia, increased DNA methylation at critical transcription regulatory regions can occur as a consequence of induced DNMT1 expression (63) through a variety of mechanisms, including p53- or AP1-dependent gene transcription (64). An early consequence of increased ROS levels is also recruitment of the DNMT1 enzyme to key growth-regulatory genes like p16, thereby silencing their transcription (65). Besides DNA methylation, increased methylation of lysine 27 in the tail of histone 3 (H3K27me3) at a specific set of cell regulatory genes has also been recently reported to be a consequence of inflammation in a mouse model of colon carcinogenesis (66).

Genes involved in DNA damage repair can also be epigenetically silenced in epithelial cancer fields (67). DNA instability, with increased microsatellite variability and telomere shortening, is not limited to tumors but occurs in the surrounding epithelial tissue (68–73). Mitochondrial DNA mutations are also a hallmark of cancer found in neighboring epithelia and, to an even greater extent, underlying stroma (74, 75). As both epigenetic gene silencing and mitochondrial dysfunction are trademarks of aging (76), they likely provide an important link between the age-related increase in cancer risk and field cancerization events.

**Inflammation: a primary or secondary determinant?**

The determining role of inflammation in cancer development has been postulated since the 1850s (77) and is a subject of intense investigation. Many premalignant and malignant lesions are associated with an inflammatory reaction, which can have both cancer-promoting and -suppressing effects. This is clinically illustrated in the skin, in which development of actinic keratoses, very common precursors of SCC and/or in situ SCC, is intimately connected with chronic inflammation. On the other hand, these lesions can be effectively reversed by treatment with TLR agonists that trigger a potent acute inflammatory reaction (78). Many studies have been dedicated to understanding how a cancer-promoting inflammatory environment can be “re-educated” to become cancer suppressing (79).

Macrophages and T cells are the primary determinants of inflammatory processes. The behavior of macrophages is very plastic and fulfills substantially different functions in acute versus chronic inflammation. These cells can be polarized into a “killing” M1 phenotype for microbe and cancer cell elimination and a permissive M2 phenotype aimed at resolving or containing acute toxic inflammation (80). It is tempting to equate M1 macrophages with an acute inflammatory reaction that can eradicate incipient tumor formation, while M2 cells can be linked with “smoldering” chronic inflammation, which promotes the carcinogenic process. This categorization is most likely an oversimplification, as M1 cytokines can have tumor-promoting effects, while M2 cytokines, such as IL-10, can be tumor suppressive (79).

Strong epidemiological and experimental evidence points to an important role for inflammation in the initial stages of cancer development. However, an important distinction needs to be made between a permissive/promoting function in expansion of “initiated” (i.e., mutated) cancer cells and a primary cancer trigger. In human skin, risk of SCC is substantially increased in clinical conditions associated with chronic inflammation (81). In mouse skin, susceptibility to chemically induced carcinogenesis is greatly influenced by transgenic expression or deletion of proinflammatory cytokines, like IL-1α (82), or enzymes, like COX-2 (83–85). However, few or no tumors developed in all these cases without prior treatment with a mutagenic carcinogen. Similarly, in classical chemical skin carcinogenesis studies, repeated treatments with proinflammatory but nonmutagenic agents, such as the tumor-promoting phorbol esters, are not sufficient for tumor initiation (81).

In contrast to the studies described above, studies in the gastrointestinal system suggest that inflammation alone can trigger cancer development. Patients with inflammatory bowel disease have a highly increased risk of colon cancer and colitis-associated cancer; a similar condition can be experimentally induced in mice by oral administration of proinflammatory tissue-damaging agents, like dextran sodium sulfate (86–89). A strong causative link exists also between H. pylori infection and stomach cancer, in which inflammation has been implicated as an underlying cause (90). Direct evidence in support of this possibility was provided by the finding that transgenic overexpression of a proinflammatory cytokine, IL-1β, in the gastric mucosa was sufficient to elicit cancer development through a cascade of NF-κB–activating cytokines and associated recruitment of immune modulatory cells (91). Interestingly, increased expression of IL-1β resulted, not only in increased proliferation and transformation of the gastric epithelium, but also in atrophy of the underlying stroma. As discussed below, stromal atrophy and associated fibroblast senescence can contribute significantly to the field carcinization process and can even have a primary determining function.

**Mesenchymal stromal alterations**

Epithelial cells covering the surface of organs are primary sensors of exogenous insults that trigger an inflammatory reaction. Resident cells of the stromal compartment are usually assumed to play a more secondary reactive role; however, a number of stimuli can directly affect the stroma, inducing changes that promote or even initiate the carcinogenic process. Long-wave UV (UVA) is thought to be a major cause of UV-induced skin cancer. It accounts for about 95% of total UV light exposure and, because of its greater penetration power, can directly affect the dermal compartment (92). Chronic UVA exposure leads to solar elastosis, a condition characterized by dermal atrophy and cellular and extracellular matrix alterations that, in the clinic (93) as well as a mouse model of skin field carcinization (94), precede keratinocyte tumor development. Smoking is another major cause of cancer, leading to a substantially increased risk, not only in lung, but in other organs, such as oral mucosa (22), bladder (95), and breast (96). A recent report has raised the possibility that chemicals in smoke can diffuse through the surface lung epithelium, directly targeting stromal cells of various organs (97). Interestingly, metabolites produced by the obesity-associated microbiome were recently implicated in liver cancer (98). In each of these cases, stromal cell senescence in the target organ is a putative tumor-promoting or -triggering mechanism.

Fibroblast senescence induces a program of gene expression, overlapping with that of cancer-associated fibroblasts (CAFs), including production of several diffusible growth factors and cytokines, like IL-6 (99, 100), whose increased expression can promote inflammation and proliferation of neighboring epithelial cancer cells.
The role of mesenchymal stroma alterations in cancer initiation was proposed several years ago in the context of colon (128) and prostate (129) cancers. An important distinction needs to be made between mesenchymal outgrowths as a primary consequence of genetic alterations and secondarily increased risk of epithelial cancer and less evident stromal changes, resulting from aging and/or exogenous insults, whose main consequences are epithelial dysplastic and neoplastic lesions. Examples of genetically determined mesenchymal outgrowths with consequently increased risk of epithelial cancer are intestinal polyposis syndromes, which are characterized by multiple hamartomas and associated inflammation (130). For example, Peutz-Jeghers syndrome (PJS) results from inactivating mutations of the serine/threonine kinase 11 (LKB1) gene (131). Available evidence suggests that LKB1 plays an important function in both stromal mesenchyme and the overlying epithelium (132–134). In fact, while loss of homozygosity occurs in the adenocarcinomas that develop in patients with PJS (130), LKB1 mutations are haplosufficient for polyp formation and are found equally in mice with global myofibroblast-specific haploinsufficiency (134). While LKB1 is best known for control of AMPK/mTOR signaling (132), its role in the colonic mesenchyme is connected to decreased TGF-β expression and function (134, 135).

TGF-β signaling is a key regulator of fibroblast behavior and, depending on conditions, can induce fibrosis and/or CAF activation (136). To genetically probe the role of this pathway in the stromal mesenchyme, TGF-β type II receptor (Tgfr2) was ablated in this compartment (137). The resulting phenotype is noteworthy for both observed alterations and those that were not found. Mice developed epithelial tumors in stomach and prostate that were associated with increased proliferation and density of surrounding fibroblasts (137). Loss of Tgfr2 in gastric and/or prostate fibroblasts resulted in increased expression of hepatocyte growth factor, Wnts, and a number of proinflammatory genes that are associated with paracrine mechanisms for cancer development (137–140). In contrast to stomach and prostate, all other examined organs were normal in this mouse model, including the skin, in which effective and dermal fibroblast-specific deletion of Tgfr2 was also demonstrated (137). Given the important role of TGF-β in mesenchymal compartments of skin and other organs, this lack of effect is very surprising and may reflect the already mentioned differences of stromal fibroblasts at various body sites.

Developmental fields play a key role in tissue and organ morphogenesis. Their establishment depends on various forms of direct and indirect cell-cell communication and gradients of
diffusible morphogens that instruct initially equivalent cells to assume different cell fates (141, 142). Selective adhesion of cells and physical force can also be a driving force in the morphogenetic process (143). Besides organ morphogenesis, these mechanisms may be implicated in maintenance and repair of already formed organs, for what has been termed “organ morphostats” (144). Notch/CSL signaling is an important developmental pathway and form of direct cell-cell communication (145). In the skin, a substantial body of evidence has shown that the Notch pathway promotes keratinocyte differentiation and suppresses tumor formation (146, 147). In contrast to that in the epidermis, the role of this pathway in the mesenchymal compartment of the skin has not been investigated until recently. Mice with mesenchymal deletion of CSL, the key effector of canonical Notch signaling, exhibited hair follicle abnormalities (148) and a skin phenotype with features of field cancerization, including early and widespread dermal atrophy, followed by expanding areas of inflammation and, by 2 to 4 months, multifocal keratinocyte tumors with features of actinic keratosis or in situ SCCs that eventually progressed into invasive cancer (94). Development of neoplastic lesions was significantly delayed by inhibition of inflammation, indicating that this process is an important mediator of the mesenchyme-induced epithelial lesions (94).

Further studies showed that compromised Notch/CSL signaling is likely to play a central role in intrinsic control of CAF activation, as deletion or silencing of CSL in dermal fibroblasts of either murine or human origin was sufficient to induce expression of many CAF effector proteins and transcription factors of the AP1 family (94), major determinants of skin photo-aging and cancer (149–151). The CSL protein exerts an intrinsic transcription-repressing function and binds to specific target genes in a dynamic manner (152, 153). As a result, expression of genes to which CSL binds with high affinity can be induced by either of two possible mechanisms: (a) transcriptional or posttranscriptional downregulation of CSL expression and function; (b) conversion of CSL from a transcriptional repressor into a transcriptional activator.
Conclusions and future directions

While metastatic spread is the principal cause of cancer-related deaths, field cancerization, with multifocal and recurrent tumors, is another clinical condition of major morbidity and lethality. As discussed above, multiplicity of lesions not amenable to surgical treatment and recurrent cancer after excision of the primary tumor are problems of foremost significance. Using a metaphor from the botanical garden, the situation can be analogous to that of a bad plant that is difficult to eradicate, because of the many roots deeply embedded in the terrain or the spreading of multiple bad seeds (Figure 3A). There is, however, another possibility—that of a bad soil that could corrupt properties of otherwise perfectly good plants (Figure 3B). In this case, unless the soil is corrected, various forms of intervention are of little or no use. Two main challenges need to be addressed. There is an urgent need to identify markers of stromal as well as epithelial alterations to guide the surgeon in tissue excision procedures and the clinician in decisions of therapeutic intervention. In this context, new in vivo imaging approaches for detection of stromal tissue alterations, including inflammation, abnormal matrix composition, proteolytic activity, fibroblast senescence, and/or altered density, could lead to important breakthroughs.

A second important goal is the elucidation of signaling pathways more closely connected with field cancerization as a necessary step to devise novel preventive and therapeutic treatments. In this respect, use of antiinflammatory agents is a promising venue to retard the process, even if it is probably not sufficient to totally prevent it. Targeting specific developmental signaling pathways provides another possible approach to counteract the spread of cancer fields. Equally attractive is the possibility of interfering with CAF activation (102) and, at an earlier stage, tissue aging and associated stromal cell senescence (156). In this respect, we note the complex relation between metabolism and aging (76) and the significantly increased cancer risk associated with obesity and diabetes (157, 158). One possibility is that a number of metabolism-modulatory drugs used to ameliorate these conditions could also be of substantial benefit for prevention and even reversal of field cancerization. Finally, a number of treatment regimens are already used for cancer field therapy in skin (159), and a better understanding of their mechanism of action may be instrumental in extending such treatments to other organs in which field cancerization takes place.

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