Genomic Alterations in Tumor Stroma

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Abstract

It was traditionally believed that the tumor was the seed that lay in the passive soil of the microenvironment, with the latter providing “permissive elements” for the tumor to grow and invade. Subsequently, it was recognized that both neoplasia and its microenvironment interacted as equal partners. Recent advances addressing genomic alterations in the tumor microenvironment, relevant to clinical outcome and treatment choices, are summarized. These include microenvironmental genomic alterations not only in different solid tumors, but also, rather surprisingly, in inflammatory bowel disease. These observations promise new biomarkers of prognosis and a new compartment to target therapy.

Introduction

It is now well accepted that the tumor and its microenvironment (or stroma) have a bidirectional relationship at multiple levels to elicit carcinogenesis, invasion, and progression. However, just several decades ago, a tumor was believed to be monocellular and monoclonal. The groundbreaking work of such investigators as Cunha and Matrisian showed that cellular interactions occurred between neoplastic epithelial cells and tumor stromal cells, and that secreted substances, such as growth factors, emanated from epithelial and stromal cells with each influencing the other. The tumor stroma itself is not a uniform entity, and is comprised of several elements, including chiefly fibroblasts, neovessels (endothelial cells), immune cells, and blood cells (Fig. 1). Indeed, Judah Folkman’s leadership on using antiangiogenic agents as an anticancer strategy represents one of the first approaches that took advantage of the tumor microenvironment. Tumor angiogenesis has been comprehensively reviewed recently by Robert Kerbel (1), and will not be covered in this brief review. Even though the concept of such tumor stromal cross-talk was accepted, and stepwise accumulation of somatic genetic alterations in solid tumors was well-established as a model for transformation and carcinogenesis (2), just a decade ago, few would have imagined that somatic genetic and genomic alterations could also occur in stromal cells. One such pioneer who dared to “think outside the box”, Fattaneh Tavassoli, showed, for the first time, that genetic events, specifically loss-of-heterozygosity (LOH) at microsatellite markers, could occur in breast cancer stroma (3). Taking advantage of their formal training and practical experience in anatomic pathology, Tavassoli and her team could consistently procure breast cancer epithelial cells and tumor stromal cells with great precision and accuracy using laser capture microdissection (LCM; ref. 3). As experienced molecular pathologists, the Tavassoli team was able to extract high-quality DNA from these procured malignant epithelial cells and tumor stromal cells from 11 breast samples with ductal carcinoma in situ, of which 5 also contained invasive carcinoma. These investigators also used LCM to obtain ductal epithelium and normal stromal cells from formalin-fixed, paraffin-embedded normal breast specimens obtained from reduction mammoplasties to act as controls. As a pilot, they selected 12 polymorphic microsatellite markers. They were able to observe LOH of markers common to both epithelium and stroma, epithelium only, and stroma only (Fig. 2). The stroma-specific LOH occurred at markers on 11q21-q23, 3p14.2, 16q23-q24, and 17q24 (3). The frequencies of stromal LOH in the invasive carcinomas was higher than those in the ductal carcinoma in situ, which suggests that genetic alterations in the tumor stroma accumulate to contribute to tumorigenesis (3). No LOH at any of the 12 markers were found in the DNA extracted from LCM-processed epithelium or stroma from formalin-fixed, paraffin-embedded normal breast samples (3). Thus, the seed was planted that somatic genetic alterations could not only occur in tumor stroma, but also play an important role in the development and/or progression of solid tumors.

Somatic Genomic Alterations in Tumor Stroma of Different Types of Primary Malignancies and Nonmalignant Neoplastic Conditions

Confirming Tavassoli’s observations using a xenograph model, David Botstein and his team used a nonmicrosatellite-based genomewide approach to analyze for somatic copy number variation in the mouse stromal cells in the microenvironment of xenografted human carcinoma-epithelial cells (4). These investigators found numerous amplifications and deletions of genomic regions in the host (murine) stroma surrounding the xenografted human carcinoma cells. Yet another independent confirmation of Tavassoli’s initial observations came when the LOH of markers were found in the tumor stroma in which 13 markers were examined in 41 noncultured invasive carcinomas of the breast (5). One dogma in the field of cancer genetics states that a region with LOH signals the presence of one or more tumor suppressor genes, the loss-of-function of which plays some role in the development or progression of neoplasia. Because LOH in the breast cancer stroma was found to involve 10q23 markers in the vicinity of PTEN and 17p13-p15 markers in the vicinity of TP53, two tumor suppressor genes known to play roles in a range of malignancies including

8 For a video showing an example of LCM, see http://content.nejm.org/cgi/content/full/357/25/2543/DC2.
heritable and sporadic breast cancers, direct mutation analyses of these two genes were performed on genomic DNA derived from LCM-procured epithelium and stroma of 50 invasive breast carcinomas (6). Somatic \( TP53 \) mutations were found in the epithelium and/or stroma of 50% of the breast carcinomas. This approximates the frequencies detected in analyses of whole breast tumors. Somatic \( PTEN \) mutations were found in epithelium and/or stroma in 30% of the samples. No mutations were found in normal epithelium and stroma from separate blocks containing normal breast tissue, and no somatic mutations were found in a control gene, \( SDHB \), in breast cancer epithelium and tumor stroma from all 50 samples. With one exception, somatic \( TP53 \) mutation and somatic \( PTEN \) mutation were mutually exclusive within any single compartment (6). This observation is consistent with functional data as well. p53 has been found to be a transcription factor for \( PTEN \) (7, 8). Additionally, PTEN protein and p53 protein have been found to physically interact; when they interact, p53 is stabilized, but PTEN is destabilized, in part due to caspase degradation, at least \( in vitro \) (8, 9).

Somatic genetic, genomic, and chromosomal alterations have been found in a broad variety of solid tumors and nonmalignant conditions. LOH (Fig. 3), microsatellite instability, consistent cytogenetic abnormalities, and/or telomere attrition in stroma have been independently described by many different investigators for a broad variety of solid tumors and nonmalignant conditions, such as head and neck squamous cell carcinomas, colorectal adenomas and carcinomas, Barrett esophagus and esophageal adenocarcinomas, esophageal squamous cell carcinomas, and carcinomas of the cervix, ovary, bladder, and prostate, as well as inflammatory bowel disease (10–22). However, do these genomic alterations in the stroma have any clinical implications for patients with cancers? A total genome LOH scan using almost 400 microsatellite markers on epithelium and stroma from 134 sporadic invasive breast carcinomas revealed that the frequencies of LOH in the epithelium were higher than those in the corresponding markers in stroma, but the number of markers affected by LOH in the stroma was significantly higher than that in the epithelium (23). One plausible interpretation of this observation is that genetic alterations in limited chromosomal regions (genes) in epithelium initiate carcinogenesis, and that genetic alterations in stroma affecting a variably large number

![Figure 1. Tumor microenvironment (stroma). A, reductionist view of cancer comprising a single type of cell. B, cancer comprising carcinomatous epithelium (cancer cells) and tumor stroma. Tumor stroma comprises several cell types, chief of which are fibroblasts, various types of immune cells, and endothelial cells within the neovasculature.](image)

![Figure 2. Somatic LOH. Germ line heterozygosity (presence of two different alleles at a polymorphic marker) is manifested by two peaks on the LOH scan (left). LOH is manifested as loss of one of the two peaks, representing deletion of regional genetic material (right).](image)
of chromosomal regions would affect the biological behavior of the carcinoma. Thus, one hypothesis stemming from these observations and interpretation would be that genetic and genomic alterations within tumor stroma would affect clinical outcome.

Total genome LOH scan and TP53 mutation analysis from neoplastic epithelium and tumor stroma compared with the germ line in 175 patients with sporadic invasive breast carcinomas revealed somatic TP53 mutations in tumor stromal fibroblasts associated with increased regional nodal metastases (Fig. 4; ref. 24). When TP53 was wild-type in the tumor stroma, then LOH at five specific markers in the tumor stroma were found to be associated with regional nodal metastases (Fig. 4).

Concordant with the biology, the five markers lay within chromosomal regions that harbored genes encoding proteins in the p53 pathway or were p53 targets. In a mouse model of prostate cancer, in which the prostate-epithelium transformation was driven by Rb deficiency, the tumor stroma was shown to be subjected to proliferative pressure which selected for TP53 deletion in the stromal fibroblasts (25).

Similarly, total genome LOH analysis of epithelial neoplasia and tumor stroma from 122 patients with head and neck squamous cell carcinoma revealed that three specific chromosomal loci that had significant LOH in tumor stroma were associated with pathologic tumor size and regional nodal metastases (26). In contrast, two different loci with significant LOH in the tumor-epithelium were associated with tumor size and clinical stage.

From these two studies, we can conclude that stromal genetic alterations, including somatic mutation and/or LOH, are associated with clinical outcome. These specific markers, if confirmed by independent and prospective study, might be useful in prognostication in breast carcinomas and head and neck squamous cell carcinoma.

Differences in Genomic Alterations in the Microenvironment of Heritable and Sporadic Carcinomas

It may be predicted that stromal genomic alterations should be different between heritable and sporadic carcinomas. Little data exists in this regard and is limited to heritable breast and ovarian cancer syndromes. A total genome LOH scan was performed on DNA derived from the epithelium and stroma of 51 BRCA1/2-related breast cancers using 372 microsatellite markers (27). These hereditary breast cancer stroma samples showed LOH in 59.7% of all loci analyzed, similar to the average observed in the epithelium (66.2%). This is markedly different from sporadic breast cancers, in which the average epithelial LOH frequency of 36.7% far exceeds that of the stroma (28.4%; ref. 27). Based on the roles of BRCA1 and BRCA2 in DNA damage and repair, the high LOH frequencies in both epithelium and stroma of the breast tumors

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**Figure 3.** LOH in epithelium and/or stroma. Genotyping chromatograms illustrate that in a single sample, LOH (star) can occur in discordant alleles of a single marker (D7S1799) between epithelium and stroma or exclusively in one compartment (D14S617 in epithelium; D9S2157 in stroma). Adapted with permission from Weber F, Xu Y, Zhang L, et al. Microenvironmental genomic alterations correlate with clinico-pathologic behavior in head and neck squamous cell carcinomas. JAMA 2007;297:187–96.

**Figure 4.** Somatic TP53 mutation or LOH/AI at 5 stromal markers associated with increased loco-regional lymph node metastases in sporadic breast carcinomas. Adapted with permission from Patocs A, Zhang L, Xu Y, et al. Stromal TP53 mutation or 5-locus allelic imbalance and nodal metastases in breast cancer. N Engl J Med 2007;357:2543–51.
makes teleologic sense. However, there seems to be two subsets of tumors, one with a higher LOH frequency and the other with a lower frequency. This observation, albeit based on small sample sizes, may suggest that there are two progression pathways in BRCA1/2-related breast cancers. Similarly, almost half of all BRCA1-related breast carcinomas showed LOH patterns that were similar between the epithelium and the stroma, compared with only 15% of BRCA2-related breast cancers (27). These observations may suggest that the manner of progression in the subset of these heritable breast cancers with similar epithelial-stromal LOH patterns is epithelial-to-mesenchymal transition. If this is true, then this pathway is favored in BRCA1-related breast cancers. In contrast, those with distinct epithelial-stromal LOH patterns represent a pathway of progression in which the genomic alterations in the epithelium evolve in parallel and independently from those in the corresponding stroma. This pathway is favored in BRCA2-related breast cancers.

Interestingly, but perhaps not surprisingly, the stromal markers associated with clinicopathologic features for sporadic breast cancers do not apply for BRCA1/2-related breast cancers. Neither stromal TP53 mutation nor LOH of the five specific markers (see above) in the stroma were related to nodal metastases in BRCA1/2-related breast cancers (24). The most attractive explanation would be biological differences, and there is ample evidence that the biology of sporadic and BRCA1/2-related breast carcinomas is distinct. However, there are alternative explanations as well. For example, the sample size (n = 51) of the heritable breast cancers is relatively small compared with the sporadic cases, and the effect cannot be seen. More plausibly, germ line BRCA1/2 mutations leading to repair deficiency already provide a repair-defective background leading to high LOH frequencies. This is particularly germane as the frequencies of somatic TP53 mutations, whether in the epithelium or stroma, in BRCA1/2-related breast cancers is relatively high. That TP53 mutations occur in almost all BRCA1/2-related basal-like breast cancers has been recently observed independently (28).

Juvenile polyposis syndrome is caused by germ line mutations in SMAD4 or BMPRIA (29–31). This syndrome is characterized by hamartomatous polyposis and a high risk of gastrointestinal carcinomas (32). Histopathologic examination of the gastrointestinal tract in human juvenile polyposis syndrome reveals a prominent stroma including proliferative inflammatory cell infiltrates underlying a grossly normal-appearing epithelium, suggesting that the stroma plays a key role in initiating epithelial neoplasia. Indeed, knockout of Smad4 in T cells results in intestinal carcinomas in this mouse model (33). However, epithelial-specific knockout of Smad4 resulted in normal mice which do not develop intestinal cancers (33). This observation may suggest that in some heritable cancer syndromes, incorrect signaling from the microenvironment might initiate carcinogenesis.

Tumor Stroma in Metastatic Disease

Students of tumor stroma always query the origin of the stromal components of a metastasis. Virtually nothing is known in this regard. A recent study suggests that for colorectal carcinomas, only the epithelial component metastasizes (34). Once at the metastatic site, in this case the liver, the carcinoma recruits resident fibroblasts from the portal region (34). Compared with nonneoplastic skin fibroblasts, such metastasis-associated stromal fibroblasts show differential expression of adhesion molecules, growth factors, transforming growth factor-β, and cyclooxygenase-2 (35). These resident fibroblasts form the metastasis’ tumor stroma, which in turn, recruits an inflammatory microenvironment (34).

To mechanistically investigate metastatic solid tumor–metastatic microenvironment interactions, an in vitro three-dimensional coculture system was constituted with prostate cancer epithelium and bone stroma, mimicking metastatic prostate cancer to bone (20). Led by Leland Chung, these investigators showed that permanent consistent cytogenetic and epigenetic alterations occurred in the bone stroma when cocultured with prostate cancer epithelium (20). Importantly, they also showed that these stromal-specific alterations were secondary to reactive oxygen species. These prostate cancer–influenced bone stromal cells were highly inductive of human prostate cancer growth in mice, and were shown to express high levels of extracellular matrix and chemokine genes, such as those encoding versican, BDNF, CCL5, CXCL5, and CXCL16 (20), all of which have been found to be elevated in human metastatic prostate cancer as well.

Further research is required to determine whether the colorectal liver metastasis observation and the corroborative data from the in vitro three-dimensional coculture model of prostate cancer and bone stroma are generalizable to all solid tumors and all metastatic sites. Such data would be important in tailoring stroma-directed therapeutics and preventatives against metastatic disease.

Conclusions and Future Directions

Identifying and characterizing the genomic, epigenomic, and functional role of the tumor microenvironment represent an advance in oncology that will help prognosticate and tailor therapies. For example, somatic TP53 mutations in breast cancer stroma are associated with regional nodal metastases (24), but TP53 mutations in breast cancer stromal fibroblasts sensitize the cancer to doxorubicin and cisplatin (36). It is interesting to surmise that tumor stroma is subjected to proliferative pressure selecting for TP53 deletion/mutation in the stromal fibroblasts (25), yet doxorubicin or cisplatin induces senescence in p53-deficient stromal fibroblasts which release paracrine factors detrimentally affecting the epithelial malignancy (36). In vitro and in severe combined immunodeficiency mouse models, long-term cocultures of pancreatic cancer epithelial cells with tumor myofibroblasts results in chemoresistance secondary to downregulation of caspases by promoter hypermethylation (37). Exposing these cocultures to demethylating agents reverses this chemoresistance.

Because the cells that comprise the tumor stroma are heterogeneous (Fig. 1B), there will likely be different alterations and different types of alterations affecting the different cells types comprising the stroma. Furthermore, even within a single cell type (e.g., fibroblasts), many are beginning to suspect that they are not derived from a single clone with uniform somatic alterations. Thus, different alterations might occur in various subsets of a single cell type. Epigenomic alterations would most likely fall under this latter category. Nonhistologic fractionation of a single cell type within stroma would be the next step for analysis of the different sets of alterations occurring in various fractions of a cell type within stroma. Further refinement of the environment surrounding tumors, such as the concept of the tumor microenvironment (38), would be important as well.
Because neither the malignant epithelium nor the tumor stroma occur in isolation, it would be important to integrate the genomic and epigenomic information from tumor and stroma with those of the host, i.e., the germ line (Fig. 5; ref. 39), and eventually, with the so-called macroenvironment (38) and external environmental exposures as well. The ideal future practice of medicine would integrate clinical phenotype, genomic and epigenomic information from the germ line and somatically from both tumor and stroma, and environmental exposures to accurately prognosticate and select the best combination therapies for the longest durable responses and the lowest likelihood of toxicity (Fig. 5).

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No potential conflicts of interest were disclosed.

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References

In this manuscript, Eng and colleagues provide a summary of the studies reporting the existence of clonal somatic alterations in cancer-associated fibroblasts. However, the review is biased because there is no acknowledgement of the published studies which do not support this view. Furthermore, the supporting publications which are cited are taken at face value, and are not critically assessed in terms of their strengths and weaknesses. For example, the study by Pelham and colleagues (2006), in which alterations in DNA copy number were detected in the mouse host stromal cells is a rather synthetic model (20). Alters in DNA copy number are not analyze equivalent noncancer-associated stroma, and therefore, cannot exclude the possibility that such alterations are common in proliferative stroma, particularly those of severely immunocompromised nude mice. The controversy in this field relates to the inherent technical flaws in the assays used to detect somatic mutations, and to the mathematical arguments supporting the selection which would allow the clonal expansion of stromal cells with extensive genetic changes without those cells themselves becoming tumors, or even just the probability of recurrent changes without underlying genetic instability. This review does not address any of these issues. The authors should rebut the technical limitations of somatic mutation detection in poor-quality and low-abundance DNA templates as described by Kern and Winter (1) and Winter and colleagues (2).

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