Stromal Responses among Common Carcinomas Correlated with Clinicopathologic Features

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Abstract

Purpose: We have previously characterized a tumor stroma expression signature in a subset of breast tumors that correlates with better clinical outcome. The purpose of this study is to determine whether this stromal signature, termed the “DTF fibroblast” (desmoid-type fibromatosis) signature, is specific to breast cancer or is a common stromal response found in different types of cancer.

Experimental Designs: The DTF fibroblast signature was applied to gene expression profiles from five ovarian, five lung, two colon, and three prostate cancer expression microarray datasets. In addition, two different tissue microarrays of 204 ovarian tumors and 140 colon tumors were examined for the expression of previously characterized protein markers of DTF fibroblast signature. The DTF fibroblast stromal response was then correlated with clinicopathologic features.

Results: The DTF fibroblast signature is robustly present in ovarian, lung, and colon carcinomas. Both expression microarray data and immunohistochemistry show that the subset of ovarian tumors with strong DTF fibroblast signature expression has statistically significant, worse survival outcomes. No reproducible survival differences were found in either the lung or the colon cancers. The prostate cancers failed to show a DTF fibroblast signature. Multivariant analysis showed that DTF fibroblast signature was significantly more prognostic than the proliferation status in ovarian carcinomas.

Conclusions: Our results suggest that the DTF fibroblast signature is a common tumor stroma signature in different types of cancer, including ovarian, lung, and colon carcinomas. Our findings provide further insight into the DTF fibroblast stromal responses across different types of carcinomas and their potential as prognostic and therapeutic targets. Clin Cancer Res; 19(18); 5127–35. ©2013 AACR.

Introduction

Tumor stroma plays an important role in cancer development and progression. Our previous studies have shown that gene signatures derived from desmoid-type fibromatosis (DTF), a soft-tissue tumor composed of homogenous fibroblasts, can be used as a surrogate to recapitulate the expression features of some tumor stroma. We use gene expression signatures of soft-tissue tumors as surrogates for expression signatures of nonneoplastic stromal cell types in the tumor microenvironment. Similar to lymphomas, in which many tumors retain markers specific for a particular lymphoid cell type, some types of soft-tissue tumors can also be regarded as a clonal outgrowth of a particular connective tissue cell type. In multiple studies, we have found that the DTF fibroblast signature, when applied to breast cancers, identifies a subset of breast cancers with favorable clinical outcomes (1, 2).

In previous work, analysis of the stromal expression patterns of synchronous breast cancers and a comparison of matched primary and metastatic tumors have suggested that the DTF fibroblast response is host specific (3) and that the genesis of the response originates within the stromal compartment and not the malignant epithelium (4). If the DTF fibroblast response is derived from the stromal cells, it is quite possible that the signature occurs in other carcinomas aside from breast cancer.

To determine whether the DTF fibroblast signature is specific to breast cancer or more widely present in different types of cancer, we conducted a survey of common cancers using gene expression profiling datasets of lung, colon, prostate, and ovarian tumors. These carcinomas have been extensively expression profiled with publicly available datasets and are clinically well annotated.

Using publicly available datasets, we examined the DTF fibroblast gene signature in a total of 1,127 ovarian tumors in five datasets (5–9), three datasets of 279 prostate tumors (10–12), three datasets of 573 colon tumors (13–15), and five datasets of 519 lung tumors (16–20). Tissue
Translational Relevance
This study examines the stromal responses in ovarian, lung, colon, and prostate carcinomas with the DTF fibroblast gene signature, which was previously found to stratify breast tumors and identify patients with breast cancer who have better clinical outcomes. The stromal responses defined by this fibroblast gene signature can stratify tumors in ovarian, lung, and colon tumors and identify patients who have ovarian cancer with worse clinical outcomes. In addition, the DTF fibroblast stromal response was found to be more prognostic than the proliferation status association with survival outcome in patients with ovarian cancer. The broader survey provided here offers characterized genetic information in stromal responses of different types of tumors from a large number of cancer patients, which can lead to better understanding of individual tumors and aid in the design of targeted therapy in the future.

Materials and Methods
Cancer datasets
We searched publicly available databases to find carcinoma datasets containing not only gene expression profiles but also clinical annotations with at least one of the following records: overall survival (OS), disease-free survival (DFS), and disease-specific survival (DSS). Datasets without this information were excluded in our analysis. Following this inclusion–exclusion criteria, a total of five ovarian cancer datasets containing not only gene expression profiles but also clinical annotations with at least one of the following records: overall survival (OS), disease-free survival (DFS), and disease-specific survival (DSS). Datasets without this information were excluded in our analysis. Following this inclusion–exclusion criteria, a total of five ovarian cancer datasets (GSE14333; ref. 13, GSE17538; ref. 14, GSE5851; ref. 15) that contained gene expression data of 1,127 patient tumors and the clinical follow-up in 1,105 cases among them. The ovarian tumors profiled in these datasets were all pretreatment samples except in 18 out of 285 tumors of GSE9891 that were from patients who had neoadjuvant, platinum-based chemotherapy. These tumors were acquired from the primary debulking surgery of patients. Three colon cancer datasets were identified (GSE14333; ref. 13, GSE17538; ref. 14, GSE5851; ref. 15) that contained gene expression data on 573 patient tumors and the clinical follow-up in 538 cases among them. Five lung cancer datasets were identified (caArray-beer-00153; ref. 16, GSE4573; ref. 17, GSE10245; ref. 18, GSE10445; ref. 19, GSE11969; ref. 20), containing gene expression data of 519 patient tumors and the clinical follow-up in 492 cases among them. Three prostate cancer datasets (GSE1431; ref. 10, GSE3933; ref. 11, GSE25136; ref. 12) were identified, including 279 prostate tumors.

Data analysis
Raw expression data were log2 normalized with Robust Multi-array Average (RMA; ref. 21). Unsupervised hierarchical clustering was carried out using Cluster3.0 with correlation (uncentered) and average linkage clustering. Treeview and Java Treeview were used to view the resulting heatmap and dendrograms.

Determination of DTF fibroblast–core gene-like (DTF-positive) case clusters
Expression values of the DTF fibroblast core genes were extracted and tumors were clustered using the program Cluster 3.0. Tumors were clustered on the basis of similar levels of expression of the DTF fibroblast core gene signature. We were interested in tumors showing highly coordinated expression of this group of genes and selected the correlation value of 0.6 among tumors with upregulated expression of these genes to identify the clustered nodes of tumors from different gene expression datasets with similarly upregulated DTF fibroblast gene expression patterns, similar to the correlations examined in our previous studies on tumor stroma in gene expression profiling datasets (1, 2, 4).

Analysis of clinicopathologic variables
Clinical annotations were collected from GEO or as indicated in the original publication for each dataset. Kaplan–Meier survival curves, log-rank (Mantel–Cox) test, Gehan–Breslow–Wilcoxon test, and HR calculations were conducted with GraphPad Prism 5. Multivariant analysis, Pearson $\chi^2$ test, Wilcoxon test, and $t$ test were carried out with R.

Case material
For immunohistochemical studies, we used 204 high-grade serous ovarian carcinomas and 140 colon carcinomas distributed over two different TMAs. The TMAs were constructed using 0.6 mm cores with a manual tissue arrayer from Beecher Instrument. For each specimen, a pathologist reviewed the hematoxylin and eosin (H&E) slides. A block with viable tumor was chosen. Scarred, necrotic, and hypocellular areas were avoided. A 0.6 mm tissue core was taken from these areas in each of the blocks and inserted into the TMA block. We did not take duplicate cores. Survival information was available for all of the cases with a mean follow-up of 5.4 (0.4–16.5) years for high-grade serous ovarian carcinomas and 3.5 (0.08–11.9) years for patients with colon cancer.

Immunohistochemistry
Slides were cut at 4 $\mu$m, deparaffinized in xylene, and hydrated in a graded series of alcohol. For immunohistochemistry, the primary antibodies used were SPARC (osteonectin; mouse monoclonal antibody, 1:1,000, Invitrogen, catalog no. 33–5500, Clone ON1-1), and CSPG2 (versican; mouse monoclonal, 1:150, Santa Cruz Biotechnology Incorporated, catalog no. 47777, Clone 5C1). Stains were interpreted as negative when the staining was less than
30% of the spindle stromal cells and positive when the staining was greater than 30%. A tumor was considered DTF positive when it had dual positivity of SPARC and CSPG2. The immunohistochemical results were scored by a pathologist (I. Espinosa) in a semiquantitative manner, on the basis of previous experience with these antibodies (1–4). In our experience, manual scoring of robustly staining markers such as the ones used in this study functions as reliably as automated, fully quantitative techniques (22), and we believe the findings from manual scoring are more generalizable as this is the practice most commonly used in the clinical setting.

Statistical analysis of tumors on the TMAs

The Kaplan–Meier method was used to estimate survival distributions. DSS was calculated from the time of diagnosis until death from the specific disease. OS was defined as the time interval from the diagnosis to death. Recurrence-free survival (RFS) was calculated from the time of diagnosis to the date of recurrence. Multivariate Cox regression analysis was used to study the relationship between survival and different covariates.

Use of the proliferation signature to examine the proliferation status of microarray-profiled tumors

The proliferation gene signature that was previously developed (23) was used to examine the proliferation status of breast and ovarian tumors with differential DTF fibroblast stromal responses. Expression values of the DTF fibroblast core genes and the proliferation genes were extracted, and tumors were clustered using the program Cluster 3.0. The clustered patterns of tumors were examined in both breast and ovarian cancer datasets to determine the relative associations between the proliferation status and the DTF fibroblast stromal responses.

Results

DTF fibroblast signature in ovarian, lung, colon, and prostate carcinomas

To examine the DTF fibroblast signature across different types of carcinomas, we selected publicly available gene expression datasets of common carcinomas, which included data from at least 250 samples (total) on high-quality arrays and clinical annotation. Datasets of studies of ovarian, lung, colon, and prostate cancers met these criteria.

We examined each dataset for the presence of DTF fibroblast-positive samples by clustering the tumors using the previously determined DTF fibroblast core gene list (2). We found a subset of the ovarian tumors with highly coordinated DTF fibroblast core gene expression in each of the five ovarian cancer datasets examined (Fig. 1). This subset of ovarian tumors with robust DTF fibroblast response accounted for 28.1% of the total number of 1,127 microarray-profiled ovarian tumors in the five datasets.

The pattern of highly coordinated DTF fibroblast core gene expression was also observed in each of the lung and colon cancer datasets. The five independent subsets of lung tumors with strong DTF fibroblast response accounted for 21.6% of the total number of 519 lung tumors (Supplementary Fig. S1). The DTF fibroblast pattern was also observed in each of the two colon cancer datasets and accounted for 28.5% of the total number of 493 colon tumors (Supplementary Fig. S2). In prostate carcinoma, however, the fibroblast stromal response was not identified in any of three datasets (Supplementary Fig. S3).

Clinical associations of the subset of tumors identified by DTF fibroblast signature

We found previously that the subset of breast tumors identified by DTF fibroblast signature with high DTF fibroblast stromal responses was correlated with a lower tumor grade, estrogen receptor expression, and good outcome in patients with breast cancer (1, 2). In this study, we looked for similar correlations in the ovarian carcinoma datasets.

The DTF-positive tumors in four of five ovarian datasets showed associations with worse OS and/or worse PFS (TCGA, GSE9891, GSE26712, and GSE31245; Fig. 2). In one ovarian dataset, GSE17260, we did not observe any survival association, although this was also the dataset with the fewest samples (110). Thus, among the five ovarian datasets, of the 1,019 tumors with available clinical information, a total of 909 cases showed the trend that tumors with highly coordinated DTF fibroblast core gene expression were associated with worse survival outcomes while 851 of these tumors from four datasets had statistically significant associations. We found that ovarian cancer in patients with tumors that had a robust DTF fibroblast stromal response was also correlated with higher tumor stage in the TCGA dataset (Pearson \( \chi^2 \) test: \( x^2 = 8.2468; df = 2; P = 0.01619 \)) and GSE9891 cohort (Pearson \( \chi^2 \) test: \( x^2 = 18.2699; df = 3; P = 0.0003869 \)). However, there were no associations between high DTF stromal responses and the tumor grade.

In contrast with the ovarian carcinomas, we found no statistically significant clinical associations between the subset of DTF–fibroblast-positive lung tumors and survival either for individual datasets or the aggregate set of lung cancers (Supplementary Fig. S4). In colon carcinomas, we found discordant results between datasets. The subset of DTF–fibroblast-positive colon tumors in the GSE14333 dataset of 255 tumors were associated with better DFS, whereas the DTF–fibroblast–positive subset of the colorectal tumors in the GSE17538 dataset of 238 tumors were significantly associated with worse OS, worse DFS, and worse DSS (Supplementary Fig. S2). No clear differences between the datasets in terms of cohort characteristics could explain these different outcomes. There were no correlations of DTF status with tumor stage (Pearson \( \chi^2 \) test: \( x^2 = 4.5957; df = 3; P = 0.2039 \)), tumor grade (Pearson \( \chi^2 \) test: \( x^2 = 0.1165; df = 2; P = 0.9434 \)), and age (Wilcoxon test, \( P = 0.0773 \)) in GSE17538. There was also no correlation of DTF status with the Duke stage of tumor in GSE14333 (Pearson \( \chi^2 \) test: \( x^2 = 2.2041; df = 3; P = 0.5311 \)). However, there was significant association between DTF status and age in GSE14333 (Wilcoxon test, \( P = 0.01661 \)).
Comparison of DTF fibroblast features in breast and ovarian carcinomas

Our current data showing worse outcome in ovarian cancer when the DTF fibroblast signature is present contrasts with a previously found correlation with good outcome in breast cancer. To address the difference between these two carcinomas, we examined the correlation between the proliferation status of breast and ovarian tumors and the DTF fibroblast stromal responses. Across a wide variety of cancers, proliferation is a dominant feature of many signatures that predict outcome. To identify tumors with a high and low proliferation index in the context of gene expression profiling data, we used a proliferation gene signature to stratify cases by proliferation index. Several proliferation signatures have been discovered in gene expression profiling studies published by different groups which have significant overlap in genes and biology (23–27). We used the proliferation gene signature developed by Dai and colleagues (23), which showed reproducible and significantly

Figure 1. The expression profiles of DTF fibroblast core gene signature in five ovarian cancer datasets, including TCGA dataset (A), GSE9891 (B), GSE26712 (C), GSE31245 (D), and GSE17260 (E). The selected subgroup of ovarian tumors highlighted by pink in the clustered array trees are the ones with highly coordinated DTF fibroblast core gene expression and are thus categorized as DTF$^+$ ovarian tumors.
worse survival associations of breast tumors with high proliferation.

In breast cancer datasets (GSE3494, GSE4922, and NKI), the DTF fibroblast stromal response was negatively correlated with the proliferation signature [Fig. 3]. Breast tumors with a strong DTF fibroblast stromal response had low expression of the proliferation genes compared with non-DTF tumors (6.9% vs. 19.7% in GSE3494, 7.1% vs. 22.8% in GSE4922, and 5.3% vs. 30% in the NKI; all \( P < 0.01 \)), which was consistent with the finding that a positive DTF fibroblast stromal response selected for better survival. However, in the ovarian cancer datasets, tumors with high DTF fibroblast stroma expression consisted of a mixture of tumors with high and low proliferation signatures (Fig. 3; 17% vs. 13.6% in GSE9891, 7.2% vs. 12.3% in TCGA, and 15.4% vs. 6.2% in GSE26712). Multivariate analysis revealed that proliferation status was not as strong as the DTF fibroblast signature in predicting the survival of patients with ovarian carcinomas (Table 1).

**Localization of the DTF fibroblast signature expression in ovarian and colon tumor stroma**

We have previously used a set of immunohistochemical markers as surrogates for the DTF fibroblast gene expression signature, including SPARC and CSPG2, to localize the expression of the signature to the tumor stroma. We assayed the expression of SPARC and CSPG2 on TMAs of ovarian and colon cancer, which contained 204 high-grade serous ovarian carcinomas and 140 colon carcinomas, respectively. These protein markers were expressed in the stromal compartment by cells with a fibroblast-like morphology (Fig. 4). As found in the gene expression datasets, patients who had ovarian cancer with coexpression of DTF fibroblast markers (SPARC...
Table 1. Multivariate analysis for ovarian tumor group status versus clinical risk factors, including proliferation, stage, grade, age, and survival

<table>
<thead>
<tr>
<th>Dataset</th>
<th>Tissue type</th>
<th>Analysis group</th>
<th>Survival data</th>
<th>Statistical significance*</th>
<th>HR (95% CI)</th>
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<td>GSE9891</td>
<td>Ovarian</td>
<td>Tothill</td>
<td>OS</td>
<td>0.05481</td>
<td>1.9 (0.9869–3.657)</td>
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<td></td>
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<td></td>
<td></td>
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<td>0.71203</td>
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<td></td>
<td></td>
<td>Stage</td>
<td>OS</td>
<td>0.00372**</td>
<td>1.774 (1.2043–2.612)</td>
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<tr>
<td></td>
<td></td>
<td>Grade</td>
<td>OS</td>
<td>0.74833</td>
<td>1.059 (0.7472–1.5)</td>
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<tr>
<td></td>
<td></td>
<td>Age</td>
<td>OS</td>
<td>0.00758**</td>
<td>1.029 (1.0075–1.05)</td>
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<tr>
<td>GSE9891</td>
<td>Ovarian</td>
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<td>RFS</td>
<td>0.066</td>
<td>1.6546 (0.9673–2.83)</td>
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<td></td>
<td>DTF</td>
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<td>0.284</td>
<td>1.2744 (0.8179–1.986)</td>
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<td>0.827</td>
<td>0.9642 (0.6272–1.452)</td>
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<td>1.9462 (1.4674–2.581)</td>
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<tr>
<td></td>
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<td>Grade</td>
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<td>1.121 (0.8625–1.457)</td>
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<td></td>
<td>Age</td>
<td>RFS</td>
<td>0.078</td>
<td>1.0144 (0.9984–1.031)</td>
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<tr>
<td>TCGA</td>
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<td>Tothill</td>
<td>OS</td>
<td>0.165918</td>
<td>0.7029 (0.4269–1.157)</td>
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<tr>
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<td>PFS</td>
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<td>PFS</td>
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<td>1.0033 (0.9925–1.0143)</td>
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</tbody>
</table>

* * * P < 0.05; ** P < 0.01; *** P < 0.001.

and CSPG2) had worse prognosis than patients who did not coexpress the DTF fibroblast markers in the tumor stroma (Fig. 4). Multivariate analysis was conducted using clinical features of age, Federation Internationale de Gynecologie et d’Obstetrique (FIGO) stage, Eastern Cooperative Oncology Group (ECOG), grade, and the DTF signature. We found that only DTF signature was prognostic [DSS: HR, 2.4; 95% confidence interval (CI), 1.14–5.05; P = 0.02]. The results in the colon carcinoma array also showed a worse outcome for DTF–fibroblast-positive cases, consistent with one, but not the other, of the results from the colon cancer gene expression dataset.

Discussion

The importance of stroma in tumor development has been observed and recognized in a number of experimental models. Activated stroma comprised inflammatory cells and fibroblasts found to be essential in supporting neoplasia proliferation and survival (28, 29). Irradiated stroma produces more mammary carcinomas compared with non-irradiated stroma in mouse models (30). In addition, higher expression of TGF-β1 in irradiated stroma fibroblasts enhances cancer progression in the mammary gland as well as in the pancreas (31).

We have previously shown that gene signatures generated from soft-tissue tumors such as desmoid-type fibromatosis and solitary fibrous tumors, highly homogeneous neoplasms with different fibroblastic phenotypes, can identify differential stromal responses in breast tumors with different associated survival outcomes (1). In this study, we sought to extend our findings in cancer stroma gene expression signatures by surveying some of the most common carcinomas for which numerous well-annotated datasets were available. We looked for the presence of the DTF fibroblast gene signature in ovarian, lung, colon, and prostate cancer datasets. We discovered the DTF fibroblast gene signature was present in a subset of ovarian, lung, and colon tumors. However, only significant and reproducible outcome differences were identified in ovarian tumors.

No stromal gene expression signature was identified in prostate carcinoma. This is interesting because the prostatic stroma is quite different from the stroma of the other organs in that it has a pronounced myofibroblastic phenotype. One possible explanation for the lack of the DTF fibroblast signature is that the myofibroblastic nature predisposes the tumor microenvironment to a different stromal signature that is not covered by our fibroblast-derived expression profiles. Tothill and colleagues (6) found a "poor prognosis subtype" identified by k-means clustering of six subtypes of
ovarian tumors. This subtype had a reactive stromal gene expression signature, which was composed of upregulated expression of 289 stroma genes. Forty-five of these 289 genes overlapped with the DTF fibroblast core gene signature of 63 genes. Although the two different gene signatures containing different gene numbers were originally generated by different methods and groups of researchers, both of them proved that stromal responses in ovarian carcinoma were associated with worse survival outcomes. These results provided solid evidence for the critical role of cancer stroma in the prognosis of ovarian cancer patients. An RNA interference screening of the common stromal genes proposed by these two groups for their functions and effects in ovarian cancer is worth pursuing. Other work on ovarian cancer stroma has studied morphologic differences between cancers. Labiche and colleagues (32), focusing on proportional representation of stroma to malignant epithelium, have shown that the stromal compartment influences ovarian cancer invasiveness and histologic differentiation. They used an automated image-analysis software approach to quantify the stromal proportion and found that higher stromal content (>50%) is associated with poor prognosis. From our study, we found that the subgroup of microarray-profiled tumors showing strong DTF fibroblast stromal signature was associated with worse survival of patients. However, a collection of ovarian tumors to be simultaneously examined for their stromal abundance and genetically profiled for differential stromal gene expression will be required to establish the direct relationship between the signature score and stromal abundance, which remains to be an interesting study in the future.

Although we found that the DTF fibroblast stromal signature is evident in both breast and ovarian cancers, the presence of this signature was associated with different outcomes for these two types of tumors: Positive breast cancers had a good outcome whereas positive ovarian cancers had a poor outcome. These results could partly be explained by differences in proliferation. Breast tumors with high DTF fibroblast stromal response had a low proliferation gene expression signature. In ovarian carcinoma, however, there was no significant correlation between DTF stromal response and proliferation. In survival multivariate analysis, only the DTF fibroblast signature, but not the proliferation signature, has a prognostic value in ovarian cancer. These differences suggest that cancer cell proliferation and stromal responses might be closely related in breast tumors. In addition to the closely related role of proliferation and stromal responses in breast carcinoma but not

Figure 3. The expression profiles of DTF fibroblast core gene signature and the proliferation signature in breast cancer datasets of GSE3494 (A), GSE4922 (B), and NKI (C) as well as ovarian cancer datasets of GSE9891 (D), TCGA dataset (E), and GSE26712 (F).
ovarian carcinoma, the contrasting associations between signature score and survival in breast and ovarian cancer may also come from differences of tissue specificity, the stage and grade of the ovarian and breast tumor samples profiled, and the potentially different cancer–stroma interaction in these two types of cancers. Further analyses to investigate chromosomal changes and methylation status on breast and ovarian tumors of differential stromal responses will help identify potential regulating factors, in addition to the fundamental differences brought by tissue specificity.

We have shown that the DTF fibroblast signature is present in multiple cancer types, including breast (1, 2), ovarian, lung, and colon carcinomas, which suggested a recurring relationship between cancer cells and the surrounding stroma. These findings suggest that the DTF fibroblast signature is a common stromal response to cancer. It is interesting to consider that this signature is found in only a subset of the carcinomas from any given organ system. It is unclear why the same stromal response is not present in every tumor, rather than just a subset. An a priori hypothesis might be that different tumors form different stromal signatures based on the intrinsic biology of the tumor. However, this does not seem to be the case, based on our earlier synchronous breast cancer study (3), in which clinically distinct cancers from the same patient tended to have the same DTF fibroblast response despite having other pathologic differences. Further work to define the genesis of the tumor stromal response is needed. In addition to the DTF fibroblast stromal signature, several other stromal signatures discovered in breast cancer (33, 34) may also be present in other types of malignant carcinomas. It would be interesting to further examine and compare these stromal signatures and their responses in a variety of cancers to develop a more complete understanding of the stromal patterns in cancer.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Conception and design: J.L-Y. Chen, M. van de Rijn, R.B. West
Development of methodology: J.L-Y. Chen, M. van de Rijn, R.B. West
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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J.L-Y. Chen, O.Y-W. Liao, R.B. West
Writing, review, and/or revision of the manuscript: J.L-Y. Chen, I. Espinosa, A. Lin, M. van de Rijn, R.B. West
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): J.L-Y. Chen, R.B. West
Study supervision: J.L-Y. Chen, M. van de Rijn, R.B. West

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