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Original contribution

Fibroblast activation protein expression by stromal cells and tumor-associated macrophages in human breast cancer☆,☆☆,★

Julia Tchou[a,b,*], **Paul J. Zhang**[c], **Yingtao Bi**[d], **Celine Satija**[a], **Rajrupa Marjumdar**[d], **Tom L. Stephen**[d], **Albert Lo**[d], **Haiying Chen**[a], **Carolyn Mies**[c], **Carl H. June**[b,c,e], **Jose Conejo-Garcia**[d], **Ellen Puré**[d]

[a]*Division of Endocrine and Oncologic Surgery, Department of Surgery, and the Rena Rowan Breast Center, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA*
[b]*Abramson Cancer Center, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA*
[c]*Department of Pathology and Lab Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA*
[d]*The Wistar Institute, Philadelphia, PA*
[e]*Abramson Family Cancer Research Institute, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA*

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Summary Fibroblast activation protein (FAP) has long been known to be expressed in the stroma of breast cancer. However, very little is known if the magnitude of FAP expression within the stroma may have a prognostic value and reflect the heterogeneous biology of the tumor cell. An earlier study had suggested that stromal FAP expression in breast cancer was inversely proportional to prognosis. We, therefore, hypothesized that stromal FAP expression may correlate with clinicopathologic variables and may serve as an adjunct prognostic factor in breast cancer. We evaluated the expression of FAP in a panel of breast cancer tissues (n = 52) using a combination of immunostain analyses at the tissue and single-cell level using freshly frozen or freshly digested human breast tumor samples, respectively. Our results showed that FAP expression was abundantly expressed in the stroma across all breast cancer subtypes without significant correlation with clinicopathologic factors. We further identified a subset of FAP-positive (or FAP$^+$) stromal cells that also expressed CD45, a pan-leukocyte marker. Using freshly dissociated human breast tumor specimens (n = 5), we demonstrated that some of these FAP$^+$CD45$^+$ cells were CD11b$^+$CD14$^+$MHC-II$^+$, indicating that they were likely tumor-associated macrophages (TAMs). Although FAP$^+$CD45$^+$ cells have been demonstrated in the mouse tumor stroma, our results demonstrating that human breast TAMs expressed FAP were novel and suggested that existing and future FAP-directed therapy may have dual-therapeutic benefits targeting both stromal mesenchymal

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* Corresponding author. Perelman Center for Advanced Medicine, 3rd Floor West, 3400 Civic Center Boulevard, Philadelphia, PA 19104-1693.
E-mail address: Julia.tchou@uphs.upenn.edu (J. Tchou).

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cells and immune cells such as TAMs. More work is needed to explore the role of FAP as a potential targetable molecule in breast cancer treatment.

1. Introduction

Although cancer drug development has traditionally targeted tumor cells, emphasis is beginning to shift toward inclusion of the tumor microenvironment for discovery of novel therapeutic approaches [1]. Among the cellular components in the tumor microenvironment, various nonimmune cells such as stromal mesenchymal cells have emerged as critical players in promoting tumor proliferation, neovascularization, invasion, and metastasis [2]. Because cancer stromal cells lack the genetic instability that is characteristic of tumor cells, they may exhibit a reduced capacity for development of drug resistance and immune escape and are, therefore, appropriate targets for drug development, complementing and, likely, enhancing efficacy of conventional cancer therapy.

Cancer-associated fibroblasts are one of the most common stromal cell types within the tumor. These cancer-associated fibroblasts characteristically adopt spindle-shaped cell morphology when placed in culture and express fibroblast markers such as fibroblast-specific protein [3], α-smooth muscle actin (α-SMA), and fibroblast activation protein (FAP) [4–7]. FAP is a homodimeric integral membrane serine proteinase. Unlike fibroblast-specific protein and other common fibroblast markers, FAP is a cell surface protein, rendering it a targetable molecule whereby novel therapeutic strategies that either (1) inhibit the FAP function (ie, protease activity) or (2) deplete cells that express FAP within the tumor microenvironment have shown promising antitumor effects [8–12]. Therefore, in this study, we sought to characterize the expression pattern of FAP in a cohort of breast cancer with heterogeneous characteristics to determine whether the expression of FAP within breast cancer stroma may correlate with conventional prognostic factors and thus have prognostic value. Our hypothesis was based on a previous study that suggested that FAP expression in breast cancer was inversely correlated with breast cancer prognosis [13].

Using immunohistochemistry (IHC) to characterize FAP expression in a panel of breast cancer samples (n = 52), we found that FAP was abundantly expressed in most (85.4% ± 13.8%) stromal cells in all of our tumor samples [13]. We did not observe a significant correlation between the levels of FAP expression and clinicopathologic variables in this study. Further characterization of these FAP-expressing stromal cells revealed a subpopulation that also expressed CD45, a pan-leukocyte marker. Using flow cytometry to analyze single-cell suspensions derived from freshly procured human breast cancer samples, we demonstrated that some of these FAP$^+$CD45$^+$ cells also expressed markers for macrophages including CD14, CD11b, CD114, and HLA-DR, suggesting that some of these FAP$^+$CD45$^+$ cells may represent tumor-associated macrophages (TAMs). Our finding that TAMs express FAP suggested that existing targeted strategies against FAP [9–12,14–18] may have dual effects against stromal fibroblasts and TAMs.

2. Materials and methods

2.1. Patients and clinical characteristics of study cohort

Women with newly diagnosed breast cancer undergoing breast cancer surgery at the Hospital of the University of Pennsylvania were asked to participate in a tissue bank protocol that had been approved by our institutional review board. Our study cohort included 52 patients treated at our institution between 2008 and 2011. Breast tumors were stratified into 3 subgroups according to receptor expression: group 1 comprised hormone receptor (HR) positive (or HR+) breast cancer, which is defined by the expression of either estrogen receptor (ER) or progesterone receptor (PR) and lacks Her2-neu (Her2) expression (n = 25); group 2 (n = 7) comprised Her2-neu positive (or Her2+) breast cancer, which expressed Her2-neu as determined by IHC and/or fluorescence in situ hybridization with (n = 2) or without expression (n = 5) of ER or PR; and group 3 comprised triple-negative breast cancer (TNBC), which lacked expression of ER, PR, or Her2 (n = 20). To ensure a balanced proportion of the less common TNBC, which typically represents only about 20% of all diagnosed patients with breast cancer, we included all banked TNBCs (n = 20) from our entire banked tumor samples during our study period from 2008 to 2011. We then included 25 unselected consecutively banked ER+ breast cancers and 7 Her2+ breast cancers in our analyses. All data collection and analyses were adherent to institutional review board–approved protocols.

2.2. IHC analyses

ER, PR, and Her2 expressions were evaluated by standard IHC staining techniques on formalin-fixed, paraffin-embeded (FFPE) breast cancer tissue samples for all participants as part of standard pathology evaluation at our institution. The Food and Drug Administration–approved PharmDx ER and PR test kits and HercepTest (DAKO, Carpinteria, CA) were used to evaluate ER, PR, and Her2 expression strictly following the manufacturers' guidelines on FFPE section at the time of the routine pathologic evaluation of the surgical specimen. The tests were reported as negative if Allred score was 2 or less for ER and PR and 0 or 1+ for Her2. Only cases with sufficient tumor tissue were studied. Fluorescent in situ hybridization was performed using PathVision HER2 DNA