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Article

Cancer-Associated Fibroblasts Are Activated in Incipient Neoplasia to Orchestrate Tumor-Promoting Inflammation in an NF-κB-Dependent Manner

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SUMMARY

Cancer-associated fibroblasts (CAFs) support tumorigenesis by stimulating angiogenesis, cancer cell proliferation, and invasion. We demonstrate that CAFs also mediate tumor-enhancing inflammation. Using a mouse model of squamous skin carcinogenesis, we found a proinflammatory gene signature in CAFs isolated from dysplastic skin. This signature was maintained in CAFs from subsequent skin carcinomas and was evident in mammary and pancreatic tumors in mice and in cognate human cancers. The inflammatory signature was already activated in CAFs isolated from the initial hyperplastic stage in multistep skin tumorigenesis. CAFs from this pathway promoted macrophage recruitment, neovascularization, and tumor growth, activities that are abolished when NF-κB signaling was inhibited. Additionally, we show that normal dermal fibroblasts can be "educated" by carcinoma cells to express proinflammatory genes.

INTRODUCTION

Although tumorigenesis has classically been viewed as a largely cell-autonomous process involving genetically transformed cancer cells, the importance of stromal cell types populating the neoplastic microenvironment is now well accepted (reviewed in Bissell and Radisky, 2001; Hanahan and Weinberg, 2000; Tlsty and Coussens, 2006). Studies investigating the link between inflammation and cancer have been particularly revealing, demonstrating that inflammatory immune cells are recruited to neoplasias, where they in many cases markedly promote tumor progression by facilitating multiple hallmark capabilities (de Visser et al., 2006; Karin and Greten, 2005; Mantovani et al., 2008). Immune cells have been documented to supply soluble growth and survival factors, matrix remodeling enzymes, reactive oxygen species, and other bioactive molecules that variously influence cancer cell proliferation, angiogenesis, invasion, and metastasis (De Marzo et al., 1999; Kuper et al., 2000; van Kempen et al., 2006).

A second stromal component of importance for tumorigenesis is cancer-associated fibroblasts (CAFs). CAFs are phenotypically and functionally distinguishable from their normal counterparts in their increased rate of proliferation and differential expression of extracellular matrix (ECM) components and growth factors (Bhowmick et al., 2004b; Kalluri and Zeisberg, 2006). Several studies have demonstrated that normal fibroblasts have a role in maintaining epithelial homeostasis by suppressing proliferation and oncogenic potential of adjacent epithelia (Begley et al., 2008; Bhowmick et al., 2004a; Trimboli et al., 2009). However, following neoplastic transformation of epithelia, CAFs have been shown to promote tumor growth by inducing angiogenesis, recruiting bone marrow–derived

Significance

Chronic inflammation is highly correlated with many types of human cancer. Here we show that cancer-associated fibroblasts (CAFs) express a proinflammatory gene signature in skin, breast, and pancreatic cancers. Proinflammatory CAFs from a transgenic mouse model of squamous cell carcinoma mediated immune cell recruitment, angiogenesis, and tumor growth. Inhibition of NF-κB signaling abolished these tumor-promoting effects, demonstrating that NF-κB is critical for tumor-enhancing inflammation mediated by CAFs. These results not only demonstrate a role for CAFs in promoting tumorigenesis, but also have important implications for therapeutic targeting of CAF regulatory or effector circuits, suggesting that inhibiting stromal NF-κB signaling could be of value in treating certain forms of human cancer.

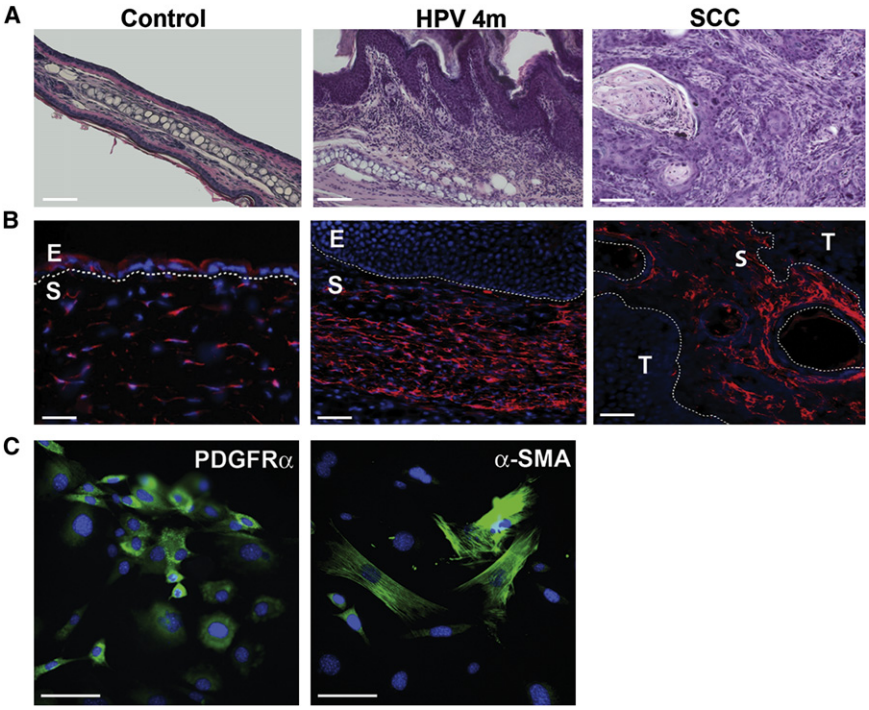

Figure 1. PDGFRα is Broadly Expressed in Normal and Neoplastic Skin Fibroblasts

(A) H&E staining of skin from nontransgenic control ears (left), a 4-month-old HPV16 mouse with dysplasia (middle), or a squamous cell carcinoma (SCC) (right).

(B) PDGFRα expression is restricted to the stromal compartment in skin and tumors. Immunohistochemical analysis of PDGFRα (Rhodamine-X, red) in the same tissue as in (A). E, epithelium; S, stroma. DAPI staining is shown in blue. The panels are representative of multiple fields of skin sections from two control and four HPV mice.

(C) Immunofluorescent staining of cultured fibroblasts sorted from dysplastic ear. All fibroblasts express PDGFRα (FITC). Only a subset of PDGFRα$^+$ fibroblasts expresses α-SMA (FITC). Scale bar, 100 μM. See also Figure S1.

endothelial progenitor cells, and remodeling the ECM (Allinen et al., 2004; Olumi et al., 1999; Orimo et al., 2005; Pietras et al., 2008). Interestingly, CAFs can even mediate resistance to antiangiogenic therapy (Crawford et al., 2009). It is increasingly apparent that CAFs are a diverse cell population that can have different characteristics in different tumor types and tissue locales. Some CAFs are related to myofibroblasts, an activated form of fibroblast that plays an important role in wound healing and is characterized by expression of α-SMA. Not all CAFs, however, express α-SMA. Increasingly, fibroblasts in tumor tissues are being recognized as a diverse population of myofibroblastic cells intermixed with other fibroblastic cells that do not express α-SMA but may be tumor promoting nevertheless (Desmouliere et al., 2004; Micke and Ostman, 2004; Sugimoto et al., 2006).

To better understand the characteristics and functions of CAFs, we set out to investigate CAFs in the K14-HPV16 mouse model of multistep squamous skin carcinogenesis. This model displays premalignant and malignant stages that are stereotypical of carcinogenesis in humans. K14-HPV16 transgenic mice have proven useful for investigating multiple aspects of carcinogenesis, including tumor-stroma interactions (Arbeit et al., 1994; Coussens et al., 1996). These mice express the HPV16 early-region that includes the E6/E7 oncogenes, under the control of the human keratin-14 promoter/enhancer. Animals develop hyperplastic and then dysplastic lesions that progress to invasive squamous cell carcinomas, typically within the epidermis of the ear or on the chest and truncal skin; by 4 months of age, 100% of transgenic animals have hyperplastic/dysplastic lesions on their ears. The preneoplastic stage is characterized by extensive remodeling of the underlying dermal stroma, which facilitates both angiogenesis and eventual tumor cell invasion. This extensive stromal remodeling develops early at the dysplastic stage in all animals, well before malignant conversion, and is characterized by a chronic inflammatory response.

In this study, we performed expression profiling of fibroblasts from dysplastic skin of K14-HPV16 transgenic mice and investigated the functional implications of tumor-promoting inflammation mediated by CAFs. Furthermore, we studied the molecular mechanism underlying activation of naive, stromal fibroblasts into proinflammatory, tumor-promoting CAFs beginning at the earliest stages of multistep skin tumorigenesis.

RESULTS

Fibroblasts from Skin Dysplasias are Proinflammatory

Stromal changes in neoplastic skin of K14-HPV16 (HPV) mice precede progression to invasive carcinomas. By 4 months of age, at sites where skin tissue is focally dysplastic, the dermis is transformed into a reactive stroma, composed of blood and lymphatic vessels, inflammatory cells, and fibroblasts, which persists during neoplastic progression to cancer (Coussens et al., 1999) (Figure 1A). We sought to identify genes whose expression changed in CAFs in early neoplastic skin lesions compared to their normal dermal counterparts. We chose to purify fibroblasts from normal and neoplastic tissues by FACS. Although the myofibroblast marker α-SMA is commonly used to identify CAFs, it is intracellular, and it is not certain that all CAFs are α-SMA positive (Sugimoto et al., 2006). We surveyed a number of surface markers for robust expression in skin fibroblasts and found PDGFRα to be the best (see Figure S1A available online). PDGFRα, which is reportedly expressed by up to 90% of stromal fibroblasts in solid tumors (Micke and Ostman, 2004), was found by immunostaining to be expressed exclusively in the stromal compartments of normal skin, dysplastic HPV tissue, and HPV skin tumors (Figure 1B). The purity of sorted PDGFRα$^+$ cells was verified by expression of fibroblast-specific genes and by a lack of expression of immune cell markers (Figure S1B). When α-SMA expression was assessed in sorted skin fibroblasts, we observed that only a subset of PDGFRα$^+$