

AD _____

Award Number: W81XWH-09-1-0468

TITLE: Fibroblast TGF-Beta Signaling in Breast Development and Cancer

PRINCIPAL INVESTIGATOR: Aubie Shaw, Ph.D.

CONTRACTING ORGANIZATION: The Vanderbilt University
Nashville, TN 37203

REPORT DATE: September 2012

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release; distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE (DD-MM-YYYY) September 2012		2. REPORT TYPE Annual Summary		3. DATES COVERED (From - To) 15 August 2009 - 14 August 2012	
4. TITLE AND SUBTITLE Fibroblast TGF-Beta Signaling in Breast Development and Cancer				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-09-1-0468	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Aubie Shaw E-Mail: vumc.research.grants@vanderbilt.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The Vanderbilt University Nashville, TN 37203				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Hypothesis: This proposal will address the hypothesis that TGFβ signals in fibroblasts allow normal mammary gland development; and prevents breast cancer growth and progression. Specific Aims: We will (1) determine the effect of loss of TGFβ signaling within stromal cells on mammary gland development and (2) determine the effect of loss of TGFβ signaling within stromal cells on mammary carcinoma development. Study Design: This will be accomplished using transgenic mice with an inducible deletion of the type II TGFβ receptor (TGFBRII), which is required for TGFβ signaling. Cre recombinase driven by Fibroblast-Specific Protein (FSP1) and Pro-Collagen Ia2 will delete TGFBRII within fibroblasts. To understand the normal biology of fibroblast TGFβ signaling, we will first examine mammary gland development in floxed TGFBRII, inducible fibroblast specific-Cre mice. Then we will examine carcinogenesis and tumor progression in floxed TGFBRII, inducible fibroblast-specific Cre, MMTV-PyVmT mice. This is the first proposed model to examine the biology of TGFβ signaling in fibroblasts in the intact mammary gland. Since TGFβ can promote or suppress cancer, understanding the roles of fibroblast TGFβ signaling in breast cancer will allow us to identify patients for which anti-TGFβ therapy is appropriate.					
15. SUBJECT TERMS Transforming Growth Factor β, mammary gland development, TGFβRII, inducible Cre, FSP1, CollagenIa2, MMTV-PyVmT, mammary tumorigenesis					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	19	19b. TELEPHONE NUMBER (include area code)

Table of Contents

Cover.....	1
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	4 - 9
Key Research Accomplishments.....	9
Reportable Outcomes.....	9 - 10
Conclusions.....	10 - 11
References.....	N/A
Appendices.....	11

INTRODUCTION

With the recognized roles of TGF β and fibroblasts in mammary gland development and breast cancer formation, and the dual role of TGF β signaling in breast cancer, it is important to understand the role of TGF β signaling in tumor stroma. Several studies have already elucidated that autonomous TGF β signaling within epithelial cells suppresses carcinoma formation early, but promotes cancer formation later. To understand the role of TGF β signaling in stromal cells, we must examine a model in which TGF β signaling can be modified at certain points in mammary gland development and before or after epithelial oncogenesis. Careful studies of the tumor microenvironment in breast cancer will allow us to determine the dual role of TGF β in breast cancer. A complete understanding of the roles of TGF β in epithelial and stromal cells in breast cancer will direct us to the appropriate roles of TGF β activation or inhibition in patient therapy.

Hypothesis: This proposal will address the hypothesis that TGF β signaling within stromal cells of the mammary gland alters mammary gland development and drives breast cancer progression in situ.

Specific Aim 1: Determine the effect of loss of TGF β signaling within stromal cells on mammary gland development.

Specific Aim 2: Determine the effect of loss of TGF β signaling within stromal cells on mammary carcinoma development.

BODY

The research findings of three years will be detailed according to the approved Statement of Work as outlined below.

STATEMENT OF WORK

Task 1. Generate FSP1-CreER, TGFBRII^{ff}, R26R^{ff} mice (timeframe months 1-6)
(Coll-CreER, TGFBRII^{ff}, R26R^{ff} mice have already been generated)

1a. Breed FSP-CreER animals to TGFBRII^{ff}, R26R^{ff} mice to generate FSP1-CreER, TGFBRII^{ff}/wt, R26R^{ff}/wt animals (months 1-3)

1b. Backcross Task 1a animals to TGFBRII^{ff}, R26R^{ff} to generate FSP1-CreER, TGFBRII^{ff}, R26R^{ff} mice (months 2-6)

The FSP-CreER strain that was created in our lab showed non-specific expression in tissues other than fibroblasts. We were able to obtain another FSP-CreER mouse that was created in Terry Van Dyke's lab at UNC-Chapel Hill that she had shown to work in the prostate and mammary gland. This model has not been published yet. We removed our FSP-CreER strain and bred Dr. Van Dyke's FSP-CreER model into our colony.

Experiments to demonstrate TGFBRII Cre recombination using the R26R-stop^{ff}- β gal reporter line have not shown Xgal conversion or immunohistochemical staining in

fibroblasts in the mammary gland. Recombination is found in the prostate and skin fibroblasts, but the methods we have used to test it so far have not shown recombination in the mammary gland. In our first experiment with mammary gland tumors, I saw that mice with FSP1-CreER had a dramatic reduction in tumor size after the mice were treated with Tamoxifen, suggesting that FSP1-CreER is affecting tumor growth or cell survival. This suggests that there is fibroblast-specific TGFBR11 recombination in the mammary gland, but we must adapt our technical methodology to demonstrate this in the mammary gland. We have used several other Cre reporter lines, including the R26R-stop^{f/f}-YFP, actin-stop^{f/f}-GFP, actin-stop^{f/f}-dsRed and actin-GFP-stop^{f/f}-mTomato reporters. Each of these Cre reporter mouse lines was bred to the FSP1-CreER, Coll-CreER or MMTV-Cre mice to examine recombination. Of these, the R26R-stop^{f/f}-YFP, actin-stop^{f/f}-GFP, actin-stop^{f/f}-dsRed did not show recombination in fibroblasts using fluorescence or immunohistochemistry of the fluorescent reporter molecule.

In a newly developed Cre reporter model, the mT/mG mouse, Cre activity removes membrane-targeted Tomato and activates expression of membrane-targeted EGFP. The fluorescent reporter molecules are visualized in frozen sections of tissue. Our earliest test of the mT/mG mouse showed recombination in epithelial cells when bred to the MMTV-Cre mouse. To test the model in fibroblasts, it was bred to the non-inducible FSP-Cre mouse where it showed recombination in mammary gland fibroblasts (Figure 1). The mT/mG Cre reporter line was then bred to the FSP1-CreER and Coll-CreER lines to examine recombination in fibroblasts after Tamoxifen induction. FSP1-CreER, mT/mG mice show recombination in fibroblasts after Tamoxifen is administered in mouse chow (Figure 2). To examine induction and recombination in mammary gland tumors, FSP1-CreER, mT/mG, MMTV-PyMT mice were treated with Tamoxifen by IP injection after tumor palpation. Fibroblast-specific recombination is seen in subsets of stroma within the tumors (Figure 3). Tomato expression remains in epithelial cells, showing cell-specific recombination within fibroblasts.

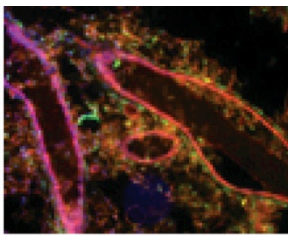


Figure 1. Recombination in fibroblasts of normal mammary gland by FSP-Cre. In FSP-Cre, mT/mG mouse, Tomato is silenced and EGFP is induced by Cre activation in fibroblasts of the normal mammary gland.

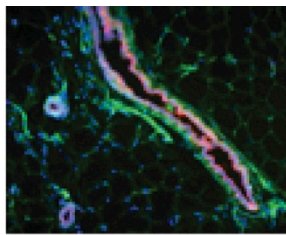


Figure 2. Induced recombination in fibroblasts of normal mammary gland by FSP-CreER. In FSP-CreER, mT/mG mouse, Tomato is silenced and EGFP is induced by Cre activation in fibroblasts after Tamoxifen treatment in mouse chow.

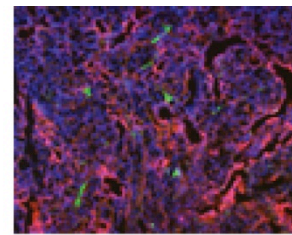


Figure 3. Induced recombination in fibroblasts of mammary gland tumor by FSP-CreER. In FSP-CreER, mT/mG, MMTV-PyVMT mouse, Tomato is silenced and EGFP is induced by Cre activation in fibroblasts after Tamoxifen treatment by IP injection after tumor palpation.

Coll-CreER, TGFBRIIf/f, mT/mG and FSP-CreER, TGFBRIIf/f, mT/mG mice were generated. The FSP-CreER mouse arrived to our lab on a mixed strain background. FSP-CreER, TGFBRIIf/f, mT/mG mice were backcrossed 5 generations to C57BL6 background. Coll-CreER, TGFBRIIf/f, mT/mG were backcrossed at least 10 generations to the inbred C57BL6 background.

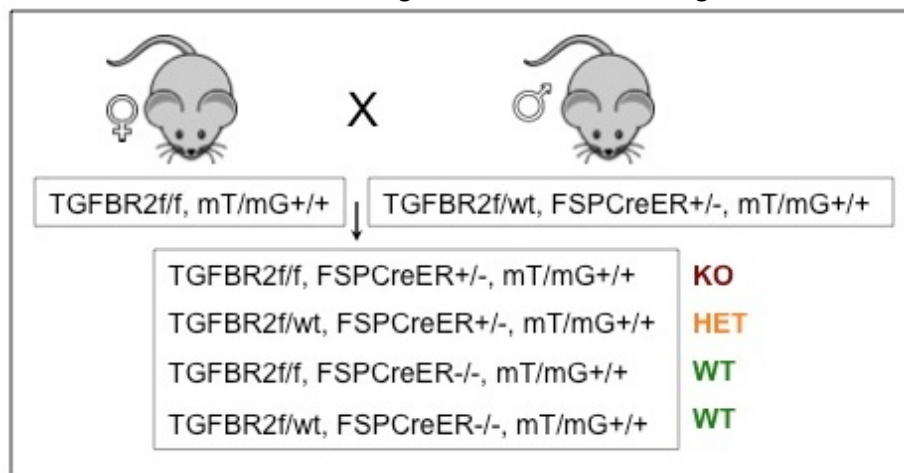
Task 2. Generate animals for Aim 1 (months 4-12)

2a. Generate 228 Coll-CreER, TGFBRIIf/f, R26Rf/wt female mice (months 4-10)

2b. Generate 228 FSP1-CreER, TGFBRIIf/f, R26Rf/wt female mice (months 6-12)

Animals: To generate Coll-CreER, TGFBRIIf/f, R26Rf/wt female mice, 154 dams and 52 sires will be mated to produce 2304 adult mice. A similar breeding strategy will generate the FSP1-CreER, TGFBRIIf/f, R26Rf/wt female mice (total animals for both Cre strains = 5020). Approximately 80 cages will be maintained for 9 months on this task.

Animals were bred according to the scheme in Figure 4.



Task 3. Tamoxifen treat animals and collect tissues for Aim 1 (months 6-18)

3a. Treat and collect tissue from Coll-CreER, TGFBRIIf/f, R26Rf/wt female mice (months 6-16)

3b. Treat and collect tissue from FSP1-CreER, TGFBRIIf/f, R26Rf/wt female mice (months 8-18)

Animals: 576 Coll-CreER, TGFBRIIf/f, R26Rf/wt female mice, and 576 FSP1-CreER, TGFBRIIf/f, R26Rf/wt female mice will be treated with Tamoxifen (total animals for both Cre strains = 1152). Approximately 40 cages will be maintained for 12 months on this task.

FSP-CreER, TGFBRIIf, mT/mG animals were treated with Tamoxifen at 3 weeks of age for 5 consecutive days by intraperitoneal injection. Mammary glands were collected from virgin female mice at ages 6 weeks, 12 weeks and 16 weeks.

Task 4. Complete western blots, Northern blots and immunohistochemistry for Aim 1 (months 7-20)

- 4a. Test *Coll-CreER*, *TGFBR1lf/f*, *R26Rf/wt* mammary glands (months 7-17)
 4b. Test *FSP1-CreER*, *TGFBR1lf/f*, *R26Rf/wt* mammary glands (months 9-19)
 4c. Analyze data (months 7-20)

Milestone #1: National meeting presentations

Milestone #2: Publication regarding Aim 1

Whole mount inguinal mammary glands were imaged with carmine-alum staining. Fibroblast *TGFBR1* homozygous or heterozygous loss did not result in defects in branching morphogenesis or ductal outgrowth. At 6 weeks, all glands had grown to the edges of the fatpad regardless of genotype (Figures 5 & 7) and exhibited the same number of terminal end buds (Figure 6). Images of 12 and 16 week mammary glands did not show changes in branching distance or number of branches (Figure 7). Histopathological analysis of tissue sections did not show any changes in mammary gland structure or acinar development (Figure 8).

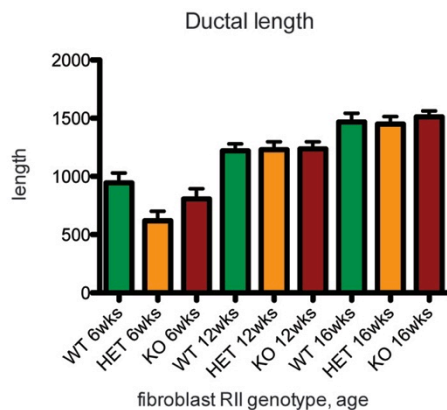


Figure 5. Length of mammary ducts on whole mount carmine-alum stained inguinal glands.

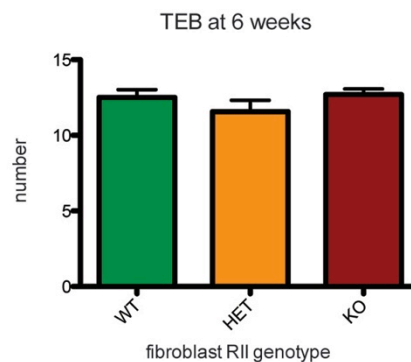


Figure 6. Number of terminal end buds (TEB) on whole mount carmine-alum stained inguinal glands.

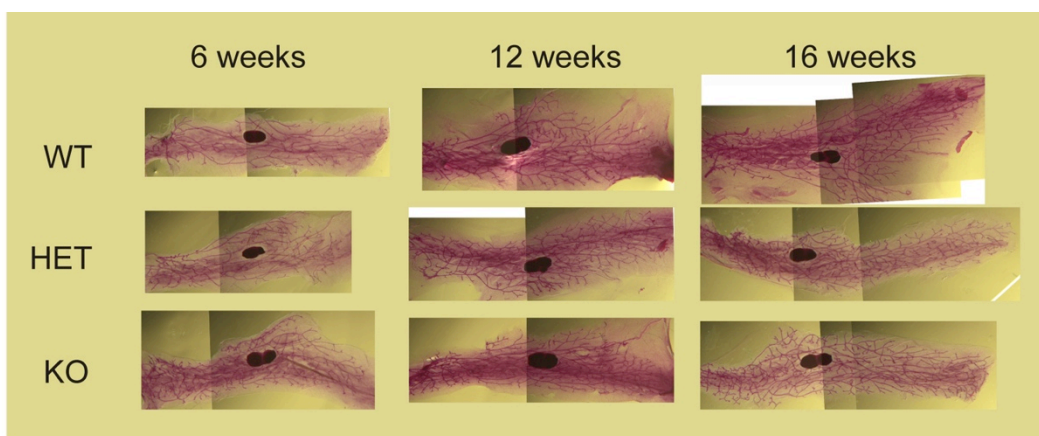


Figure 7. Carmine-alum stained whole mount inguinal mammary glands at different ages.

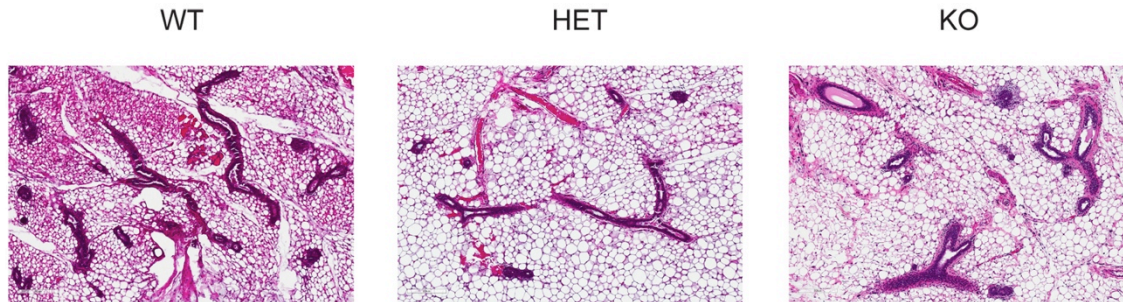


Figure 8. H&E stained mammary glands at 16 weeks.

Since mammary glands did not show differences in whole mount ductal growth or acinar development, glands were not further examined using molecular analysis.

Task 5. Generate animals for Aim 2 (months 11-22)

5a. Generate *TGFBR1f/f*, *R26Rf/f*, MMTV-PyVmT^{+/+} male mice (months 11-14)

5b. Generate 36 *Coll-CreER*, *TGFBR1f/f*, *R26Rf/wt*, MMTV-PyVmT female mice by breeding mice generated in Task 2a and Task 5a (months 13-19)

5c. Generate 36 *FSP1-CreER*, *TGFBR1f/f*, *R26Rf/wt*, MMTV-PyVmT female mice by breeding mice generated in Task 2b and Task 5a (months 16-22)

Animals: To generate *Coll-CreER*, *TGFBR1f/f*, *R26Rf/wt*, MMTV-PyVmT female mice, 52 dams and 18 sires will be mated to produce 768 adult mice. A similar breeding strategy will generate the *FSP1-CreER*, *TGFBR1f/f*, *R26Rf/wt*, MMTV-PyVmT female mice (total animals for both Cre strains = 1676). Approximately 40 cages will be maintained for 12 months on this task.

FSP-CreER, *mT/mG*, MMTV-PyMT mice treated with Tamoxifen after tumor palpation showed interesting changes in size relevant to *TGFBR1* status. Tumors with *TGFBR1* deleted (*TGFBR1f/f*, *FSP-CreER*⁺, last 2 bars in Figure 9) were larger than tumors with *TGFBR1* intact (3rd bar in Figure 9). However, the opposite trend was noticed when tumors were treated with Tamoxifen prior to tumor palpation. Tumors with *TGFBR1* deleted (1st bar in Figure 9) were smaller than tumors with heterozygous deletion of *TGFBR1* (2nd bar in Figure 9). This suggests that fibroblast *TGFβ* signals have opposing effects in developing tumors versus late stage tumors.

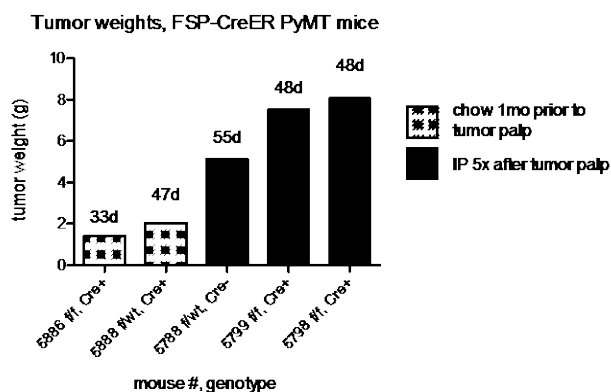


Figure 9. Tumor sizes in *FSP-CreER*, MMTV-PyMT mice. Mice were treated with Tamoxifen prior to tumor palpation (hatched bars) or after tumor palpation (solid bars). Numbers above the bars show the age of the tumor after palpation in days.

Task 6. Tamoxifen treat animals and collect tissues for Aim 2 (months 12-29)

6a. Treat and collect tissue from Coll-CreER, TGFBRIIf/f, R26Rf/wt, MMTV-PyVmT female mice (months 14-26)

6b. Treat and collect tissue from FSP1-CreER, TGFBRIIf/f, R26Rf/wt, MMTV-PyVmT female mice (months 17-29)

Animals: 96 Coll-CreER, TGFBRIIf/f, R26Rf/wt, MMTV-PyVmT female mice and 96 FSP1-CreER, TGFBRIIf/f, R26Rf/wt, MMTV-PyVmT female mice will be treated with Tamoxifen (total animals for both Cre strains = 192). Approximately 11 cages will be maintained for 18 months on this task.

As noted in Task 1, Cre mouse strains needed to be changed and it took 1 year to breed this onto our colony. The Cre reporter was changed from R26R to mT/mG and it took an additional 6 months to breed this into our colony. In addition, preliminary experiments showed that Tamoxifen treatment delayed mammary tumor development. We were not able to breed the MMTV-PyVmT oncogenic strain into our mouse line. This task could not be completed during the time of this award.

Task 7. Complete western blots, Northern blots and immunohistochemistry for Aim 2 (months 15-36)

7a. Test Coll-CreER, TGFBRIIf/f, R26Rf/wt mammary glands (months 15-27)

7b. Test FSP1-CreER, TGFBRIIf/f, R26Rf/wt mammary glands (months 18-30)

7c. Analyze data (months 15-36)

Milestone #3: National meeting presentations

Milestone #4: Publication regarding Aim 2

This task could not be completed.

KEY RESEARCH ACCOMPLISHMENTS

- The classically used R26R-βgal Cre reporter model cannot be used to demonstrate Cre activity in fibroblasts of the mammary gland.
- mT/mG Cre reporter mouse can be used to visualize recombination in fibroblasts.
- Loss of TGF signaling within fibroblasts does not alter development of the virgin mammary gland.

REPORTABLE OUTCOMES

Abstracts and Presentations:

Shaw A, Novitskiy S, Owens P, Moses HL (2012) Myeloid-derived Suppressor Cells Promote Cancer-Associated Fibroblast Migration and Carcinoma Cell Invasion. Poster at American Association for Cancer Research Annual Meeting, Chicago, IL

Shaw A, Novitskiy S, Moses HL (2011) Myeloid-derived Suppressor Cells Direct Fibroblast Migration and Mammary Carcinoma Cell Invasion. Poster at Department of Defense Era of Hope Meeting, Orlando, FL.

Shaw A, Novitskiy S, Moses HL (2010) Myeloid-derived Suppressor Cells Direct Fibroblast Migration and Mammary Carcinoma Cell Invasion. Poster at AACR Joint Conference on Metastasis and the Tumor Microenvironment, Philadelphia, PA

Kennedy J, Boutte A, Cargen L, Crowe S, Hanson C, Hardin G, Hallock K, Lancaster D, Lee P, Markham N, Martin P, McCollum D, McCullough N, Moses J, Obenaus A, Piper G, Piper J, Shaw A, Weiss J (2010) Mythbusters: Cancer Research in Jeopardy? Poster at American Association for Cancer Research Annual Meeting, Washington, DC –*this abstract was chosen for press release*

Shaw A, Johnson R, Sterling JA, Mundy GR, Moses HL (2010) A Novel Mouse Model of Breast Cancer Metastasis to Bone. Mini-symposium at American Association for Cancer Research Annual Meeting, Washington, DC

Awards:

- 2012 2nd Prize for Outstanding Poster Presentation in Vanderbilt Postdoctoral Research and Shared Resources Symposium \$150
- 2011 1st Prize for Outstanding Poster Presentation in Vanderbilt Postdoctoral Research and Shared Resources Symposium \$300
- 2010 AACR-Susan G. Komen Scholar-in-Training Award \$1,000

Manuscripts:

Franco OE, Shaw AK, Strand DW, Hayward SW (2010) Cancer Associated Fibroblasts in Cancer Pathogenesis. Sem Cell Dev Biol 21: 33-39. Featured on the cover.

Johnson MD, Shaw AK, O'Connell MJ, Sim FJ, Moses HL (2010) Analysis of transforming growth factor β receptor expression and signaling in higher grade meningiomas. J Neurooncol, Sept 19, 2010.

Xu BJ, Yan W, Jovanovic B, Shaw AK, An QA, Eng J, Chytil A, Link AJ, Moses HL. (2010) Microdialysis combined with proteomics for protein identification in breast tumor microenvironment in vivo. Cancer Microenviron. 4(1): 61-71.

Shaw A, Gipp J, Bushman W. (2010) Exploration of Shh and BMP paracrine signaling in a prostate cancer xenograft.. Differentiation, 79 (1): 41-47.

CONCLUSIONS

Cre recombination within fibroblasts of the mammary gland cannot be shown using the classical R26R transgenic mouse model with either Xgal substrate conversion or bgal immunohistochemistry. We have found a Cre reporter mouse model, mT/mG, that can be used to visualize fibroblast-specific recombination. Although progress on this

proposal was delayed, we generated FSP-CreER mice that were used to examine Aim 1. We found that fibroblast-specific inducible loss of TGF β signaling does not alter development of the mammary gland. FSP-CreER containing oncogenic MMTV-PyVmT were generated for preliminary experiments to examine Aim 2, however we found that Tamoxifen treatment significantly delays tumor development. We could not examine fibroblast-specific inducible loss of TGF β signaling in mammary tumors because of delays in generating the mouse model.

APPENDIX

Manuscript

(I) Franco OE, Shaw AK, Strand DW, Hayward SW (2010) Cancer Associated Fibroblasts in Cancer Pathogenesis. Sem Cell Dev Biol 21: 33-39. Featured on the cover.

Contact Information:

Address:

Oregon Health & Science University
Department of Cell & Developmental Biology
Mail code L215
3181 SW Sam Jackson Park Rd
Portland, OR 97239-3098

Email: shawau@ohsu.edu

Phone: (503)-494-9859



This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

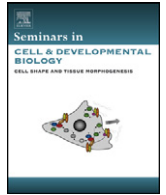
<http://www.elsevier.com/copyright>



Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb



Review

Cancer associated fibroblasts in cancer pathogenesis

Omar E. Franco^a, Aubie K. Shaw^b, Douglas W. Strand^a, Simon W. Hayward^{a,b,*}

^a Department of Urologic Surgery, Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, Nashville, TN 37232-2765, USA

^b Department of Cancer Biology, Vanderbilt-Ingram Cancer Center, Nashville, TN 37232, USA

ARTICLE INFO

Article history:

Available online 5 November 2009

Keywords:

Cancer associated fibroblast
Stromal–epithelial interaction
Tumor heterogeneity
Tumor stroma
Activated fibroblasts
Myofibroblasts

ABSTRACT

In the past century, gradual but sustained advances in our understanding of the molecular mechanisms involved in the growth and invasive properties of cancer cells have led to better management of tumors. However, many tumors still escape regulation and progress to advanced disease. Until recently, there has not been an organized and sustained focus on the “normal” cells in the vicinity of tumors. Interactions between the tumor and these host cells, as well as autonomous qualities of the host cells themselves, might explain why tumors in people with histologically similar cancers often behave and respond differently to treatment. Cells of the tumor microenvironment, variously referred to as cancer stroma, reactive stroma or carcinoma-associated fibroblasts (CAF), exist in close proximity to the cancer epithelium. Both stromal and epithelial phenotypes co-evolve during tumorigenesis and it is now becoming clear that these stromal cells may not be the innocent bystanders they had been widely thought to be, but rather may be active contributors to carcinogenesis. Our group and others have shown the important role that CAF play in the progression of cancer. In this article we will address current trends in the study of the interactions between cancer stroma and tumor cells in different organs. We will also highlight perceived knowledge gaps and suggest research areas that need to be further explored to provide new targets for anticancer therapies.

© 2009 Elsevier Ltd. All rights reserved.

Contents

1. Introduction.....	33
2. Stromal influence on adult tissue homeostasis.....	34
3. Tumor-promoting ability of carcinoma-associated fibroblasts in different organs.....	34
3.1. Prostate.....	34
3.1.1. Genetic stability and hormones.....	34
3.1.2. Soluble factors secreted by CAF.....	35
3.2. Breast.....	36
3.3. Pancreas.....	36
4. Heterogeneity of stromal cells.....	37
5. Summary and future opportunities.....	37
Acknowledgments.....	38
References.....	38

1. Introduction

For many years malignant tumors were viewed as being composed of transformed cells that acquired cell-autonomous

hyperproliferative and invasive survival capacities. However, tumors are complex organ facsimiles composed not only of the founder neoplastic cells, but also of tumor-associated stromal cells and an extracellular milieu that includes matrix components, chemokines and cytokines, all of which increase the complexity of the tumor microenvironment. Fibroblasts are an important component of the tumor stroma. The term fibroblast encompasses a number of stromal cells with a broadly similar phenotype. Most tumors incorporate an obvious biologically active, fibroblastic cell type known variously as reactive fibroblasts, myofibroblasts, or

* Corresponding author at: Department of Urologic Surgery, A-1302 Medical Center North, 1161 21st Ave South, Nashville, TN 37232-2765, USA.

Tel.: +1 615 343 5984; fax: +1 615 322 5869.

E-mail address: simon.hayward@vanderbilt.edu (S.W. Hayward).

simply carcinoma-associated fibroblasts. These cells have received increased attention because of their participation in tumor development, including invasion and metastasis and their ability to act as markers of patient prognosis [1]. Thus, elucidation of the critical cellular and/or molecular events that orchestrate tumor evolution is important as these represent potentially attractive targets for therapeutic intervention. The purpose of this review is to summarize our current understanding of these cells, highlighting recent advances and analyzing the therapeutic potential of targeting CAF.

2. Stromal influence on adult tissue homeostasis

Maintenance of the anatomical *status quo*, or tissue homeostasis, is crucial for the preservation of normal tissue morphology and the proper function of organs. In adult tissue, this task is tightly controlled by the stromal compartment through interactions between the different cell types and the production of extracellular matrix (ECM) components that provide the structure for proper tissue architecture and function.

The identification of multipotent mesenchymal stem cells (MSC) derived from adult human tissues, including bone marrow stroma and a number of connective tissues, has provided a new understanding of tissue homeostasis. These cells have the potential to differentiate into chondrocytes, osteoblasts, adipocytes, fibroblasts, marrow stroma, and other tissues of mesenchymal origin. Interestingly, these MSC reside in a diverse host of tissues throughout the adult organism and possess the ability to 'regenerate' cell types specific for these tissues, reviewed in [2]. Given the wide distribution of the sources of MSC, the bone marrow stroma may be considered to be the source of a common pool of multipotent cells that gain access to various tissues via the circulation, subsequently adopting characteristics that meet the requirements of maintenance and repair of a specific tissue type. In fact, the presence of MSC in tissues other than the marrow stroma strongly suggests the existence of cell populations with more limited capacity for differentiation; specifically, monopotent or bipotent cells may have differentiation potentials developmentally adapted to (and perhaps restricted to) the tissues in which they reside and help to the maintain tissue architecture.

It is well known that during the wound healing process, fibroblast-to-myofibroblast differentiation is a key event in the physiological reconstruction of the connective tissue. Myofibroblasts remodel the ECM and induce angiogenesis [3]. Smooth muscle α -actin (α SMA) is the most common marker used to identify myofibroblasts, but other markers such as vimentin, desmin, or smooth muscle myosin can be used in combination with α SMA to distinguish between different subpopulations of myofibroblasts and normal fibroblasts or smooth muscle cells [4,5]. Myofibroblast differentiation is induced by paracrine signals generated by repairing or injured tissues. Among those signals, transforming growth factor beta (TGF- β) is among the most potent. TGF- β activates myofibroblast differentiation of prostate fibroblasts [6]. The identification of myofibroblasts in prostate cancer patient tissue predicts tumor relapse [7]. Under normal circumstances once the tissue is repaired, myofibroblasts and other resident cells undergo apoptosis, and the remaining collagen-rich tissue is slowly remodeled to a normal phenotype [8]. In cancers, sometimes described as resembling chronic wounds, the origin of myofibroblasts remains controversial; however, their presence may be essential for much invasive growth and is translated into poor clinical prognosis [9]. Soluble factors secreted by myofibroblasts have been shown to mediate the invasive growth of breast and colon cancer cells *in vitro* and *in vivo* [10]. Thus, management of stromal reaction through prevention of the formation of myofibroblasts is a potentially attractive opportunity for therapy.

3. Tumor-promoting ability of carcinoma-associated fibroblasts in different organs

The role of cancer-associated fibroblasts in tumor progression is now well accepted. Many "*in situ*" cancers never progress to an invasive type, most likely due to host factors that prevent this development, a phenomenon termed "cancer without disease". This highlights the fact that a transformed cell *per se* is not enough to cause lethal cancer, but that carcinogenesis requires the recruitment of a tumor microenvironment permissive of further tumor growth and metastasis [11]. The stromal microenvironment is complex and contains many cell types including fibroblasts, smooth muscle cells, immune and inflammatory cells, adipocytes and endothelial cells (Fig. 1). Of utmost significance for tumor progression, as the primary stromal cell type that produces ECM, fibroblasts can bi-directionally 'sense' signals between ECM and cells by means of their integrin-dependent cell-matrix adhesions, eliciting changes in stromal dynamics and composition.

Changes in stromal characteristics can be perceived as an initial attempt to 'repair the damage' induced by transformed epithelium. There is a complex interplay of reciprocal stromal-epithelial interactions that essentially fools repair mechanisms resulting in changes in the ECM and the tumor stroma, a phenomena called "stromogenesis" [5]. Carcinogenesis then results from the loss or breakdown of biological organization induced by perturbed stromal-epithelial interactions, rather than from epithelial or stromal mutations alone [12]. There is substantial evidence for the contention that cancer-associated fibroblasts produce a tumor supportive ECM, which promotes the growth, expansion and dissemination of the pre-neoplastic epithelial cell population, creating a permissive 'pasture' for the emerging malignant cell [4]. Stromal cells are thought to be critical drivers of tumor progression in a number of organs.

3.1. Prostate

Prostate cancer is the most common non-skin malignancy in men in the USA and the second leading cause of male cancer death [13]. The stromal component of prostate cancer co-evolves with tumor cells and several lines of experimental and clinical evidence show that a dynamic interaction between stroma and epithelium plays a critical role in this progression. Using a xenograft model of human cells, we and others have shown that recombination of CAF with non-tumorigenic epithelial cells results in permanent malignant transformation of non-tumorigenic epithelial cells [14,15]. More recently, it has been shown that inhibition of the retinoblastoma gene function in epithelial cells can induce a selective expansion of a subpopulation of highly proliferative p53-null stromal cells through a paracrine mechanism [16].

3.1.1. Genetic stability and hormones

Although the stroma is usually genetically stable, lesions on chromosome 8p have been detected, and a high incidence (33%) of loss of heterogeneity (LOH) has been reported in the tumor stroma of prostate cancer patients [17] although this mechanism is not universally accepted. Several studies support the occurrence of a field effect for gene silencing in the stroma by cancer cells through hypermethylation. Among the genes implicated are GSTP1, APC, RASSF1A, H1N-1 and RAR β 2 [18,19]. More research is needed to clarify the contribution of any of these candidates in prostate cancer.

In a switch from its activity in the developing and normal adult organ, androgens promote prostate cancer growth through direct activation of the epithelial androgen receptor (AR) [20]. However, the role of AR in the tumor stroma has received little attention. Recent studies have shown that elevated AR expression in cancer

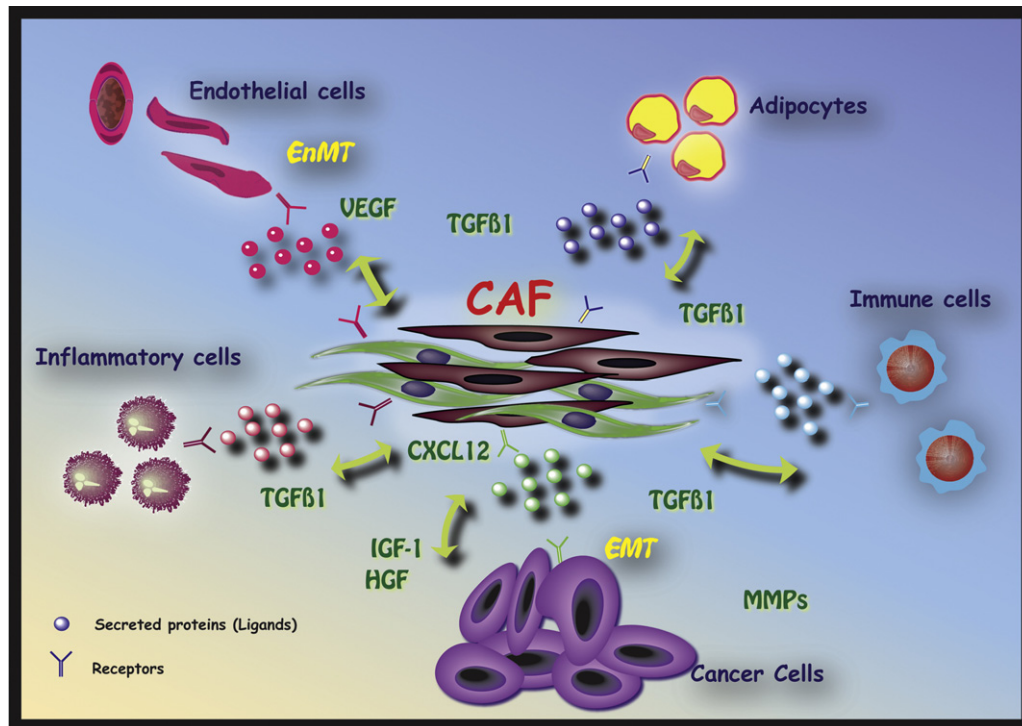


Fig. 1. Carcinoma-associated fibroblasts play a central role in the modulation of cancer growth. CAF are composed of a mixture of fibroblasts which might have different origins. When activated, these cells interact with cancer cells and start expressing several mitogenic and pro-invasive factors that create a favorable milieu for tumor cells to proliferate and invade into the surrounding tissue. Chemokines and cytokines can act in a paracrine manner binding to their cognate receptors resulting in bidirectional or multi-directional communication.

cells concurrent with loss of AR in the surrounding stroma was correlated with higher clinical stage, higher PSA levels, and earlier and higher relapse rates after radical prostatectomy [21,22]. It has been suggested that CAF could be derived from AR-negative fibroblasts that surround normal glands or from dedifferentiated smooth muscle cells [23]. More experimental studies are needed to further investigate the mechanism of AR loss within the stroma and its potential role in the progression of prostate cancer.

3.1.2. Soluble factors secreted by CAF

Stromal–epithelial interactions in prostate cancer involve a number of soluble factors and their receptors. These factors can act in a paracrine manner and work in coordination with other signaling molecules, such as ECM and integrins, which facilitate not only carcinogenesis, but cancer progression towards metastasis [24].

In cancer, transforming growth factor beta 1 (TGF-β1) is expressed at elevated levels to a greater extent than TGF-β2 or TGF-β3. In normal prostate epithelial cells, TGF-β controls homeostasis by eliciting differentiation, inhibiting proliferation and inducing apoptosis. However, TGF-β signaling in fibroblasts modulates the growth and the oncogenic potential of adjacent epithelia. For example, inactivation of the TGFβR2 in mouse fibroblasts results in the appearance of prostatic intraepithelial neoplasia in prostate tissue in association with an increased number of stromal cells [25]. TGF-β often suppresses transformation and early tumorigenesis but, as cancer progresses, cancer cells become insensitive to the growth inhibitory effects of TGF-β [26]. Signaling mediated by TGF-β involves the activation of direct downstream effectors including PI3K/AKT, MAPK, TAK1, Ras, Rho. However, the only downstream targets that are thought to be TGF-β-specific are SMAD molecules. Loss of regulation of Wnt expression resulting from reduced TGF-β function has been implicated in prostate cancer progression [27]. TGF-β2 can promote cancer cell survival

and resistance to apoptosis, effects that are attributable to the activation of nuclear factor-kappaB (NF-κB) [28]. These observations provide evidence supporting the clear association with cancer progression and makes TGF-β a potential target for therapeutic intervention.

Insulin-like growth factor-1 (IGF-1) is expressed primarily in prostatic stroma. Paracrine action of IGF includes proliferation of prostate epithelial cells through upregulation of MAPK, Akt and Cyclin D1 and downregulation of p27. Overexpression of IGF-1 has been shown to elicit neoplastic transformation of murine epithelium and significantly increased the invasive capacity of prostate cancer cell lines *in vitro*. Blockade of the IGF-1R or the use of specific inhibitors of the MAPK and PI3K pathways can decrease IGF-mediated tumor invasion [29]. Inhibition of IGF-1R signaling results in cytoplasmic AR retention accompanied by significant changes in androgen-regulated gene expression [30]. Another important interaction is that observed between IGF-1 and TGF-β1. Significantly, activation of the IGF-1R interferes with TGF-β1 activation of Smad3 thereby blocking TGF-β-mediated apoptosis in prostate epithelial cells [26]. Interactions between IGF-1, TGF-β1 and androgens highlight the importance of these factors during cancer progression and may account for the conversion to androgen-independence in the absence of androgen ligand.

HGF/scatter factor is expressed throughout the prostate stroma and acts in a paracrine fashion on epithelial cells where it binds to its receptor c-met. C-met is expressed in about 50% of localized prostate cancer and in almost all metastatic lesions [31]. Elevated expression of HGF/SF was shown to regulate invasion and growth of prostate cancer cells [32]. HGF can induce bone morphogenetic protein-7 (BMP-7), both *in vitro* and *in vivo* in prostate cancer models [33]. Androgen signaling downregulate c-met expression and androgen withdrawal can activate c-met indicating that c-met signaling may have a role in androgen independent progression of prostate cancer [34].

Recently several groups, including ours, have started to focus on the role of stromal-derived chemokines in prostate cancer progression. The pleiotropic actions of chemokines include the modulation of growth, angiogenesis, invasion, and metastasis [35]. While cancer cells can express all of the ligands, stromally derived chemokines include CXCL2, CXCL5, CXCL6 and CXCL12 [35].

In a recent study, we demonstrated that elevated TGF- β expression in the tumor stroma can activate CXCR4 expression in adjacent epithelial cells allowing the cognate ligand for this receptor, SDF1/CXCL12 (which is also expressed at elevated levels in the tumor stroma) to activate the receptor and thus stimulate phosphorylation of Akt in the epithelial cells [27,36]. In addition, phosphorylation of Akt in human prostatic epithelium inhibits the nuclear translocation of the activated Smad2,3,4 complex thus allowing cells to ignore the growth inhibitory effects of TGF- β [37]. CXCR4 is elevated in localized and metastatic cancer and is a marker of poor prognosis [38].

3.2. Breast

Breast cancer is the most common form of cancer and is the second most common cause of cancer death in women [13]. The stroma of the breast is composed of fibroblasts, adipocytes, endothelial cells, pericytes, immune components and nerves. Stromal–epithelial interactions are crucial for proper breast development and breast tumor stroma can alter the proliferation, survival, differentiation, invasion and metastatic ability of cancer cells.

In breast tumors, the normal stromal microenvironment is drastically changed. These changes include increased numbers of fibroblasts, including myofibroblasts, infiltration of inflammatory cells, angiogenesis and ECM remodeling. This is coupled with changes in epithelial cell activities such as proliferation, differentiation, invasion and survival. Many studies have examined the ability of the altered stromal cell types to induce the observed changes in epithelial cell activity. In one study, mammary fibroblasts that are unable to respond to TGF- β were grafted with breast carcinoma cells and implanted into mice. Tumor growth, invasion and metastasis were increased [39,40]. This shows that molecular alterations of tumor stromal cells alone can significantly alter the behavior and progression of tumors. Lethally irradiated fibroblasts or inclusion of fibroblast-conditioned medium in breast cancer cell grafts increased tumorigenicity and tumor growth, showing that soluble secreted products from fibroblasts promote tumors [41]. Of these soluble, secreted factors, CXCL12 and CXCL14 are secreted by myofibroblasts in breast tumors and increase proliferation, invasion and metastasis of breast cancer cells [42,43]. It has been suggested that myofibroblasts in tumors may arise from infiltration of bone marrow-derived mesenchymal stem cells (MSC). Subcutaneous grafts of MSC mixed with breast cancer cells enhanced lung metastasis. Breast cancer cells from these grafts were re-derived from the grafts and subcutaneously grafted again. These grafts did not have the same increased metastatic rate, showing that there are no irreversible changes in the cancer cells and that enhanced metastasis is dependent on the paracrine interaction with MSC. This study went on to show that this activity is dependent on CCL5–CCR5 interactions [44]. The Hedgehog pathway, which is essential for normal mammary gland development, is also essential for maintenance of the CD44⁺/CD24^{low} subpopulation of breast cancer cells that resemble mammary stem cells [45]. Expression of the Hedgehog target gene Gli1 correlates with poor prognosis in breast cancer [46]. In this study, Gli1 expression was primarily noted in cancer cells and correlated with another Hedgehog ligand, Sonic Hedgehog. This suggests that maintenance of a stem cell-like population of breast cancer cells operates on a pathway that is independent of the *Ihh*–Gli2 pathway that controls epithelial and fibroblast pro-

liferation in ductal development. These studies demonstrate the links between normal developmental pathways and those active in cancer. Future studies should focus on the pathways, such as TGF- β , Hedgehog and chemokines, in breast cancer development and progression.

Examination of the molecular changes in stromal cells that control cancer development and progression in the breast have shown that these include alterations in expression of many genes. This includes genes that remodel ECM, alter growth factor and chemokine signaling, stimulate inflammation and increase migration and invasion [47]. Since many of these changes in gene expression are maintained for long periods, including in cell culture, it is possible that these changes are genetically heritable. These may include gene amplification and deletion or microsatellite instability and point mutations [48–51] as well as promoter methylation. However, this concept of heritable changes in CAF is not widely accepted since some studies have shown the absence of such aberrations in breast cancer stroma [52]. Future studies will clarify this and should delineate possible differences in breast cancer sub-types.

3.3. Pancreas

Pancreatic cancer is the most lethal abdominal malignancy. Mortality reaches an alarming 95% of patients within 5 years and on average, patients live only 6 months after diagnosis [53]. One of the defining characteristics of pancreatic ductal adenocarcinoma (PDAC) is the presence of extensive stromal response called desmoplasia. However, the precise role of the stroma in pancreatic cancer is poorly understood. The desmoplastic stroma consists of proliferating fibroblasts and pancreatic stellate cells, inflammatory cells, nerve fibers, and marrow-derived stem cells. The predominant mesenchymal cell within the stroma is the stellate cell, which resembles myofibroblasts commonly found in the stroma of other types of cancer. Stellate cells are rarely found in the normal pancreas but are prevalent during inflammation and in malignant disease. These cells when quiescent, express the intermediate filament protein desmin, and when activated, start expressing smooth muscle actin, produce large amounts of ECM proteins (mostly collagen I and III) and release other inflammatory mediators, including TGF- β [54]. Autocrine production of PDGF, TGF- β , cytokines (IL-1, IL-6, and tumor necrosis factor-related apoptosis-inducing ligand) and COX-2 potentiates the stromal phenotype. Despite the lack of effective *in vivo* systems to evaluate the role of the stromal reaction in pancreatic cancer, some clues have been obtained from xenograft models. Overexpression of TGF- β in Panc-1 tumor cells induced a dramatic response within the stroma in orthotopically transplanted nude mice, similar to that observed in human prostatic ductal adenocarcinoma [55].

KRAS proto-oncogene mutations are present in more than 90% of human pancreatic tumors. However, attempts to model in KRAS mutations in mice gave mixed results. Endogenous mutant expression of KRAS (G12D) alone or in combination with deletion of the tumor suppressor allele CDKN2/*Ink-4a*/Arf can induce the entire progression of pancreatic cancer, from preinvasive (PanIN-1 to PanIN-3) to invasive and metastatic disease. These tumors appear to bear a strong resemblance to human pancreatic cancer with a proliferative stromal component. Finally, one of the most compelling studies that supports a critical role for stroma in pancreatic cancer is the finding that mice with Smad7 deletion in stromal cells develop PanIN accompanied with increased fibrosis around the ductal regions [56]. Based on these observations and the aggressive behavior of this type of cancer more exhaustive research is needed to develop strategies for targeting the stroma of pancreatic cancer.

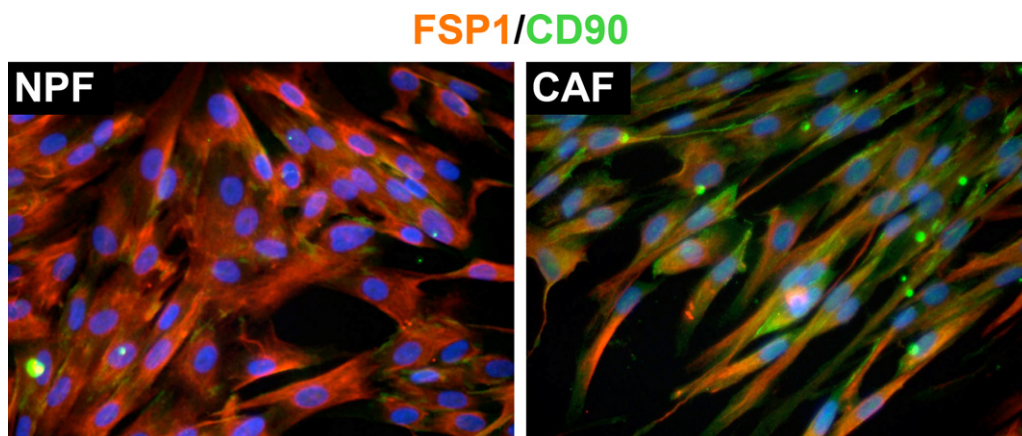


Fig. 2. Immunofluorescence analysis of fibroblast-specific protein 1 (FSP1) and the mesenchymal stem marker CD90. Human normal prostate fibroblast (NPF) and carcinoma-associated fibroblast (CAF) were isolated, double-labeled with antibodies against FSP1 and CD90 and visualized using fluorochrome-conjugated (FITC or TRITC) secondary antibodies. NPF show homogeneous expression of FSP1 and low expression of CD90. However CAF presented not only a heterogeneous expression of FSP1 and CD90 but also high intensity pattern of expression for CD90 positive cells.

4. Heterogeneity of stromal cells

Fibroblasts are highly heterogeneous [57]. *In vivo* the fibroblastic populations surrounding tumors have been shown to contain a number of distinct phenotypes [58]. Clonal modulation of fibroblast subsets is a potential mechanism underlying the pathogenesis of cancer and may play a role in modulating the proliferation of tumor cells. The stroma contains fibroblasts (normal and activated), inflammatory cells, immunocytes, macrophages, lymph and endothelial cells and nerves. Fibroblasts isolated from human cancer patients have been referred as simply carcinoma-associated fibroblasts. However, CAF remains a poorly defined group of cells commonly identified using biological assays. CAF have classically been defined by the absence of epithelial cell marker cytokeratins, and the expression of vimentin and α SMA. However, a recent study that used the vimentin, α SMA, type I collagen, FSP1 (S100A4), PDGFR β and NG2 markers to examine fibroblasts heterogeneity within breast and pancreatic carcinomas indicated that distinct fibroblasts subpopulations could be identified and quantified within each tumor microenvironment [58]. Of these markers, co-expression of α SMA and FAP were characteristic of myofibroblasts. FSP1 could identify fibroblasts, and in combination with the other markers form a picture of what constitutes the CAF populations. It still remains to be determined whether the presence of any particular population or combination of populations within the stroma is essential for tumor progression. Some clues have come from a recent work in which mammary carcinoma cells in combination with FSP1+/+ mouse embryonic fibroblasts were able to promote progression to metastasis in FSP1-/- mice [59]. Currently it is not known how many fibroblast subpopulations are present within the tumor stroma, and whether the presence of an individual population or the presence of all of these cell types are equally important for the initial steps during tumor development.

Another potentially controversial area of CAF research is focused on the origin of CAF. While local fibroblasts were originally considered to be the most likely major source of CAF, recent studies have shown that there are other additional sources depending on the type of cancer. For example bone marrow-derived MSC can contribute up to 25% of the total myofibroblast population in the tumor stroma in a mouse xenograft model of pancreatic carcinoma [60]. Once recruited, MSC become activated and start secreting matrix proteins helping cancer cells to metastasize. In a similar study, MSC had the ability to increase the metastatic potential of weakly metastatic breast cancer cells by upregulation of the chemokine CCL5 [44]. It has been shown recently that fibroblasts

isolated from human prostate cancer samples that express high levels of the MSC marker CD90 (CD90^{hi}) may have tumor-promoting potential compared to a subpopulation of low CD90 expressors (CD90^{lo}) [61] (Fig. 2). On the other hand MSC have been shown to inhibit tumor growth in a model of Kaposi's sarcoma [62]. Due to their ability to localize in tumor sites, genetically modified MSC carrying the human interferon β gene were injected in tumor bearing mice. These cells were able to decrease not only the size of the primary tumor, but also the number of metastasis compared to control animals [63]. A better understanding of the interplay between MSC and tumor cells in relation to the tumor microenvironment will be important in developing strategies for therapy. It has also been suggested that malignant epithelial cells undergoing EMT can integrate within the tumor stroma and contribute to the CAF population. While this is a plausible hypothesis definitive *in vivo* experiments and clinical correlates are not presently available. Studies on genetic alterations in tumor stroma described above have the potential to shed some light in this area.

Recently, studies have demonstrated that a phenomena called endothelial-to-mesenchymal transition occurs in areas of fibrosis and can account for up to 40% of the tumor-associated fibroblasts [64]. The molecular mechanism of EnMT in tumors has not yet been specifically studied, but it has been suggested to be mediated by TGF- β and inhibited by BMP-7 [65]. EnMT may play a role in the genesis of vascular networks composed of "tip cells" (vessels without a lumen) an important mechanism for stabilizing the neovasculature. Clearly, more research is warranted in this area to: (a) identify and characterize the biological relevant subpopulation(s), (b) identify the factors responsible for the activation/maintenance/recruitment of CAF and (c) investigate how to modulate them to possibly normalize the stroma by generation of therapeutic agents.

5. Summary and future opportunities

The current trend in cancer research is the inclusion of the microenvironment as a major contributor of cancer progression. The tumor-inducing actions of CAF start during the conversion of stressed normal epithelial cells to cancer cells and continue through progression to metastasis.

The demonstrated tumor-promoting capacity of CAF has increased interest in exploiting them as therapeutic targets for anticancer therapy. Inhibiting the secretion of tumor-promoting factors, or neutralization of these factors seems to be the first line approach. Several molecular candidates have been proposed

in this article. However a better understanding of the effects of targeting these pathways is needed. Another potential therapeutic approach is the interference with the recruitment or activation of CAF. Promising results in experimental models have been obtained when targeting growth factors such as PDGF, TGF- β , chemokines like CXCL12 and inhibitors to the Hedgehog signaling. Similar results have been documented in immunotherapy approaches when using FAP antibodies in animal models. Together, these studies emphasize the importance of cross-talk between stromal and malignant cells of the tumor. It is likely that the continued characterization of these interactions, and the molecular identification of key mediators, will provide insights into tumor biology and suggest further novel therapeutic options.

Acknowledgments

The authors acknowledge funding from the National Cancer Institute via grants U54 CA113007 and U54 CA126505. We also thank the Frances Williams Preston Laboratories of the TJ Martell Foundation for support. The content of this paper is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References

- [1] Ayala G, Tuxhorn JA, Wheeler TM, Frolov A, Scardino PT, Ohori M, et al. Reactive stroma as a predictor of biochemical-free recurrence in prostate cancer. *Clin Cancer Res* 2003;9(13):4792–801.
- [2] Kolf CM, Cho E, Tuan RS. Mesenchymal stromal cells. Biology of adult mesenchymal stem cells: regulation of niche, self-renewal and differentiation. *Arthritis Res Ther* 2007;9(1):204.
- [3] Tuxhorn JA, McAlhany SJ, Dang TD, Ayala GE, Rowley DR. Stromal cells promote angiogenesis and growth of human prostate tumors in a differential reactive stroma (DRS) xenograft model. *Cancer Res* 2002;62(11):3298–307.
- [4] Kunz-Schughart LA, Knuechel R. Tumor-associated fibroblasts. Part I. Active stromal participants in tumor development and progression? *Histol Histopathol* 2002;17(2):599–621.
- [5] Tuxhorn JA, Ayala GE, Rowley DR. Reactive stroma in prostate cancer progression. *J Urol* 2001;166(6):2472–83.
- [6] Tuxhorn JA, McAlhany SJ, Yang F, Dang TD, Rowley DR. Inhibition of transforming growth factor-beta activity decreases angiogenesis in a human prostate cancer-reactive stroma xenograft model. *Cancer Res* 2002;62(21):6021–5.
- [7] Yanagisawa N, Li R, Rowley D, Liu H, Kadmon D, Miles BJ, et al. Stromogenic prostatic carcinoma pattern (carcinomas with reactive stromal grade 3) in needle biopsies predicts biochemical recurrence-free survival in patients after radical prostatectomy. *Hum Pathol* 2007;38(11):1611–20.
- [8] Midwood KS, Williams LV, Schwarzbauer JE. Tissue repair and the dynamics of the extracellular matrix. *Int J Biochem Cell Biol* 2004;36(6):1031–7.
- [9] Dvorak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. *N Engl J Med* 1986;315(26):1650–9.
- [10] De Wever O, Demetter P, Mareel M, Bracke M. Stromal myofibroblasts are drivers of invasive cancer growth. *Int J Cancer* 2008;123(10):2229–38.
- [11] Folkman J, Kalluri R. Cancer without disease. *Nature* 2004;427(6977):787.
- [12] Weaver VM, Gilbert P. Watch thy neighbor: cancer is a communal affair. *J Cell Sci* 2004;117(Pt 8):1287–90.
- [13] Jemal A, Siegel R, Ward E, Hao Y, Xu J, Murray T, et al. Cancer statistics. *CA Cancer J Clin* 2008;58(2):71–96.
- [14] Olumi AF, Grossfeld GD, Hayward SW, Carroll PR, Tlsty TD, Cunha GR. Carcinoma-associated fibroblasts direct tumor progression of initiated human prostatic epithelium. *Cancer Res* 1999;59(19):5002–11.
- [15] Hayward SW, Wang Y, Cao M, Hom YK, Zhang B, Grossfeld GD, et al. Malignant transformation in a nontumorigenic human prostatic epithelial cell line. *Cancer Res* 2001;61(22):8135–42.
- [16] Hill R, Song Y, Cardiff RD, Van Dyke T. Selective evolution of stromal mesenchyme with p53 loss in response to epithelial tumorigenesis. *Cell* 2005;123(6):1001–11.
- [17] Macintosh CA, Stower M, Reid N, Maitland NJ. Precise microdissection of human prostate cancers reveals genotypic heterogeneity. *Cancer Res* 1998;58(1):23–8.
- [18] Grover AC, Tangrea MA, Woodson KG, Wallis BS, Hanson JC, Chuquai RF, et al. Tumor-associated endothelial cells display GSTP1 and RARbeta2 promoter methylation in human prostate cancer. *J Transl Med* 2006;4:13.
- [19] Krop I, Player A, Tablante A, Taylor-Parker M, Lahti-Domenici J, Fukuoka J, et al. Frequent HIN-1 promoter methylation and lack of expression in multiple human tumor types. *Mol Cancer Res* 2004;2(9):489–94.
- [20] Heinlein CA, Chang C. Androgen receptor in prostate cancer. *Endocr Rev* 2004;25(2):276–308.
- [21] Ricciardelli C, Choong CS, Buchanan G, Vivekanandan S, Neufing P, Stahl J, et al. Androgen receptor levels in prostate cancer epithelial and peritumoral stromal cells identify non-organ confined disease. *Prostate* 2005;63(1):19–28.
- [22] Henshall SM, Quinn DI, Lee CS, Head DR, Golovsky D, Brenner PC, et al. Altered expression of androgen receptor in the malignant epithelium and adjacent stroma is associated with early relapse in prostate cancer. *Cancer Res* 2001;61(2):423–7.
- [23] Wikstrom P, Marusic J, Stattin P, Bergh A. Low stroma androgen receptor level in normal and tumor prostate tissue is related to poor outcome in prostate cancer patients. *Prostate* 2009;69(8):799–809.
- [24] Chung LW, Baseman A, Assikis V, Zhou HE. Molecular insights into prostate cancer progression: the missing link of tumor microenvironment. *J Urol* 2005;173(1):10–20.
- [25] Bhowmick NA, Chytil A, Plieth D, Gorska AE, Dumont N, Shappell S, et al. TGF-beta signaling in fibroblasts modulates the oncogenic potential of adjacent epithelia. *Science* 2004;303(5659):848–51.
- [26] Danielpour D. Functions and regulation of transforming growth factor-beta (TGF-beta) in the prostate. *Eur J Cancer* 2005;41(6):846–57.
- [27] Li X, Placencio V, Iturregui JM, Uwamariya C, Sharif-Afshar AR, Koyama T, et al. Prostate tumor progression is mediated by a paracrine TGF-beta/Wnt3a signaling axis. *Oncogene* 2008;27(56):7118–30.
- [28] Lu T, Burdelya LG, Swiatkowski SM, Boiko AD, Howe PH, Stark GR, et al. Secreted transforming growth factor beta2 activates NF-kappaB, blocks apoptosis, and is essential for the survival of some tumor cells. *Proc Natl Acad Sci USA* 2004;101(18):7112–7.
- [29] Saikali Z, Setya H, Singh G, Persad S. Role of IGF-1/IGF-1R in regulation of invasion in DU145 prostate cancer cells. *Cancer Cell Int* 2008;8:10.
- [30] Wu JD, Haugk K, Woodke L, Nelson P, Coleman I, Plymate SR. Interaction of IGF signaling and the androgen receptor in prostate cancer progression. *J Cell Biochem* 2006;99(2):392–401.
- [31] Knudsen BS, Edlund M. Prostate cancer and the met hepatocyte growth factor receptor. *Adv Cancer Res* 2004;91:31–67.
- [32] Verras M, Lee J, Xue H, Li TH, Wang Y, Sun Z. The androgen receptor negatively regulates the expression of c-Met: implications for a novel mechanism of prostate cancer progression. *Cancer Res* 2007;67(3):967–75.
- [33] Ye L, Lewis-Russell JM, Sanders AJ, Kynaston H, Jiang WG. HGF/SF up-regulates the expression of bone morphogenetic protein 7 in prostate cancer cells. *Urol Oncol* 2008;26(2):190–7.
- [34] Maeda A, Nakashiro K, Hara S, Sasaki T, Miwa Y, Tanji N, et al. Inactivation of AR activates HGF/c-Met system in human prostatic carcinoma cells. *Biochem Biophys Res Commun* 2006;347(4):1158–65.
- [35] Vindrieux D, Escobar P, Lazennec G. Emerging roles of chemokines in prostate cancer. *Endocr Relat Cancer* 2009;16(3):663–73.
- [36] Ao M, Franco OE, Park D, Raman D, Williams K, Hayward SW. Cross-talk between paracrine-acting cytokine and chemokine pathways promotes malignancy in benign human prostatic epithelium. *Cancer Res* 2007;67(9):4244–53.
- [37] Ao M, Williams K, Bhowmick NA, Hayward SW. Transforming growth factor-beta promotes invasion in tumorigenic but not in nontumorigenic human prostatic epithelial cells. *Cancer Res* 2006;66(16):8007–16.
- [38] Akashi T, Koizumi K, Tsuneyama K, Saiki I, Takano Y, Fuse H. Chemokine receptor CXCR4 expression and prognosis in patients with metastatic prostate cancer. *Cancer Sci* 2008;99(3):539–42.
- [39] Cheng N, Bhowmick NA, Chytil A, Gorska AE, Brown KA, Muraoka R, et al. Loss of TGF-beta type II receptor in fibroblasts promotes mammary carcinoma growth and invasion through upregulation of TGF-alpha, MSP- and HGF-mediated signaling networks. *Oncogene* 2005;24(32):5053–68.
- [40] Cheng N, Chytil A, Shyr Y, Joly A, Moses HL. Enhanced hepatocyte growth factor signaling by type II transforming growth factor-beta receptor knockout fibroblasts promotes mammary tumorigenesis. *Cancer Res* 2007;67(10):4869–77.
- [41] Noel A, De Pauw-Gillet MC, Purnell G, Nussgens B, Lapiere CM, Foidart JM. Enhancement of tumorigenicity of human breast adenocarcinoma cells in nude mice by matrigel and fibroblasts. *Br J Cancer* 1993;68(5):909–15.
- [42] Orimo A, Gupta PB, Sgroi DC, Arenzana-Seisdedos F, Delaunay T, Naeem R, et al. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. *Cell* 2005;121(3):335–48.
- [43] Muller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, et al. Involvement of chemokine receptors in breast cancer metastasis. *Nature* 2001;410(6824):50–6.
- [44] Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW, et al. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. *Nature* 2007;449(7162):557–63.
- [45] Tanaka H, Nakamura M, Kameda C, Kubo M, Sato N, Kuroki S, et al. The Hedgehog signaling pathway plays an essential role in maintaining the CD44⁺CD24⁺/low subpopulation and the side population of breast cancer cells. *Anticancer Res* 2009;29(6):2147–57.
- [46] ten Haaf A, Bektas N, von Serenyi S, Losen I, Arweiler EC, Hartmann A, et al. Expression of the glioma-associated oncogene homolog (GLI) 1 in human breast cancer is associated with unfavourable overall survival. *BMC Cancer* 2009;9:298.
- [47] Sadlonova A, Bowe DB, Novak Z, Mukherjee S, Duncan VE, Page GP, et al. Identification of molecular distinctions between normal breast-associated fibroblasts and breast cancer-associated fibroblasts. *Cancer Microenviron* 2009. doi:10.1007/s12307-008-0017-0. <http://www.springerlink.com/content/313502j7g0282502>.

- [48] Allinen M, Beroukhi R, Cai L, Brennan C, Lahti-Domenici J, Huang H, et al. Molecular characterization of the tumor microenvironment in breast cancer. *Cancer Cell* 2004;6(1):17–32.
- [49] Kurose K, Gilley K, Matsumoto S, Watson PH, Zhou XP, Eng C. Frequent somatic mutations in PTEN and TP53 are mutually exclusive in the stroma of breast carcinomas. *Nat Genet* 2002;32(3):355–7.
- [50] Moinfar F, Man YG, Arnould L, Bratthauer GL, Ratschek M, Tavassoli FA. Concurrent and independent genetic alterations in the stromal and epithelial cells of mammary carcinoma: implications for tumorigenesis. *Cancer Res* 2000;60(9):2562–6.
- [51] Fukino K, Shen L, Matsumoto S, Morrison CD, Mutter GL, Eng C. Combined total genome loss of heterozygosity scan of breast cancer stroma and epithelium reveals multiplicity of stromal targets. *Cancer Res* 2004;64(20):7231–6.
- [52] Qiu W, Hu M, Sridhar A, Opeskin K, Fox S, Shipitsin M, et al. No evidence of clonal somatic genetic alterations in cancer-associated fibroblasts from human breast and ovarian carcinomas. *Nat Genet* 2008;40(5):650–5.
- [53] Lillemoe KD, Yeo CJ, Cameron JL. Pancreatic cancer: state-of-the-art care. *CA Cancer J Clin* 2000;50(4):241–68.
- [54] Aoki H, Ohnishi H, Hama K, Shinozaki S, Kita H, Yamamoto H, et al. Existence of autocrine loop between interleukin-6 and transforming growth factor-beta1 in activated rat pancreatic stellate cells. *J Cell Biochem* 2006;99(1):221–8.
- [55] Lohr M, Schmidt C, Ringel J, Kluth M, Muller P, Nizze H, et al. Transforming growth factor-beta1 induces desmoplasia in an experimental model of human pancreatic carcinoma. *Cancer Res* 2001;61(2):550–5.
- [56] Kuang C, Xiao Y, Liu X, Stringfield TM, Zhang S, Wang Z, et al. In vivo disruption of TGF-beta signaling by Smad7 leads to premalignant ductal lesions in the pancreas. *Proc Natl Acad Sci USA* 2006;103(6):1858–63.
- [57] Schor SL, Schor AM. Clonal heterogeneity in fibroblast phenotype: implications for the control of epithelial–mesenchymal interactions. *Bioessays* 1987;7(5):200–4.
- [58] Sugimoto H, Mundel TM, Kieran MW, Kalluri R. Identification of fibroblast heterogeneity in the tumor microenvironment. *Cancer Biol Ther* 2006;5(12):1640–6.
- [59] Grum-Schwensen B, Klingelhofer J, Berg CH, El-Naaman C, Grigorian M, Lukanidin E, et al. Suppression of tumor development and metastasis formation in mice lacking the S100A4(mts1) gene. *Cancer Res* 2005;65(9):3772–80.
- [60] Ishii G, Sangai T, Oda T, Aoyagi Y, Hasebe T, Kanomata N, et al. Bone-marrow-derived myofibroblasts contribute to the cancer-induced stromal reaction. *Biochem Biophys Res Commun* 2003;309(1):232–40.
- [61] Zhao HJ, Peehl DM. Tumor-promoting phenotype of CD90(hi) prostate cancer-associated fibroblasts. *Prostate* 2009;69(9):991–1000.
- [62] Khakoo AY, Pati S, Anderson SA, Reid W, Elshal MF, Rovira II, et al. Human mesenchymal stem cells exert potent antitumorigenic effects in a model of Kaposi's sarcoma. *J Exp Med* 2006;203(5):1235–47.
- [63] Studeny M, Marini FC, Dembinski JL, Zompetta C, Cabreira-Hansen M, Bekele BN, et al. Mesenchymal stem cells: potential precursors for tumor stroma and targeted-delivery vehicles for anticancer agents. *J Natl Cancer Inst* 2004;96(21):1593–603.
- [64] Zeisberg EM, Potenta S, Xie L, Zeisberg M, Kalluri R. Discovery of endothelial to mesenchymal transition as a source for carcinoma-associated fibroblasts. *Cancer Res* 2007;67(21):10123–8.
- [65] Zeisberg EM, Tarnavski O, Zeisberg M, Dorfman AL, McMullen JR, Gustafsson E, et al. Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. *Nat Med* 2007;13(8):952–61.