AWARD NUMBER: W81XWH-16-1-0190

TITLE: Untapped Therapeutic Targets in the Tumor Microenvironment

PRINCIPAL INVESTIGATOR: Sandra Orsulic, PhD

CONTRACTING ORGANIZATION: Cedars-Sinai Medical Center
8700 Beverly Boulevard
Los Angeles, CA 90048

REPORT DATE: 08/31/2018

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	TYPE OF REPORT	**3. DATES COVERED**
08-31-2018	ANNUAL	08-01-2017 – 07-31-2018

4. TITLE AND SUBTITLE
Untapped Therapeutic Targets in the Tumor Microenvironment

5a. CONTRACT NUMBER
W81XWH-16-1-0190

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Sandra Orsulic

E-Mail: orsulics@cshs.org

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Cedars-Sinai Medical Center
8700 Beverly Boulevard
Los Angeles, CA 90048

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
Most therapeutic approaches have focused on the tumor cell and its genetic alterations. However, it is becoming clear that the microenvironment plays an important role in tumor evolution. We hypothesized that conventional chemotherapy for ovarian cancer will be more effective if the microenvironment that harbors the resistant cancer cells is simultaneously targeted. Since activated carcinoma-associated fibroblasts (CAFs) have a prominent role in most aspects of tumor progression, including responses to anticancer agents by forming a physical barrier that prevents chemotherapy access and promotes resistance, we predicted that targeting CAFs would inhibit tumor progression and/or increase chemotherapeutic efficacy. Using three different approaches to targeting CAFs in an immunocompetent mouse model of ovarian cancer that was developed in our laboratory, we failed to show any significant benefits of targeting CAFs. We realized that we needed to develop a more fibrotic model of ovarian cancer to demonstrate the efficacy of anti-fibrotic agents. During this funding period, we generated two new mouse models, both of which exhibit extensive fibrosis and rapid onset of ovarian carcinomatosis. We are currently testing anti-fibrotic agents in these improved mouse models. We also made significant advances in characterizing the functional properties of COL11A1, which we previously identified as a molecular target that distinguishes CAFs from other fibroblasts.

15. SUBJECT TERMS
Ovarian cancer, tumor microenvironment, tumor progression, cancer-associated fibroblasts

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
Unclassified	Unclassified	Unclassified	Unclassified	14	**19b. TELEPHONE NUMBER** *(include area code)*

Table of Contents

Page

1. **Introduction…… 1**
2. **Keywords…… …… 1**
3. **Accomplishments…… 1**

Major goals of the project…… 1

Major accomplishments…… 4

Major activities…… 4

Specific objectives…… 4

Significant findings or key outcomes…… 4

Other achievements…… 10

4. **Impact…… 11**
5. **Changes/problems…….. 12**
6. **Products…… 12**
7. **Participants…….. 13**
8. **Special reporting requirements…… 14**
9. **Appendix…….. N/A**

1. INTRODUCTION:

High grade serous ovarian carcinoma is among the most lethal cancers affecting women in the U.S. While most therapeutic approaches have focused on malignant epithelial tumor cells and their genetic alterations, it is becoming increasingly clear that the tumor microenvironment plays an equally important role in tumor evolution. The presence of cancer cells induces a reaction in the surrounding stromal cells similar to fibrosis after an injury. These reactions can also reduce therapeutic efficacy of chemotherapy by creating a physical barrier for drug transport while providing a protective environment for cancer cells to repopulate after completion of treatment. Thus, it is thought that anti-cancer therapies should target not only malignant cancer cells but also the microenvironment that fosters tumor growth and survival. Our goal is to demonstrate that targeting processes responsible for the formation of carcinoma associated fibroblasts (also known as CAFs) in the tumor microenvironment will effectively attenuate tumor growth, improve intratumoral drug delivery and restore anti-tumor immune responses. We are using three different approaches to targeting CAFs. The first approach is to test anti-fibrotic agents for their efficacy in preventing CAF activation and increasing sensitivity to chemotherapy in a mouse model of ovarian cancer that was developed in our laboratory. The second approach is to increase the precision of targeting activated CAFs, by targeting a protein that we previously identified to be present in activated CAFs but absent from fibroblasts associated with non-cancerous conditions such as fibrosis, inflammation, and wound healing. The third approach is test several agents for their efficacy in inducing CAF-to-cartilage differentiation with the idea that a terminally-differentiated microenvironment cannot protect malignant cells from chemotherapy or foster their dormancy for future recurrence.

2. KEYWORDS:

Ovarian cancer, tumor microenvironment, cancer-associated fibroblasts, fibrosis, targeted therapy, clinical outcome

3. ACCOMPLISHMENTS:

- **What were the major goals of the project?**

Specific Aim 1 (specified in proposal)	Timeline	Percent Completed
Major Task 1 Test the therapeutic efficacy of CTGF, CTSK, FN1, and LOXL2 inhibitors	**Months**	**Cedars-Sinai Medical Center**
Subtask 1 Amend approved IACUC protocol 5318 (Mouse Models of Tumor Microenvironment, PI: Orsulic) for local approval and send related material for DoD's approval.	Upon award notice	100%
Subtask 2 Purchase FVB mice, drugs, and reagents; plan experiments.	1-2	70%
Subtask 3 Implant FVB mice with mouse ovarian cancer cells.	2-25	25%

Subtask 4 Treat mice with CTGF, CTSK, FN1, and LOXL2 inhibitors Assess therapeutic efficacy: 1. Tumor growth: tumor weight/volume, luciferase whole-animal imaging. 2. Tumor invasion and metastasis: dissection and immunohistochemistry. 3. Stromal differentiation: Masson's trichrome stain, qPCR and immunostaining for myofibroblast markers (α-SMA, fibronectin, COL11A1). 4. Chemotherapy diffusion: quantification of fluorescently-labeled dextran beads. 5. Tumor-infiltrating immune cells: flow cytometric analyses with antibody cocktail (CD3, CD4, CD8a, CD44, CD62L, CD25, Nkp46, F4/80, CD11b, Gr1, Ly6G, CD11c, and FoxP3). 6. Cancer stem cell content: flow cytometric analyses with CD133, CD44, CD24, and CD117. 7. Angiogenesis: CD31 and CD34. 8. Apoptosis, DNA damage: ApopTag, CC3 positivity, PARP cleavage, or histone H2AX phosphorylation. 9. Toxicity: histological analysis of liver, lung, and kidney injury 10. TGFβ signaling: immunodetection of phosphorylated Smad2/3.	3-30	10%
Subtask 5 Analyze data using statistical methods; replicate experiments if necessary, prepare and submit manuscripts.	3-36	5%
Milestone Achieved Verified therapeutic efficacy of CTGF, CTSK, FN1, and LOXL2 inhibitors.	32	5%
Specific Aim 2 (specified in proposal)	**Timeline**	**Site 1**
Major Task 1 **Determine the effect of COL11A1 knockdown in human carcinoma-associated fibroblasts**	**Months**	**Cedars-Sinai Medical Center**
Subtask 1 Amend approved IACUC protocol 5318 (Mouse Models of Tumor Microenvironment, PI: Orsulic) for local approval and send related material for DoD's approval.	Upon award notice	100%
Subtask 2 Knock out COL11A1 in human carcinoma-associated fibroblasts using CRISPR.	1-3	10%
Subtask 3 Co-culture COL11A1 knockout carcinoma-associated fibroblasts with	3-12	10%

ovarian cancer cells under kidney capsule of nude mice; measure cell proliferation, cell death and other parameters.		
Subtask 4 Analyze data using statistical methods; replicate experiments if necessary.	12-18	10%
Milestone Achieved Verified whether COL11A1 in carcinoma-associated fibroblasts is essential for the tumor promoting effects in a paracrine manner.	18	10%
Major Task 2 **Determine the potential of COL11A1 as a therapeutic target**	**Months**	**Cedars-Sinai Medical Center**
Subtask 1 Amend approved IACUC protocol 5318 (Mouse Models of Tumor Microenvironment, PI: Orsulic) for local approval and send related material for DoD's approval.	Upon award notice	100%
Subtask 2 Purchase FVB mice, drugs and reagents; plan experiments.	1-2	50%
Subtask 3 Implant FVB mice with mouse ovarian cancer cells.	2-25	50%
Subtask 4 Treat mice with COL11A1 neutralizing antibody. Assess therapeutic efficacy as in Aim 1, Task 4.	3-30	50%
Subtask 5 Analyze data using statistical methods; replicate experiments if necessary; prepare and submit manuscripts.	3-36	10%
Milestone Achieved Verified whether COL11A1 is promising as a therapeutic target with high specificity for activated carcinoma-associated fibroblasts.	36	10%
Specific Aim 3 (specified in proposal)	**Timeline**	**Site 1**
Major Task 1 **Assess the effect of differentiating carcinoma-associated fibroblasts into cartilage on tumor progression and chemosensitivity**	**Months**	**Cedars-Sinai Medical Center**
Subtask 1 Amend approved IACUC protocol 5318 (Mouse Models of Tumor Microenvironment, PI: Orsulic) for local approval and send related material for DoD's approval.	Upon award notice	100%
Subtask 2 Purchase FVB mice, drugs and reagents; plan experiments.	1-2	30%
Subtask 3 Implant FVB mice with mouse ovarian cancer cells.	2-25	30%

Subtask 4 Treat mice with recombinant collagen II, rAAV-FLAG-Sox9, and dexamethasone. Assess therapeutic efficacy as in Aim 1, Task 4.	3-30	30%
Subtask 5 Analyze data using statistical methods; replicate experiments if necessary; prepare and submit manuscripts.	4-36	10%
Milestone Achieved Verified whether agents that induce terminal differentiation of activated carcinoma-associated fibroblasts are effective in attenuating tumor growth and increasing chemosensitivity.	36	10%

- **What was accomplished under these goals?**

1) major activities

Using three different approaches to targeting CAFs in an immunocompetent mouse model of ovarian cancer that was developed in our laboratory, we failed to show any significant benefits of targeting CAFs. We realized that we needed to develop a more fibrotic model of ovarian cancer to demonstrate the efficacy of anti-fibrotic agents. During this funding period, we generated two new mouse models, both of which exhibit extensive fibrosis and rapid onset of ovarian carcinomatosis. We are currently testing anti-fibrotic agents in these improved mouse models. We also made significant advances in characterizing the functional properties of COL11A1, which we previously identified as a molecular target that distinguishes CAFs from other fibroblasts.

2) specific objectives

Our objectives were to: 1) generate suitable mouse ovarian cancer models for testing the efficacy of anti-fibrotic agents in improving ovarian cancer chemosensitivity to cisplatin; 2) increase the specificity of targeting activated CAFs by targeting the CAF-specific protein COL11A1; and 3) identify a method to induce bone/cartilage differentiation of CAFs.

3) significant results or key outcomes, including major findings, developments, or conclusions

AIM 1. Improve therapeutic efficacy by targeting processes involved in CAF activation

CAFs are the most prominent component of the tumor stroma in advanced ovarian cancer. However, it is still not completely understood how the presence of CAFs specifically contribute to tumor progression and therapeutic resistance in ovarian cancer (1). Studies in other solid tumors have shown that CAFs can promote tumor growth, angiogenesis, invasion, and metastasis while at the same time suppressing antitumor immunity and conferring drug resistance and/or limiting access of chemotherapeutics, anti-angiogenic therapies, and immunotherapies. Experimental mouse models that exhibit extensive cancer fibrosis, such as the K-ras^{G12D} mutation-driven autochthonous pancreatic cancer model and the xenograft 4T1 breast cancer model, have been crucial in proving that CAF-targeting therapeutic approaches can improve

tumoral immune response, intratumoral drug delivery, and therapeutic efficacy (2-12). These studies confirmed the key role of CAFs in cancer progression and demonstrated their effectiveness as a therapeutic target. However, similar studies in ovarian cancer models have not been successful due to the lack of extensive fibrosis in current ovarian cancer models. In order to test the efficacy of anti-fibrotic agents in improving chemotherapeutic efficacy in ovarian cancer, we generated two new mouse models that exhibit increased fibrosis. These models will be a valuable resource for studying the role of fibroblasts in ovarian cancer initiation and progression.

In the first model, we used tight skin (TSK) mice in which fibroblasts are permanently activated due to overexpression of fibrillin 1 (FBN1) (13, 14). The TSK mouse model has been used extensively to study fibrosis and ECM remodeling (15-18) but has not been used to study cancer progression. In comparison to the wild type (WT) littermates, TSK mice exhibited faster ovarian cancer progression when mouse ovarian cancer cells were injected intraperitoneally (data not shown) or subcutaneously (**Fig. 1**), indicating that fibroblast activation may contribute to ovarian cancer progression in this model.

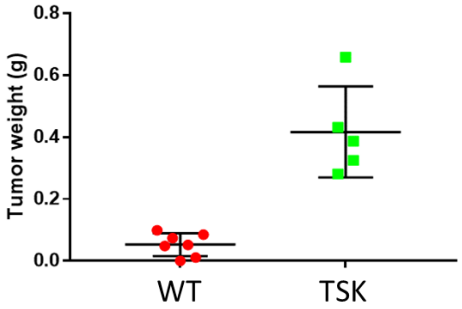

Fig. 1. Increased tumor growth in tight skin (TSK) mice. Wet weight of isolated tumors 3 weeks after injection of cancer cells into mammary fat pads of TSK mice and their WT littermates.

In the second model, we injected FVB mice with 2 ml PBS (control group) or 2 ml PBS with 0.05% sodium hypochlorite (NaOCl, also known as bleach) seven days prior to intraperitoneal injection of 10^6 BR-luc mouse ovarian cancer cells. Seven days after cancer cell injection, IVIS imaging showed a marked difference in luciferase signal intensity in the two groups (**Fig. 2A**), indicating that cancer cells in the NaOCl-pretreated mice were more efficient in surviving and/or proliferating than cancer cells in the PBS-pretreated mice. Twenty days after cancer cell injection, mice were euthanized for pathologic analysis. PBS-pretreated mice exhibited small tumor nodules confined to the omentum while NaOCl-pretreated mice exhibited cancer cell deposits on multiple intraperitoneal organs, including the peritoneal wall (**Fig. 2B**). We are currently studying the molecular mechanisms underlying the rapid development of carcinomatosis in this model. Masson's trichrome staining analysis revealed that the intraperitoneal mesothelium, which consisted of a single layer of cells in the PBS-pretreated mice, was multilayered in the NaOCl-pretreated mice (**Fig. 2C**). Cancer cells were frequently seeded on top of the multilayered fibrotic mesothelium (**Fig. 2C**). In some instances, mesenchymal cells resembling fibrocytes (mesenchymal cells of bone marrow origin) were located between the fibrotic mesothelium and the cancer cells (**Fig. 2C**). We are currently using the NaOCl mouse model of ovarian cancer to test the efficacy of the FDA-approved anti-fibrotic drug, Pirfenidone, in reducing fibrosis and reducing metastatic progression (data not shown).

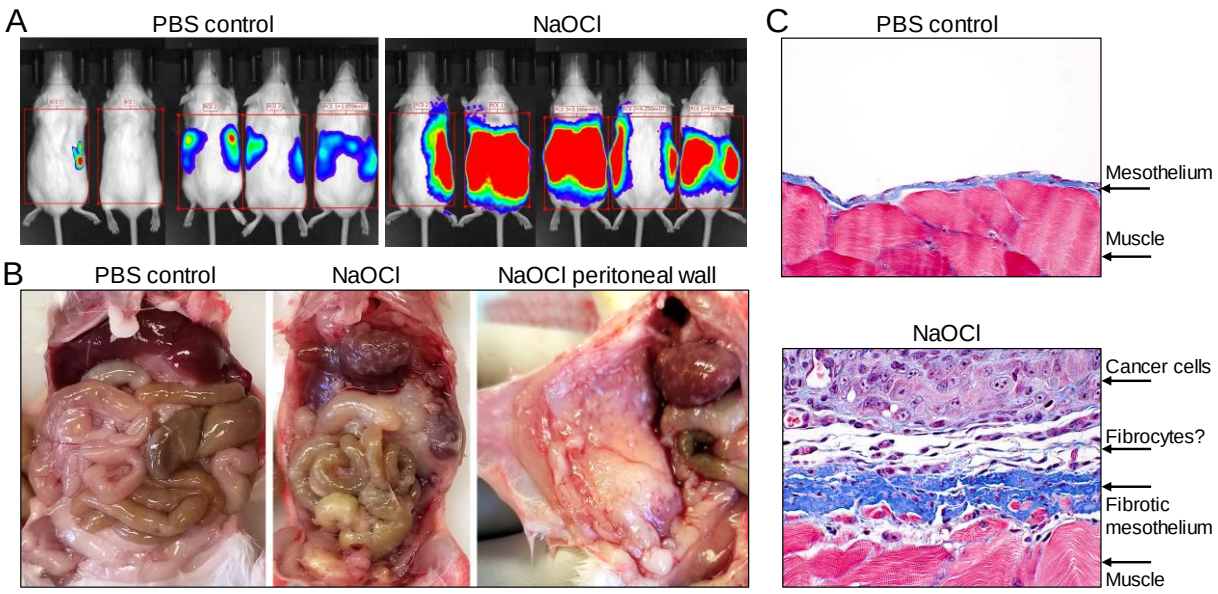

Fig. 2. A mouse model for rapid and aggressive ovarian carcinomatosis. (A) Intravital luciferase imaging of PBS-pretreated mice and NaOCl-pretreated mice 7 days after intraperitoneal injection of 10^6 BR-luc cells. **(B)** Representative images of tumor spread after euthanasia. PBS-pretreated mice exhibit small tumor nodules confined to the omentum. NaOCl-pretreated mice exhibit widespread carcinomatosis on multiple intraperitoneal organs and the peritoneal wall. **(C)** Representative Masson's trichrome-stained images of the peritoneal wall mesothelium from mice shown in (B). In the PBS-pretreated mice, the mesothelium is multilayered and frequently covered with cancer cells. In some instances, loose connective tissue that resembles fibrocytes is located between the fibrotic mesothelium and cancer cells.

AIM 2. Increase specificity of targeting activated CAFs

Our analyses identified COL11A1 as the most specific target for activated CAFs. We hypothesized that targeting COL11A1 function will disable activated CAFs with a minimal effect on normal fibroblasts. We used two approaches to inactivate COL11A1.

Approach 1: Knockout COL11A1 in human CAFs. We characterized the expression of COL11A1 mRNA in two types of hTERT-immortalized cell lines: normal ovary-associated fibroblasts (INOF; hereafter referred to as NAF) and ovarian cancer-associated fibroblasts (781T; hereafter referred to as CAF). COL11A1 levels were negligible in NAF in comparison to CAF (**Fig. 3A**), which is consistent with our published *in situ* hybridization results showing COL11A1 expression in fibroblasts adjacent to cancer cells but not in fibroblasts distant to cancer cells or in the normal ovary (Jia et al. *Cancer Letters* 2016). However, we also observed that COL11A1 levels diminish upon passaging CAF, suggesting that CAF return to the NAF state if not in the presence of cancer cells (**Fig. 3A**). We also showed that COL11A1 expression can be induced in NAF by co-culturing NAF with ovarian cancer cell lines; this effect was more prominent when CAF and cancer cells were directly mixed rather than separated with a transwell membrane (**Fig. 3B**).

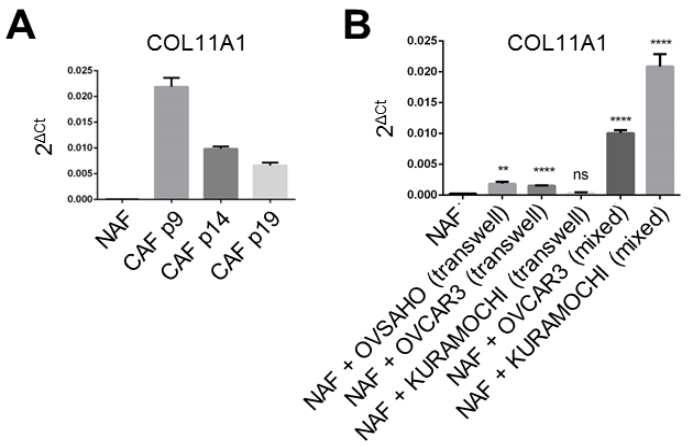

Fig. 3. COL11A1 expression in fibroblasts is induced and maintained by cancer cells.
(A) qPCR analysis of COL11A1 mRNA in normal ovary-associated fibroblasts (NAF) and in different passages of cancer-associated fibroblasts (CAF). **(B)** qPCR analysis of COL11A1 mRNA in NAF cultured alone or co-cultured with different ovarian cancer cell lines through indirect (transwell membrane) or direct contact.

To determine if fibroblasts support the growth of ovarian cancer cells in vivo, we subcutaneously injected the human ovarian cancer cell line SKOV3 alone or in combination with NAF or CAF. Based on the average tumor size and weight, we concluded that both NAF and CAF support the growth of SKOV3 cells (**Fig. 4A**). Since NAF and SKOV3 cells were labeled with Tomato and GFP, respectively, we explanted the excised tumors to observe the ratio of NAF to SKOV3 cells under a fluorescence microscope. While SKOV3 cells were positive for GFP, fibroblasts were negative for Tomato, indicating that NAF were replaced by endogenous mouse fibroblasts during tumor growth (**Fig. 4B**). In the H&E sections of the tumors, we observed significant immune infiltrates only in tumors where SKOV3 cells were co-injected with NAF or CAF (data not shown). We concluded that both NAF and CAF were able to support the growth of SKOV3 cancer cells to the same extent, possibly because both types of fibroblasts were activated by the co-injected cancer cells. The absence of injected fibroblasts and the presence of endogenous host fibroblasts and immune cells in the tumor explants indicates that injected fibroblasts support tumor growth only in the initial stages of tumor formation, possibly by recruiting the host cells, such as tumor-promoting fibroblasts, immune cells and blood vessels.

Approach 2: Neutralizing endogenous COL11A1 with a COL11A1-specific antibody. The ability of the COL11A1 antibody to suppress CAF function was tested in two *in vitro* assays. In the collagen contraction assay, we showed that CAF contract collagen more effectively than NAF (**Fig. 5A**) and that this contraction ability is abrogated in the presence of the COL11A1 antibody (**Fig. 5B**). In the second assay, we mixed NAF or CAF with KURAMOCHI ovarian cancer cells in tissue culture plates. As shown in **Fig. 1**, direct co-culture of NAF with KURAMOCHI cells can induce COL11A1 expression in NAF that is equivalent to the level of COL11A1 in CAF. Thus, in direct co-culture with KURAMOCHI cells, both NAF and CAF exhibited a contractile phenotype, which was abrogated in the presence of the COL11A1 antibody (**Fig. 5C**).

In an experiment with 10 mice treated with control IgG or COL11A1 antibody (2.3mg/ml, daily for 10 days), we did not see a statistically significant difference in tumor growth between the two groups of mice, indicating that this antibody (or this concentration) is not effective in inhibiting tumor growth (data not shown).

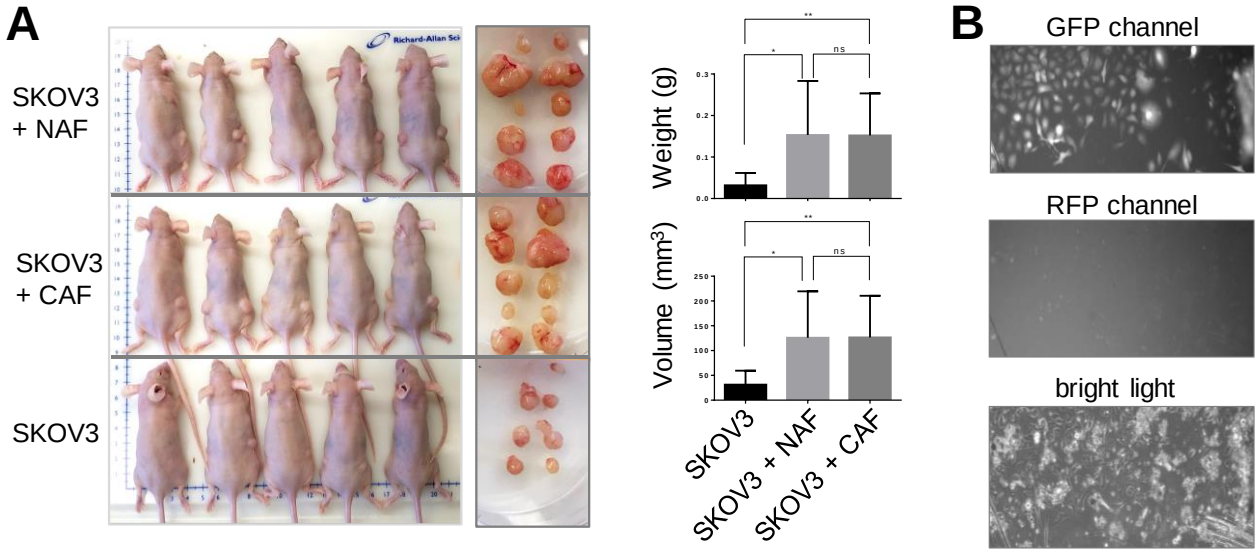

Fig. 4. CAF and NAF support the growth of ovarian cancer xenografts despite their absence in fully-formed tumors. (A) Fifteen nude mice (5 in each group) were subcutaneously injected on each hind flank with a mixture of 50 µl matrigel + 50 µl cells in PBS. Group 1: 0.5 x 10^6 SKOV3-GFP + 1.5 x 10^6 NAF-Tomato. Group 2: 0.5 x 10^6 SKOV3-GFP + 1.5 x 10^6 CAF. Group 3: 0.5 x 10^6 SKOV3-GFP. Tumors were excised 50 days post-injection to quantitate their weight and volume. **(B)** Immunofluorescence analysis of explanted tumor cells to detect SKOV3-GFP cells (GFP channel) and NAF-Tomato cells (RFP channel) among the unlabeled host-derived cells.

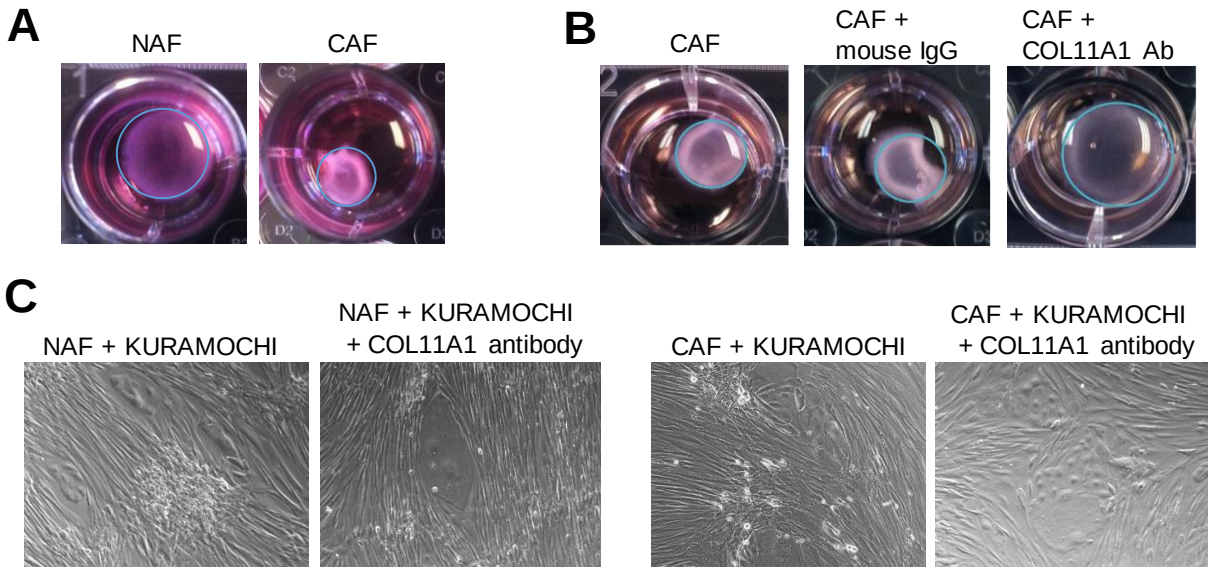

Fig. 5. The COL11A1 antibody is effective in suppressing the contractile phenotype of CAF. **(A)** Collagen contraction 48 hours after mixing NAF and CAF with collagen. **(B)** Collagen contraction 48 hours after mixing CAF with collagen in the presence of a control antibody (mouse IgG) or COL11A1 antibody. **(C)** Fibroblast contraction upon co-culture with KURAMOCHI ovarian cancer cells in the presence or absence of the COL11A1 antibody.

AIM 3. Induce activated CAF-to-cartilage differentiation.

We have analyzed data from the mouse tissues that were injected with differentiation agents. Masson's trichrome staining analysis did not show any difference in cartilage/bone formation in the control or experimental groups. One drawback of our experiment was the low level of fibroblast recruitment to the tumor site. We will repeat this experiment with our new NaOCl-induced fibrotic model of ovarian cancer.

References:

1. Naora H. Heterotypic cellular interactions in the ovarian tumor microenvironment: biological significance and therapeutic implications. *Frontiers in oncology.* 2014;4(18.
2. Loeffler M, Kruger JA, Niethammer AG, and Reisfeld RA. Targeting tumor-associated fibroblasts improves cancer chemotherapy by increasing intratumoral drug uptake. *The Journal of clinical investigation.* 2006;116(7):1955-62.
3. Provenzano PP, Cuevas C, Chang AE, Goel VK, Von Hoff DD, and Hingorani SR. Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma. *Cancer cell.* 2012;21(3):418-29.
4. Liu J, Liao S, Diop-Frimpong B, Chen W, Goel S, Naxerova K, Ancukiewicz M, Boucher Y, Jain RK, and Xu L. TGF-beta blockade improves the distribution and efficacy of therapeutics in breast carcinoma by normalizing the tumor stroma. *Proceedings of the National Academy of Sciences of the United States of America.* 2012;109(41):16618-23.
5. Liao D, Luo Y, Markowitz D, Xiang R, and Reisfeld RA. Cancer associated fibroblasts promote tumor growth and metastasis by modulating the tumor immune microenvironment in a 4T1 murine breast cancer model. *PloS one.* 2009;4(11):e7965.
6. LeBeau AM, Brennen WN, Aggarwal S, and Denmeade SR. Targeting the cancer stroma with a fibroblast activation protein-activated promelittin protoxin. *Molecular cancer therapeutics.* 2009;8(5):1378-86.
7. Feig C, Jones JO, Kraman M, Wells RJ, Deonarine A, Chan DS, Connell CM, Roberts EW, Zhao Q, Caballero OL, et al. Targeting CXCL12 from FAP-expressing carcinoma-associated fibroblasts synergizes with anti-PD-L1 immunotherapy in pancreatic cancer. *Proceedings of the National Academy of Sciences of the United States of America.* 2013;110(50):20212-7.
8. Jacobetz MA, Chan DS, Neesse A, Bapiro TE, Cook N, Frese KK, Feig C, Nakagawa T, Caldwell ME, Zecchini HI, et al. Hyaluronan impairs vascular function and drug delivery in a mouse model of pancreatic cancer. *Gut.* 2013;62(1):112-20.
9. Kraman M, Bambrough PJ, Arnold JN, Roberts EW, Magiera L, Jones JO, Gopinathan A, Tuveson DA, and Fearon DT. Suppression of antitumor immunity by stromal cells expressing fibroblast activation protein-alpha. *Science.* 2010;330(6005):827-30.
10. Wang LC, Lo A, Scholler J, Sun J, Majumdar RS, Kapoor V, Antzis M, Cotner CE, Johnson LA, Durham AC, et al. Targeting fibroblast activation protein in tumor stroma with chimeric antigen receptor T cells can inhibit tumor growth and augment host immunity without severe toxicity. *Cancer immunology research.* 2014;2(2):154-66.
11. Lo A, Wang LC, Scholler J, Monslow J, Avery D, Newick K, O'Brien S, Evans RA, Bajor DJ, Clendenin C, et al. Tumor-Promoting Desmoplasia Is Disrupted by Depleting FAP-Expressing Stromal Cells. *Cancer research.* 2015;75(14):2800-10.

12. Arnold JN, Magiera L, Kraman M, and Fearon DT. Tumoral immune suppression by macrophages expressing fibroblast activation protein-alpha and heme oxygenase-1. *Cancer immunology research.* 2014;2(2):121-6.
13. Kasturi KN, Shibata S, Muryoi T, and Bona CA. Tight-skin mouse an experimental model for scleroderma. *International reviews of immunology.* 1994;11(3):253-71.
14. Kielty CM, Raghunath M, Siracusa LD, Sherratt MJ, Peters R, Shuttleworth CA, and Jimenez SA. The Tight skin mouse: demonstration of mutant fibrillin-1 production and assembly into abnormal microfibrils. *The Journal of cell biology.* 1998;140(5):1159-66.
15. Gayraud B, Keene DR, Sakai LY, and Ramirez F. New insights into the assembly of extracellular microfibrils from the analysis of the fibrillin 1 mutation in the tight skin mouse. *The Journal of cell biology.* 2000;150(3):667-80.
16. Ito S, Bartolak-Suki E, Shipley JM, Parameswaran H, Majumdar A, and Suki B. Early emphysema in the tight skin and pallid mice: roles of microfibril-associated glycoproteins, collagen, and mechanical forces. *American journal of respiratory cell and molecular biology.* 2006;34(6):688-94.
17. Lemaire R, Farina G, Kissin E, Shipley JM, Bona C, Korn JH, and Lafyatis R. Mutant fibrillin 1 from tight skin mice increases extracellular matrix incorporation of microfibril-associated glycoprotein 2 and type I collagen. *Arthritis and rheumatism.* 2004;50(3):915-26.
18. Pablos JL, Everett ET, Harley R, LeRoy EC, and Norris JS. Transforming growth factor-beta 1 and collagen gene expression during postnatal skin development and fibrosis in the tight-skin mouse. *Laboratory investigation; a journal of technical methods and pathology.* 1995;72(6):670-8.

4) other achievements

Nothing to report.

a. What opportunities for training and professional development has the project provided?

Nothing to report.

b. How were the results disseminated to communities of interest?

We have published our results as open access articles in journals *Scientific Reports* and *Frontiers in Cell and Developmental Biology*.

c. What do you plan to do during the next reporting period to accomplish the goals?

We will analyze the molecular mechanisms underlying the rapid and aggressive ovarian carcinomatosis in our new NaOCl-induced fibrosis mouse model of ovarian cancer and publish the results in order for other ovarian cancer researchers to use this method in inducing fibrosis in their ovarian cancer models. We will use this model to test the efficacy of anti-fibrotic agents in increasing the chemotherapeutic sensitivity of ovarian cancer to cisplatin and in reducing the metastatic progression of ovarian cancer.

3. IMPACT:

a. What was the impact on the development of the principal discipline(s) of the project?

Nothing to report.

b. What was the impact on other disciplines?

Nothing to report.

c. What was the impact on technology transfer?

Nothing to report.

d. What was the impact on society beyond science and technology?

Nothing to report.

4. CHANGES/PROBLEMS:

a. Changes in approach and reasons for change.
Using three different approaches to targeting CAFs in an immunocompetent mouse model of ovarian cancer that was developed in our laboratory, we failed to show any significant benefits of targeting CAFs. We realized that we needed to develop a more fibrotic model of ovarian cancer to demonstrate the efficacy of anti-fibrotic agents. During this funding period, we generated two new mouse models, both of which exhibit extensive fibrosis and rapid onset of ovarian carcinomatosis. We believe that these new mouse models will transform future studies of the roles of fibrosis in ovarian cancer initiation and progression.

b. Actual or anticipated problems or delays and actions or plans to resolve them.
Nothing to report.

c. Changes that had a significant impact on expenditures. No

d. Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents. No

e. Significant changes in use or care of human subjects. No

f. Significant changes in use or care of vertebrate animals. No

g. Significant changes in use of biohazards and/or select agents. No

5. PRODUCTS:

Nothing to report.

a. Publications, conference papers, and presentations

i. Journal publications.

1. Jia D, Kamata Y, Katsumata M, Orsulic S. Inflammation is a key contributor to ovarian cancer cell seeding. Scientific Reports, 2018, 8:12394. (Published, acknowledged grant funding)

2. Haro M and Orsulic S. A Paradoxical Correlation of Cancer-Associated Fibroblasts with Survival Outcomes in B-Cell Lymphomas and Carcinomas. Frontiers in Cell and Developmental Biology, 2018. (In press, acknowledged grant funding)
3.

ii. Books or other non-periodical, one-time publications. N/A

iii. Other publications, conference papers, and presentations.

Oral presentation (acknowledged grant funding)
Sandra Orsulic. Tumor Microenvironment. Cancer Biology Seminar, Mayo Clinic, Jacksonville, FL. February 23, 2018.

b. Website(s) or other Internet site(s). N/A

c. Technologies or techniques. N/A

d. Inventions, patent applications, and/or licenses. N/A

e. Other Products. N/A

6. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

a. What individuals have worked on the project?

Name:	Sandra Orsulic
Project Role:	PI
Nearest person month worked:	2.00
Contribution to Project:	Dr. Orsulic oversaw projects for all three specific aims, including experimental design, execution, and data analysis and interpretation. She wrote the manuscripts (Jia et al, Scientific Reports, 2018; Haro and Orsulic, Frontiers in Cell and Developmental Biology, 2018) and prepared presentations as well as the progress report.

Name:	Beth Karlan
Project Role:	Collaborator
Nearest person month worked:	0.12
Contribution to Project:	Dr. Karlan advised on the translational aspects of the proposal and participated in experimental design.

Name:	Marcela Haro, PhD
Project Role:	Postdoctoral Fellow
Nearest person month worked:	6.50
Contribution to Project:	Dr. Haro conducted all experiments that involved testing different combinations of treatments in the immunocompetent mouse model of ovarian cancer and assisted in data acquisition and analysis. She assisted in the writing of the manuscript (Haro and Orsulic, Frontiers in Cell and Developmental Biology, 2018).

Name:	Barbie Taylor-Harding, PhD

Project Role:	Research Associate
Nearest person month worked:	3.00
Contribution to Project:	Dr. Taylor-Harding conducted all experiments that involved testing different combinations of treatments in the immunocompetent mouse model of ovarian cancer and assisted in data acquisition and analysis.

b. Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period? No

c. What other organizations were involved as partners? None

7. SPECIAL REPORTING REQUIREMENTS

a. COLLABORATIVE AWARDS: N/A

b. QUAD CHARTS: N/A

8. APPENDICES: N/A