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TITLE: Targeting Premalignant Lesions: Implications for Early Breast Cancer Detection and Intervention

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Breast cancer progression constitutes a multistep process through a series of intermediate hyperplastic and neoplastic stages to invasive carcinoma. In this study, we aimed to identify peptides that specifically recognize premalignant lesions in the mammary tissue. To achieve this goal, we utilized the power of phage display to probe hyperplastic lesions associated with premalignant disease in a transgenic MMTV-PyMT animal model. We have identified a peptide CISQ that targets to the stroma in premalignant lesions and binds to cancer-associated fibroblasts (CAFs) in MMTV-PyMT mice. Considerable numbers of CAFs are frequently observed within the tumor-associated stroma of various human cancers, including those of the breast, prostate, lung, colon and pancreas and have been also reported in the premalignant lesions. This peptide could provide us with an opportunity to therapeutically intervene to successfully inhibit or even reverse tumor progression.

15. SUBJECT TERMS
Breast cancer, Premalignant lesions, early intervention, homing peptides, nanomedicine,

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1. INTRODUCTION:

Difficulty in managing treatment of advanced stage breast cancer has led to the goal for detection and intervention of early-stage disease. However, current non-invasive methods are not specific enough to reliably detect early breast cancer. Our laboratory has successfully employed *in vivo* screening of phage libraries to develop new probes for breast tumors. Progression of breast cancer constitutes a multistep process wherein each stage is characterized by distinct phenotypic changes that occur in the mammary gland. We proposed to utilize this animal model to probe early stage (premalignant) lesions with phage libraries to identify novel peptides that specifically recognize the premalignant stage of breast cancer. These peptides and the identification of their putative receptors will help our understanding of the underlying biology of breast cancer progression. Furthermore, these probes will be used to develop targeted therapeutic nanoparticles for early intervention in breast cancer.

2. KEYWORDS:

Early breast cancer, early detection, homing peptides, premalignant lesions, targeted nanomedicine

3. ACCOMPLISHMENTS:

Major Goals and Objectives approved (and completed) for this project are as follows:

Specific Aim 1: Identify peptides that specifically home to premalignant breast lesions (Months 1-12)

Task 1. To screen phage libraries for new peptides that specifically recognize premalignant lesions (Months 1-9):

- Develop and characterize the CX7C and X7 phage libraries for screening (COMPLETED)
- Screening of libraries in MMTV-PyMT animals (COMPLETED)
- High throughput sequencing on recovered phage from these lesions (COMPLETED)
- Bioinformatics analysis (ONGOING)

Task 2. To validate the homing specificities of individual phage and synthetic peptides (ONGOING)

- Individually test homing of identified phage (COMPLETED)
- Determine phage specificity to premalignant lesions (COMPLETED)
- Phage overlay on human tissue microarrays (TO BE DONE)
- Validation of peptide homing in MMTV-NeuYD transgenic mouse model (TO BE DONE)

Specific Aim 2: Identify and characterize putative receptors in premalignant lesions (Months 12-24).

Task 1: To identify putative receptors of these peptides in these early lesions (Months 12-15) (ONGOING)

*Task 2: To characterize the identified receptor in early lesions (Months 15-18**) (ONGOING)***

Task 3: To study significance of receptor in disease progression across different stages of breast cancer (Months 18-24)

Specific Aim 3: Target premalignant lesions utilizing peptide-conjugated nanoparticles to prevent/delay progression of premalignant lesions to invasive breast cancer (Months 18-36)

Task 1: To engineer and characterize peptide conjugated therapeutic nanoparticles (Months 18-24)

- Develop peptide nanoparticle drug conjugates (Months 18-20)
- Characterize targeted nanoparticles (Months 20-24)

Task 2: Study the effect of targeted delivery of therapeutic nanoparticles on the onset of the disease (Months 24-36)

- Treat MMTV-PyMT animals with peptide nanoparticle conjugates (*Months 24-32*)
- Evaluate tumor progression (*Months 32-36*)

RESULTS:

Specific Aim 1: Identify peptides that specifically home to premalignant breast lesions

As part of this aim, we have identified a new peptide (CISQ) that targets to the stroma in premalignant lesions in MMTV-PyMT mice (Fig.1). This peptide is specific to these early lesions, as a control peptide does not show any accumulation in these early (Fig. 2). This peptide binds to fibroblasts in the premalignant lesions that stain positive for ER-Tr7 marker and vimentin (Fig.3). This suggests that these fibroblasts represent a subset termed as cancer-associated fibroblasts (CAFs). Considerable numbers of CAFs are frequently observed within the tumor-associated stroma of various human cancers, including those of the breast, prostate, lung, colon and pancreas and have been also reported in the premalignant lesions (Erez N, et. al Cancer Cell 2010). We are in the process of confirming this finding and identifying the receptor for CISQ. We have already conducted affinity chromatography experiments using tumor lysates to isolate the receptor. The analysis for these experiments is currently ongoing. This peptide could provide us with an opportunity to therapeutically intervene to successfully inhibit or even reverse tumor progression.

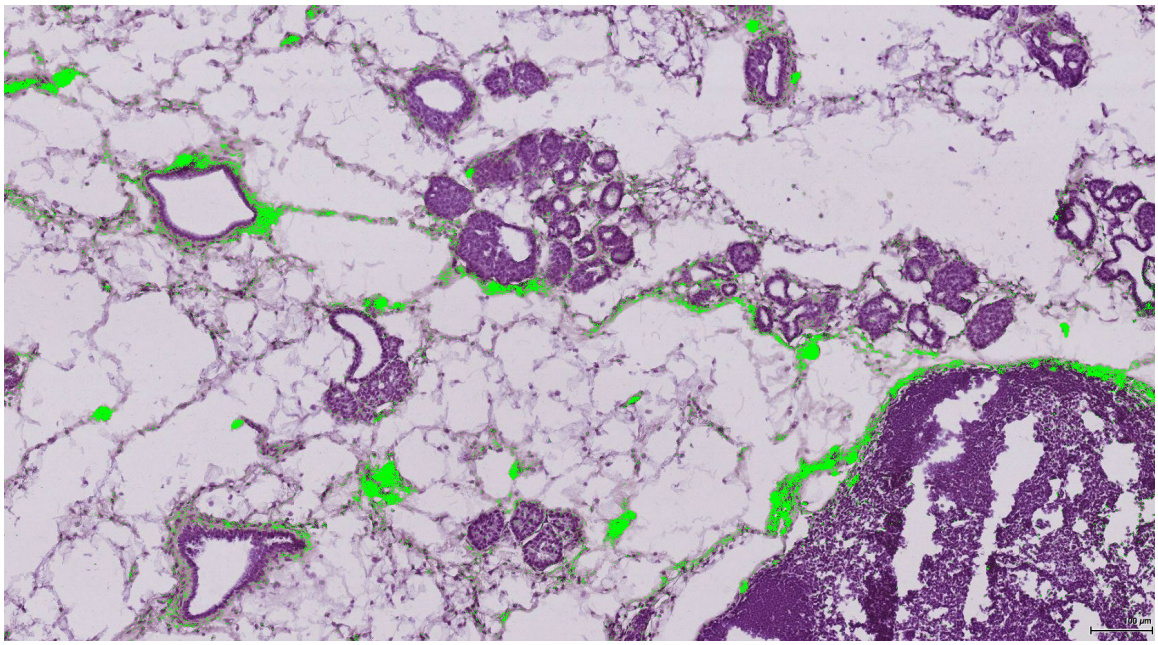

Fig. 1: CISQ accumulates in early (premalignant) hyperplastic lesions in mammary fat pad isolated from PyMT-MMTV animals. Immunofluorescence staining overlapped with H&E staining on whole mount sections of mammary fat pad isolated following FAM-CISQ injection in PyMT-MMTV mouse. Green – anti-FAM-CISQ; Purple - Nuclear Stain.

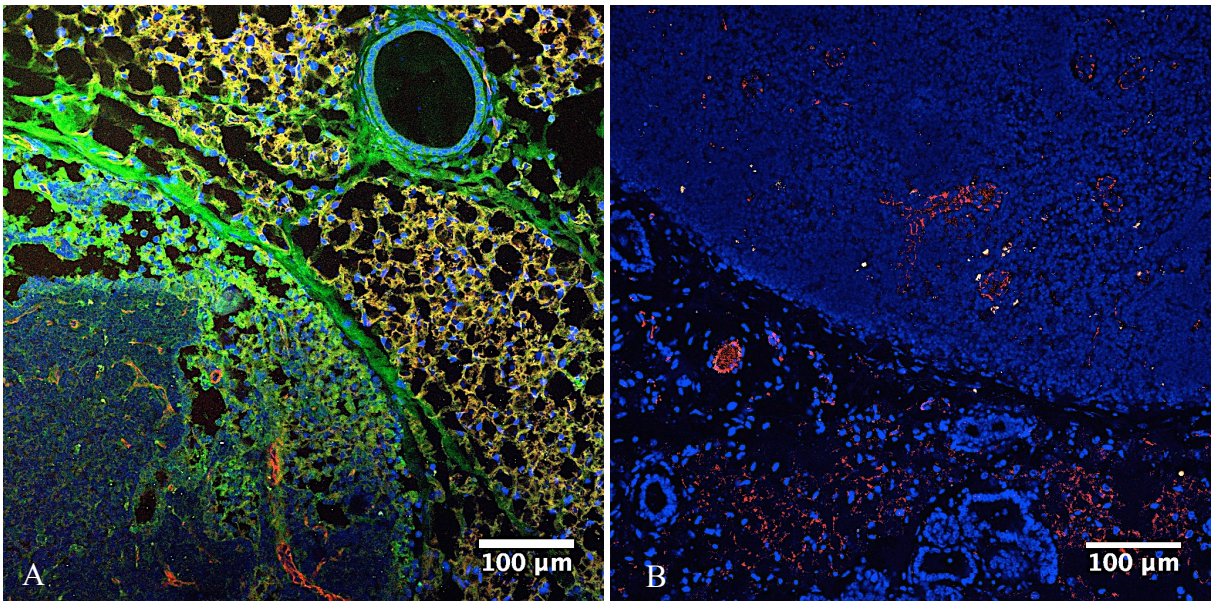

Fig. 2: CISQ homes to early (premalignant) hyperplastic lesions in mammary fat pad isolated from PyMT-MMTV animals. Immunofluorescence staining on whole mount sections of mammary fat pad isolated following 1 hour in-vivo circulation of FAM-CISQ (**A)** and control peptide **(B)** in PyMT-MMTV mouse.

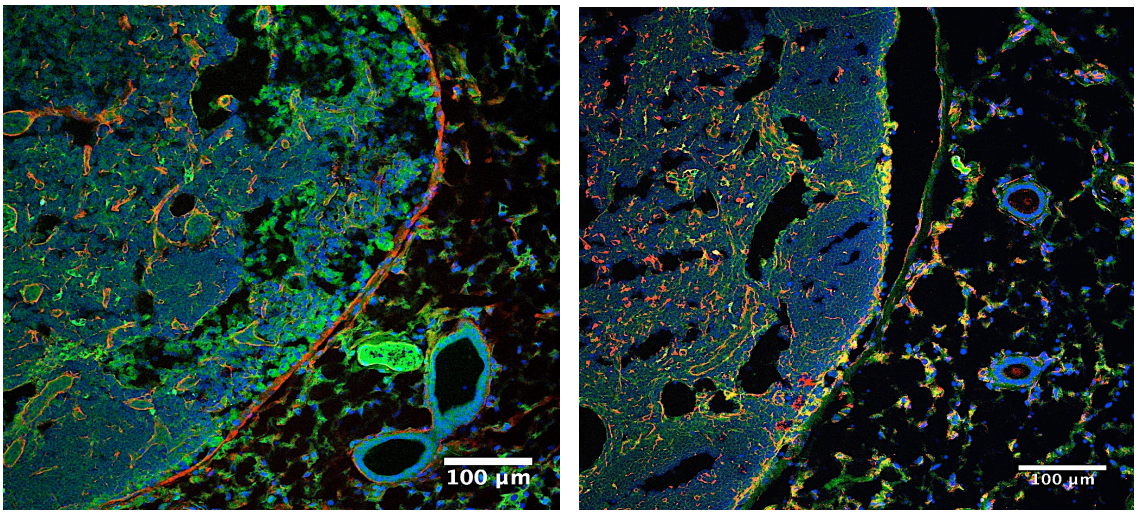

Fig. 3: CISQ colocalizes with fibroblasts in early (premalignant) lesions in mammary fat pad isolated from PyMT-MMTV animals. Immunofluorescence staining on whole mounts of mammary fat pad isolated following 1hour in-vivo circulation of FAM-CISQ stained with ER-TR7 (**A)** and vimentin **(B)**.

Opportunities for training and professional development: None

Dissemination of results:

1. Presentation at the Annual Postdoctoral symposium held at Sanford Burnham Medical Research Institute.

4. IMPACT: Nothing to report

Impact on the development of the principal discipline(s) of the project: Nothing to report

Impact on other disciplines: Nothing to report

Impact on technology transfer: Nothing to report

Impact on society beyond science and technology: Nothing to report

5. CHANGES/PROBLEMS - Nothing to report

6. PRODUCTS:

Journal publications. None

Books or other non-periodical, one-time publications: *None*

Website(s) or other Internet site(s): None

Technologies or techniques -None

Inventions, patent applications, and/or licenses - None

Other Products - None

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

Name: Aman Mann
Project Role: PI
Nearest Person Month Worked: 12
Contribution to Project: Principal Investigator and oversee all scientific, experimental and administrative aspects

Name: Erkki Ruoslahti
Project Role: Mentor
Nearest Person Month Worked: 0
Contribution to Project: Serves as a mentor to Dr. Aman Mann

8. SPECIAL REPORTING REQUIREMENTS: None

9. APPENDICES: N/A