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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. After multiple ex-vivo and in-vivo rounds of selection, we identified a peptide, Prem-1, that on intravenous administration, specifically homed to premalignant mammary lesions. Prem-1 also homed to fully developed breast tumors in the same animal model, suggesting that the putative receptor for Prem-1 is expressed throughout the progression of the disease. Interestingly, Prem-1 did not show any affinity to normal breast tissue. Furthermore, we also identified 2 other candidate peptides that showed significant homing to premalignant lesions with a very different binding pattern as compared to Prem-1. We hypothesized that all three peptides recognize early changes in the breast tissue microenvironment but each bind a different target receptor in the tissue. We are currently investigating these receptors and analyzing their expression in breast cancer progression.

15. SUBJECT TERMS

Breast cancer, Premalignant lesions, early intervention, homing peptides, nanomedicine,

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Table of Contents

Page

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 (ONGOING)
- Determine phage specificity to premalignant lesions (TO BE DONE)
- 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)

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

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 further characterized two peptides (IDG and CSG) from our panel of peptides that home to the premalignant lesions. The two peptides show very distinct localization in these early tumor lesions. For instance, IDG peptide binds to fibroblasts in the premalignant lesions as shown in Fig.1. We are in the process of confirming if these fibroblasts are the cancer associated fibroblasts (CAFs). CAFs have been reported to be present in the premalignant lesions (ref).

On the other hand, the CSG peptide binds to components in the extra cellular matrix. The matrix seems to undergo a change to facilitate the tumor initiation and progression. We analyzed the expression of two ECM components in these early lesions, Nidogen-1 and Laminin (Fig. 2). Both of these members show re-organization of the matrix in the mammary glands containing the early cancer lesions. On further analysis, we determined that CSG peptide co-localizes with Nidogen-1 (Fig. 3).

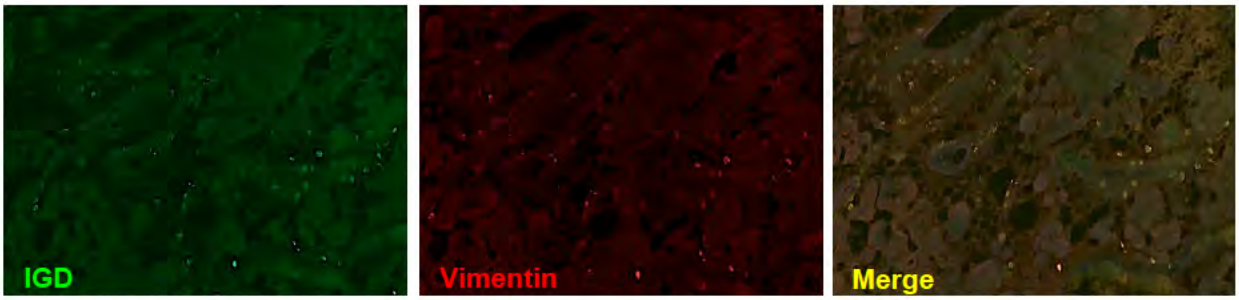

Fig. 1: IGD colocalizes with fibroblasts in early (premalignant) hyperplastic lesions in mammary fat pad isolated from PyMT-MMTV animals. Immunofluorescence staining on whole mount sections of mammary fat pad isolated following FAM-IGD injection in PyMT-MMTV mouse. Green – anti-FAM-IGD; Red – anti-Vimentin-1, Blue - Nuclear Stain.

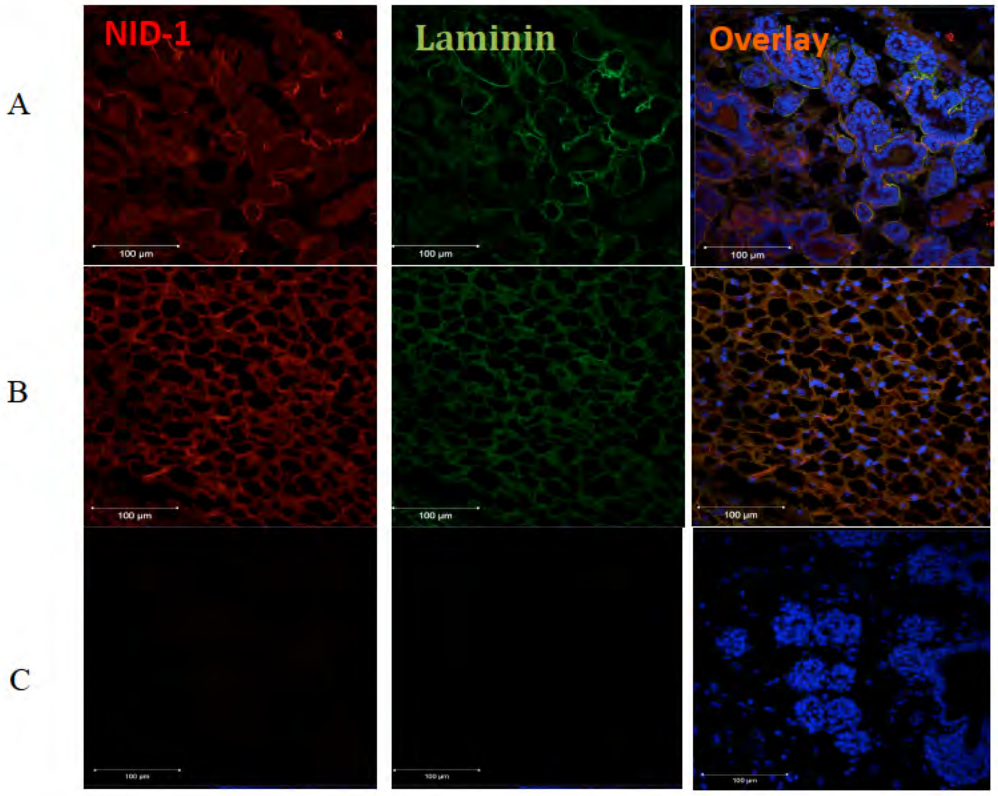

Fig. 2: Expression of ECM components in early (premalignant) hyperplastic lesions in mammary fat pad isolated from PyMT-MMTV animals. Immunofluorescence staining on whole mount sections of mammary fat pad in PyMT-MMTV mouse (**A**) or normal Blk6 mouse (**B**). Green – anti-Laminin; Red – anti-Nidogen-1, Blue - Nuclear Stain. (**C**) IgG control staining.

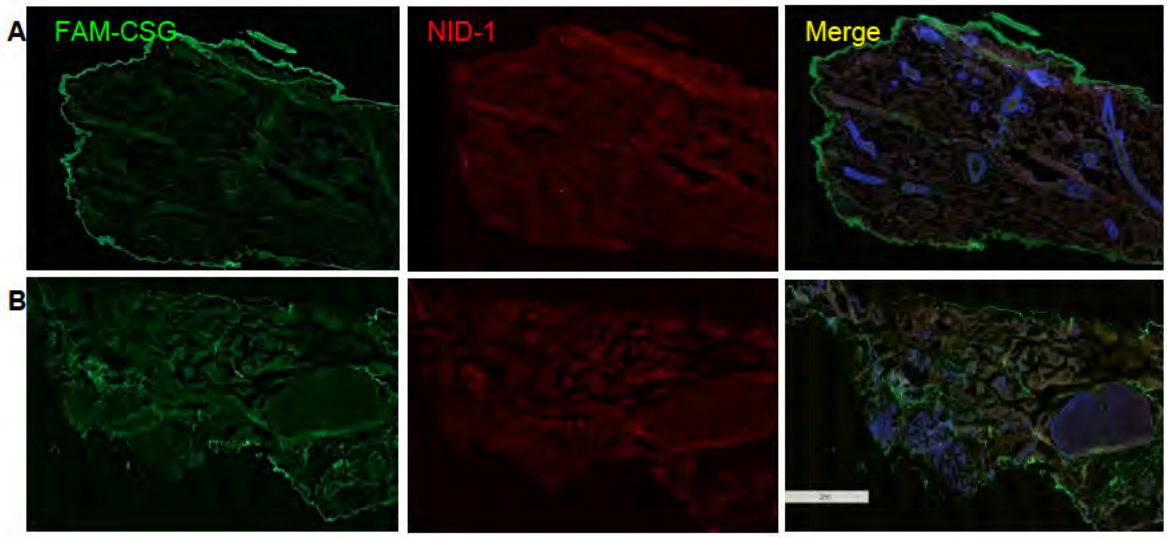

Fig. 3: CSG homes to early (premalignant) hyperplastic lesions in mammary fat pad isolated from PyMT-MMTV animals. Immunofluorescence staining on whole mount sections of mammary fat pad isolated following 1 hour in-vivo circulation of FAM-CSG in normal Blk6 mouse (**A**) or PyMT-MMTV mouse (**B**). Green – anti-FAM-CSG; Red – anti-Nidogen-1, Blue - Nuclear Stain. Scale Bar is 2 micron.

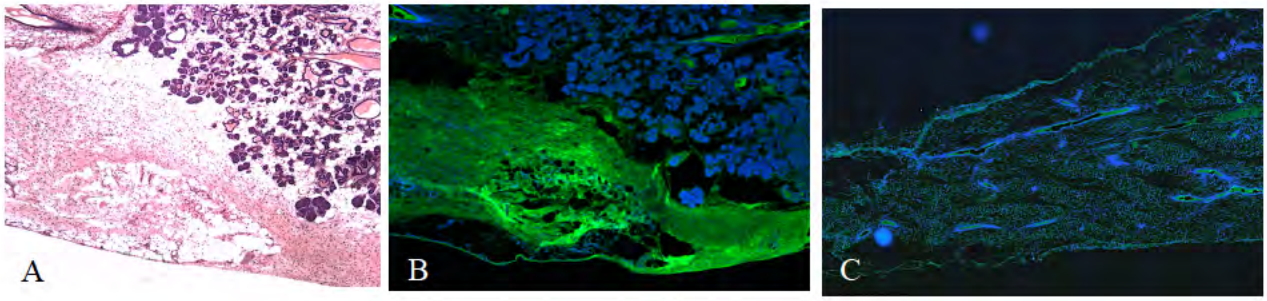

Fig. 4: CAG homes to early (premalignant) hyperplastic lesions in mammary fat pad isolated from PyMT-MMTV animals. Immunofluorescence staining on whole mount sections of mammary fat pad isolated following 1 hour in-vivo circulation of FAM-CAG in PyMT-MMTV mouse (**B**) or normal Blk6 mouse (**C**). **A** shows the H&E staining on the corresponding section in B. Green – anti-FAM-CAG; Blue - Nuclear Stain.

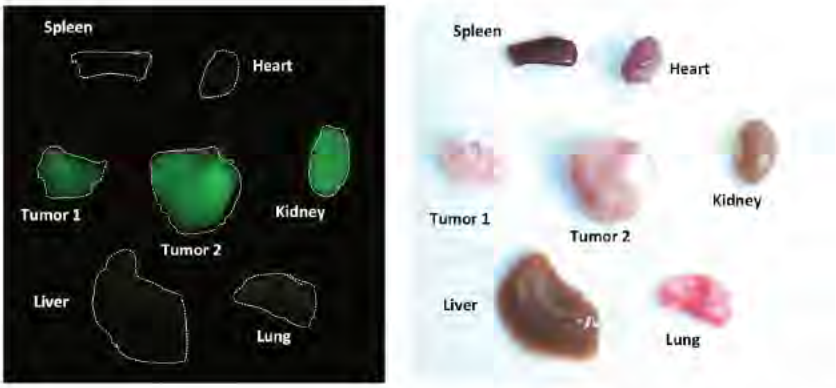

Fig. 5: CAG homes to fully developed tumors isolated from PyMT-MMTV animals. Ex vivo imaging of all major organs using a illumatool following 1 hour in-vivo circulation of FAM-CAG in PyMT-MMTV mouse. Left panel shows imaging under green channel. Right panel is bright field image.

CAG peptide that was identified from phage screening showed significantly higher homing to the early lesions as compared to other peptides as well as to normal breast tissue (Fig. 4). CAG peptide localizes in the matrix in this tissue and we are currently investigating the putative receptor for this peptide. CAG peptide also binds mature PyMT tumors as shown in Fig. 5, suggesting that the receptor for this peptide is shared across different stages of tumor in this animal model.

Additionally, as proposed in the statement of work, we carried out phage screening followed by high throughput sequencing on the phage pool recovered from screening in these animals with early breast cancer.

The screening comprised of one round ex-vivo on the mammary glands containing premalignant lesions followed by one round in-vivo selection. The bioinformatics analysis of the sequencing data is currently ongoing. This data would provide us with possible motifs that enriched in this screen.

Opportunities for training and professional development:

During this project so far the following training and professional development has been undertaken

1. "Pathobiology of the Mouse Tier 1A" online course offered through UC Davis Extension
2. "The Art of Science Communication" course offered by Sanford-Burnham and ASBMB.

Dissemination of results:

1. Presented highlights of this work on December 3, 2014, at the Annual Holiday Party of Group of 12 - a community service group in La Jolla stressing health, education, and friendship.
2. Presented at the inaugural Postdoc Open-Mic Night conducted in La Jolla by Scripps Research Institute in collaboration with The Salk Institute and UCSD.

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:

Conference Presentation and Publication in proceedings in an International Conference

Conference: American Association for Cancer Research Annual Meeting 2014
Title of Presentation: Targeting Premalignant lesions for early breast cancer detection and intervention
Authors: Aman P Mann, Venkata Ramana Kotamraju, Tambet Teesalu, and Erkki Ruoslahti
Status of publication: Published
Bibliographic information: Cancer Res October 1, 2014 74; 3258
Acknowledgement of federal support: Yes

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:
Ccontribution to Project: Principal Investigator and oversee all scientific, experimental and administrative aspect

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

8. SPECIAL REPORTING REQUIREMENTS: None

9. APPENDICES: none