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TITLE: Notch3 as a Tumor Suppressor in the Postpartum Mammary Gland

PRINCIPAL INVESTIGATOR: Keli Xu

CONTRACTING ORGANIZATION: University of Mississippi Medical Center

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Fort Detrick, Maryland 21702-5012

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Notch3 as a Tumor Suppressor in the Postpartum Mammary Gland

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6. AUTHOR(S)

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5e. TASK NUMBER

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University of Mississippi Medical Center
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Jackson, MS 39216

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U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The goal of this project is to determine the functions of Notch3 in the pathogenesis of postpartum breast cancer, focusing on the regulation of parity-induced mammary epithelial cells, as we as regulation of brown adipose tissue repopulation in the postpartum mammary gland and its potential role in modulating tumor microenvironment. We showed that parous Notch3 mutant glands exhibited an expansion of CD24HiCD49fLo cells, a subpopulation known to be enriched in bipotent lobule progenitors and multipotent stem cells, associated with upregulation of Gata3. Unexpectedly, we found that Notch3 knockout mice suppressed mammary tumor xenografts. We identified candidates of Notch3 target genes in immune cells that may regulate tumor-immune interaction. Our results suggest that Notch3 functions as a tumor suppressor in the mammary epithelium and brown adipocytes in the postpartum mammary gland while exerting an oncogenic role in the immune system.

15. SUBJECT TERMS

Postpartum breast cancer, Notch signaling, Parity-induced mammary epithelial cell, Tumor microenvironment, Brown adipocyte, Immune cell

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	19	19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

We propose to test the hypothesis that Notch3 functions as a tumor suppressor in the postpartum mammary gland by restricting the parity-associated mammary stem/progenitor cells, and by promoting brown adipocyte differentiation during post-lactational involution, which may ameliorate the pro-tumorigenic microenvironment. The specific aims of this project are: (1) to determine whether the post-lactational involuting mammary microenvironment of Notch3 knockout mice accelerates tumor growth and metastasis compared to that of wild-type mice and whether Notch3 functions in the mammary epithelium or the stroma in this context; (2) to determine the regulation of parity-identified mammary epithelial cells or other stem/progenitor populations by Notch3 in the postpartum mammary gland, and (3) to determine how Notch3 regulates brown adipocyte differentiation during involution and whether brown adipocytes have an impact on the postpartum mammary microenvironment. Our objectives are: (1) to determine the contribution of parity-identified mammary epithelial progenitors to postpartum breast cancer and its regulation by Notch signaling; and (2) to determine the mechanism underlying brown adipose tissue repopulation in the postpartum mammary gland and its potential role in modulating postpartum mammary microenvironment, which may link obesity-associated metabolic changes to the progression of postpartum breast cancer.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

postpartum breast cancer, tumor microenvironment, brown adipocyte differentiation, parity-identified mammary epithelial cells (PI-MEC), Notch signaling, tumor-infiltrating immune cells

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Major Task 1: Determine whether post-lactational involuting mammary microenvironment of Notch3 knockout mice accelerates tumor growth and metastasis compared to that of wildtype mice
Milestone/target date: 01/31/2020
Actual completion date: 12/01/2019

Major Task 2: Determine the compartment in which Notch3 regulates pro-tumorigenic mammary microenvironment.
Milestone/target date: 09/30/2020
Actual completion date: 11/30/2020

Major Task 3: Determine the mechanisms by which Notch3 regulates brown adipocyte differentiation in the involuting mammary gland
Milestone/target date: 04/30/2021
Actual completion date: 04/12/2021

Major Task 4: Determine whether brown adipocytes have an impact on pro-tumorigenic microenvironment in the postpartum mammary gland
Milestone/target date: 12/31/2021
Ongoing (50% completion)

Major Task 5: Determine alterations in the self-renewal and/or differentiation of PI-MECs or other stem/progenitor populations in parous Notch3 knockout mice
Milestone/target date: 03/31/2022
Actual completion date: 02/15/2022

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Major activities:

1) Performed lineage-tracing of parity-identified mammary epithelial cells (PI-MEC) in *Notch3*$^{\beta\text{-}Geo/\beta\text{-}Geo}$ mutant mice compared to the wild type mice (Major Task 5: Determine alterations in the self-renewal and/or differentiation of PI-MECs or other stem/progenitor populations in parous Notch3 knockout mice).
2) Examined apoptosis of mammary epithelial cells in *Notch3*$^{\beta\text{-}Geo/\beta\text{-}Geo}$ mutant mice during involution, in comparison with the wild type mice (Major Task 5: Determine alterations in the self-renewal and/or differentiation of PI-MECs or other stem/progenitor populations in parous Notch3 knockout mice).
3) Determined alterations in brown adipocytes in *Notch3*$^{\beta\text{-}Geo/\beta\text{-}Geo}$ mutant mice throughout mammary gland development (Major Task 4: Determine whether brown adipocytes have an impact on pro-tumorigenic microenvironment in the postpartum mammary gland).
4) Performed transplantation to show that postpartum microenvironment is required for stem cell activity of PI-MECs isolated from *Notch3*$^{\beta\text{-}Geo/\beta\text{-}Geo}$ mutant mice (Major Task 5: Determine alterations in the self-renewal and/or differentiation of PI-MECs or other stem/progenitor populations in parous Notch3 knockout mice).

Specific objectives:

1) To determine whether deletion of Notch3 leads to expansion of PI-MECs.
2) To determine the mechanisms underlying the expansion of PI-MECs in the Notch3 mutant mice.
3) To determine the Notch3 regulation of brown adipocyte differentiation in the mammary fat pad at different developmental stages.
4) To determine whether postpartum mammary gland microenvironment is required for stem cell activity of mammary epithelial cells isolated from parous Notch3 mutant mice.

Significant results:

1) Parity-identified mammary epithelial cells (PI-MECs), a subset of alveolar cells that do not die during involution or remodeling after lactation, have been shown to exhibit stem cell characteristics and may serve as the cellular targets for oncogenic transformation. We analyzed PI-MECs by labeling mammary epithelial cells during pregnancy using *R26^{YFP};WAP-Cre* and stained for YFP at involution day 28 post lactation. We found that deletion of Notch3 caused a significant increase in PI-MECs (Fig. 1). We also performed TUNEL assay in mammary tissues during early stage of involution, and observed decreased number of apoptotic cells in the Notch3 mutant mice (Fig. 2). Our results indicate that deletion of Notch3 leads to increased PI-MECs, which may be attributed to decreased mammary epithelial cell apoptosis during post-lactational involution.
2) Remodeling of the mammary gland during post-lactational involution is accompanied by adipocytes repopulation. We examined brown adipocyte differentiation by immunostaining for Ucp1 during involution. Brown adipocytes were found to emerge in close vicinity to alveolar epithelial cells upon the start of involution in the wild type, but not in the Notch3 mutant mice, suggesting that deletion of Notch3 caused defective brown adipocyte differentiation. We also performed Western blot analysis for Ucp1 in mammary tissues harvested from wild type and Notch3 mutant mice at various developmental stages. Although rapid decrease of Ucp1 staining was noted after the initial stage of involution (data not shown), Ucp1 expressions were detected by Western blot in mammary tissues 3 months after completion of post-lactational involution, and parous Notch3 mutant mice exhibited lower Ucp1 levels as compared to the wild type mice (Fig. 3). A recent study found that breast cancer cells co-cultured with Ucp1-deficient adipocytes had increased proportion of cancer stem cells (Zhang et al., 2020). Our results suggest that defective brown adipocyte differentiation may also contribute to the expansion of stem-like PI-MECs in the Notch3 mutant mice.
3) We tested whether postpartum microenvironment played a role in the regulation of PI-MECs, by injecting mammary epithelial cells isolated from the parous *Notch3$^{\beta\text{-}geo/\beta\text{-}geo}$* mice into the mammary glands of nulliparous wild type mice or age-matched wild type mice at involution day 21 (all on syngeneic FVB background). In three independent experiments, whole-mount X-gal staining at 8 weeks post-injection revealed incorporation of *Notch3$^{\beta\text{-}geo/\beta\text{-}geo}$* PI-MECs into the mammary glands of the postpartum but not the nulliparous hosts (Fig. 4A, B). Interestingly, the vast majority of X-gal^{+} cells are located in the ductal/lobule

buds, indicating that *Notch3*$^{\beta\text{-}geo/\beta\text{-}geo}$ PI-MECs contributed primarily to this region. For comparison, both nulliparous and parous *Notch3*$^{\beta\text{-}geo/\beta\text{-}geo}$ mice showed X-gal staining throughout the mammary ducts (Fig. 4C), and Notch3 protein levels were similar in age-matched nulliparous and parous wild type mice (Fig. 4D), indicating that parity does not alter Notch3 expression in mammary epithelium. Thus, absence of X-gal staining in the *Notch3*$^{\beta\text{-}geo/\beta\text{-}geo}$ cell-injected nulliparous hosts is most likely due to failed incorporation of injected cells into the host glands, rather than silencing of the *Notch3* locus in the nulliparous microenvironment. These results suggest that postpartum microenvironment is required for stem cell activity of PI-MECs in the parous Notch3 mutant mice.

Stated goal not met:

We were not able to complete the Major Task 4--Determine whether brown adipocytes have an impact on pro-tumorigenic microenvironment in the postpartum mammary gland.

Based on the observation of mammary alveolar cells converting to brown adipocytes in post-lactational mice (Giordano et al., 2017) and our data showing Notch3 activation in myoepithelial cell during involution, we hypothesized that Notch3 might regulate alveolar myoepithelial cell to brown adipocyte transdifferentiation during involution. However, quantitative lineage tracing revealed that less than 1% of brown adipocytes were converted from mammary epithelial cells in the post-weaning mammary gland (Li et al., 2017). A recent study identified Notch3 as the major Notch receptor that enhances brown adipogenesis in multipotent mesenchymal cells (Rodríguez-Cano et al., 2020). Given that Notch3 is deleted in all tissues in the *Notch3*$^{\beta\text{-}geo/\beta\text{-}geo}$ mice, it is likely that loss of Notch3 in the stroma, rather than mammary epithelium, caused defective brown adipocyte differentiation.

We have deleted Prdm16, a master regulator of brown adipocyte differentiation, in mammary epithelial cells using MMTV-Cre and K14-Cre. These mice exhibited normal post-lactational involution and no lesions were found after multiple pregnancies (reported in previous Annual Reports). Thus, deletion of Prdm16 in mammary epithelial cells appears to have no impact on the postpartum mammary gland. We will need to ablate brown adipocytes systemically by deleting Prdm16 using Adiponectin-Cre. However, previous study suggested a systemic effect of brown adipocytes on mammary epithelial differentiation (Gouon-Evans and Pollard, 2002), which may compromise our study of brown adipocyte function specifically in the mammary gland microenvironment.

References:

Giordano, A., Perugini, J., Kristensen, D. M., Sartini, L., Frontini, A., Kajimura, S., Kristiansen, K. and Cinti, S. (2017). Mammary alveolar epithelial cells convert to brown adipocytes in post-lactating mice. *Journal of cellular physiology* 232, 2923-2928.
Gouon-Evans V, Pollard JW. 2002. Unexpected deposition of brown fat in mammary gland during postnatal development. *Mol Endocrinol* **16**: 2618-2627.

Li, L., Li, B., Li, M., Niu, C., Wang, G., Li, T., Krol, E., Jin, W. and Speakman, J. R. (2017). Brown adipocytes can display a mammary basal myoepithelial cell phenotype in vivo. *Mol Metab* 6, 1198-1211.
Rodríguez-Cano, M. M., González-Gómez, M. J., Sánchez-Solana, B., Monsalve, E. M., Díaz-Guerra, M. M., Laborda, J., Nueda, M. L. and Baladrón, V. (2020). NOTCH Receptors and DLK Proteins Enhance Brown Adipogenesis in Mesenchymal C3H10T1/2 Cells. *Cells* 9.
Zhang, F., Liu, B., Deng, Q., Sheng, D., Xu, J., He, X., Zhang, L. and Liu, S. (2020). UCP1 regulates ALDH-positive breast cancer stem cells through releasing the suppression of Snail on FBP1. *Cell Biol Toxicol*.

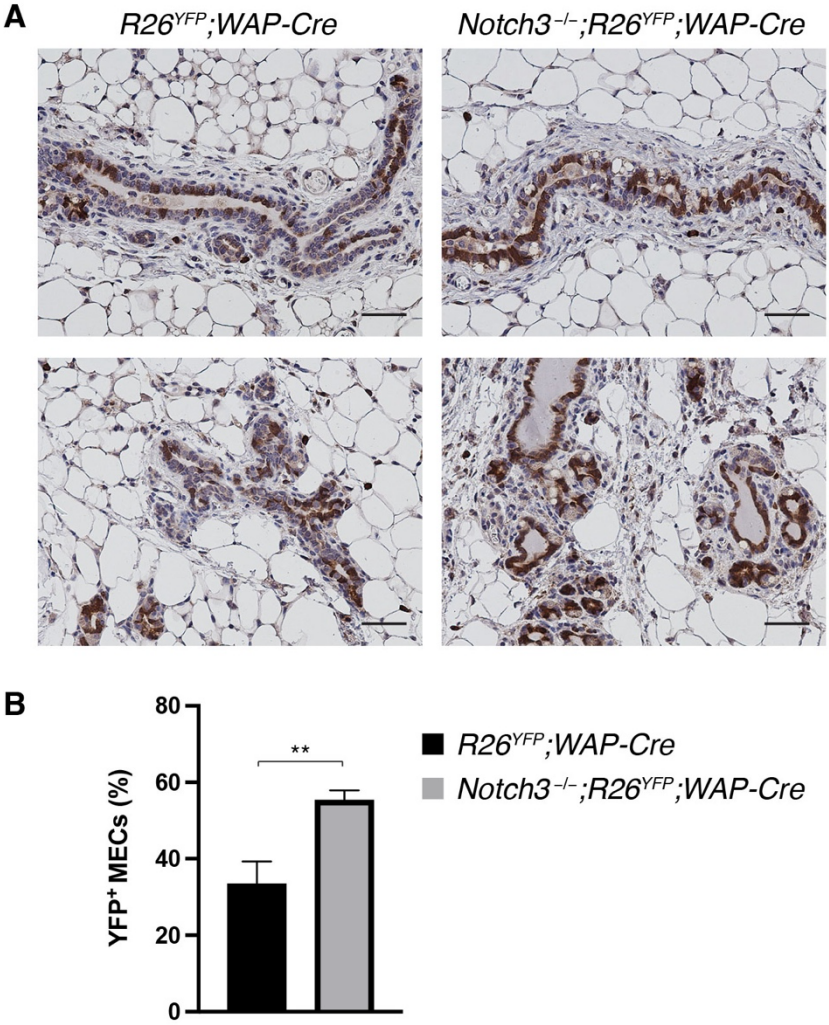

Fig. 1. Increased PI-MECs in Notch3 mutant mammary glands. (A) Anti-YFP immunostaining in mammary tissues from *R26^{YFP};WAP-Cre* and *Notch3$^{-/-}$;R26^{YFP};WAP-Cre* mice at involution day 28. (B) Quantitation of YFP-positive mammary epithelial cells in the *R26^{YFP};WAP-Cre* and *Notch3$^{-/-}$;R26^{YFP};WAP-Cre* mice at involution day 28. Scale bars: 50 μm. **p<0.01 (unpaired t test).

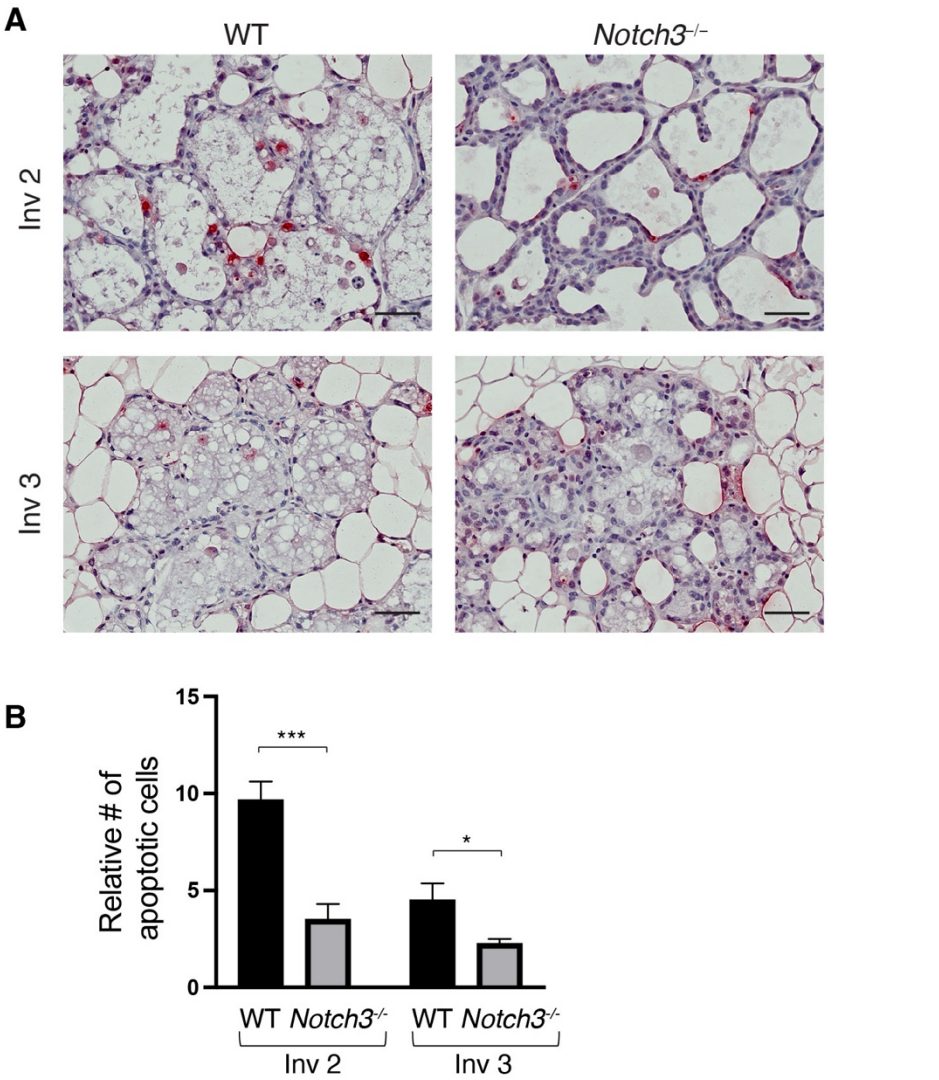

Fig. 2. Reduced apoptosis in Notch3 mutant mammary glands during involution. (A) Representative photomicrographs of TUNEL assays in the mammary tissues of wild type and *Notch3*$^{-/-}$ mice at involution day 2 and 3. (B) Quantitation of TUNEL positive cells. Scale bars: 50 μm. *p<0.05, ***p<0.001 (unpaired t test).

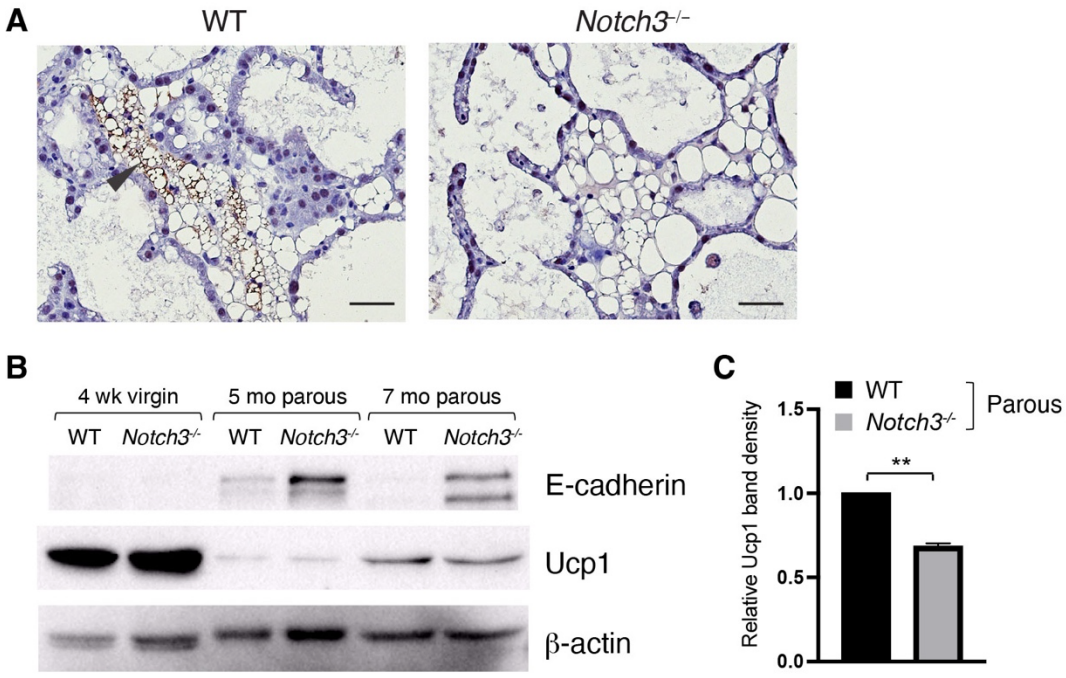

Fig. 3. Defective brown adipocyte differentiation during involution in Notch3 mutant mammary glands. (A) Immunostaining for Ucp1 in the wild type and *Notch3*$^{-/-}$ mice at involution day 1. (B) Western blot analysis of E-cadherin and Ucp1 in mammary tissues from wild type and *Notch3*$^{-/-}$ mice. (C) Relative levels of Ucp1 normalized with the level of β-actin in parous wild type and *Notch3*$^{-/-}$ mice. Scale bars: 50 μm. **p<0.01 (unpaired t test).

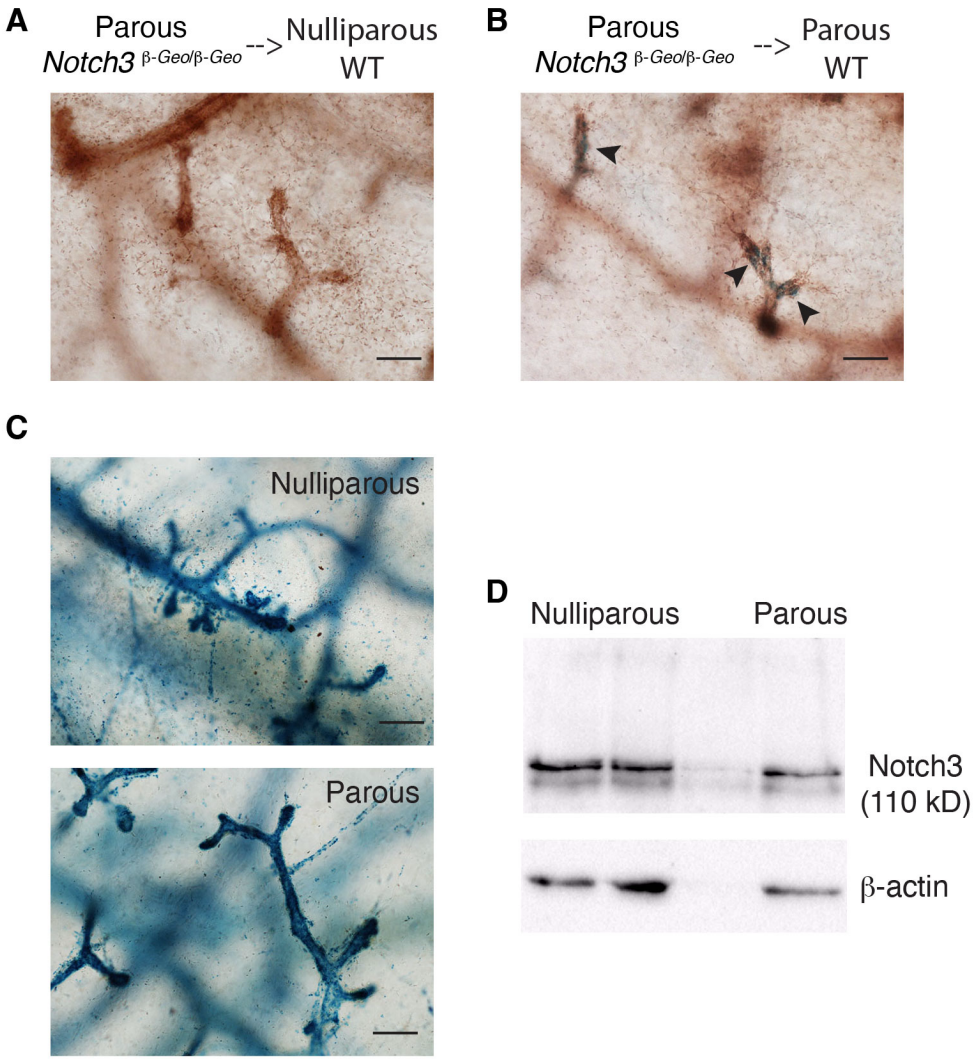

Fig. 4. Postpartum microenvironment is required for stem-cell activity of mammary epithelial cells isolated from parous *Notch3*$^{-/-}$ mice. (A) Whole-mount X-gal staining of a nulliparous wild-type mammary gland harvested at 8 weeks post transplantation of mammary epithelial cells isolated from a parous *Notch3*$^{\beta\text{-Geo}/\beta\text{-Geo}}$ donor. (B) Whole-mount X-gal staining of a parous wild-type mammary gland 8 weeks after injection of parous *Notch3*$^{\beta\text{-Geo}/\beta\text{-Geo}}$ mammary epithelial cells at involution day 21. X-Gal staining in (A) and (B) were counterstained with hematoxylin. (C) Whole-mount X-gal staining of mammary glands from nulliparous and parous *Notch3*$^{\beta\text{-Geo}/\beta\text{-Geo}}$ mice. (D) Western blot analysis for Notch3 in mammary tissues from age-matched nulliparous and parous wild type mice. Scale bars: 100 μm.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

This project was highlighted in the Annual Report of the Cancer Center and Research Institute at the University of Mississippi Medical Center that reaches to local communities for the purpose of enhancing public understanding and increasing interest in cancer research.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

It remains to be determined whether Notch3 exerts a tumor-suppressive function in part through the promotion of brown adipocyte differentiation in the postpartum mammary gland. We tried experiments outlined in SOW Major Task 4 to determine the impact of brown adipocytes on postpartum mammary gland. Since deletion of Prdm16 in mammary epithelial cells showed no effect, we will need to delete Prdm16 using Adiponectin-Cre to ablate brown adipocytes systemically.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style

1) The observation that mammary epithelial cells from the postpartum Notch3 mutant mice contributed specifically to the ductal/lobule buds in the host mice suggests that PI-MECs in Notch3 mutant mice are enriched in stem-like cells. Ductal/lobule buds in the mouse mammary glands are structurally similar to the terminal ductal lobular units in humans, the primary anatomical source of most breast cancers. This may represent a new avenue for the investigation of cellular origin and initiation of postpartum breast cancer.

2) Results from transplantation experiments suggest that postpartum microenvironment is required for the repopulating activity of PI-MECs isolated from parous Notch3 mutant mice, highlighting the importance of mammary gland microenvironment, which may include adipocytes, immune cells, fibroblasts, and blood and lymphatic vasculatures, for mammary stem cell activity and tumorigenesis.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to Report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:
- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to Report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:
- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to Report.

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

There were no significant changes in the project or its direction during this reporting period. However, we found that Notch3 knockout mice suppressed (rather than promoted) mammary tumor xenografts. We have identified candidates of Notch3 downstream genes that potentially function in the immune system. Thus, in addition to the proposed tumor-suppressive functions in the mammary epithelium and brown adipocytes, our results suggest an oncogenic role for Notch3 in tumor-infiltrating immune cells.

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to Report.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

COVID-19 pandemic had a negative impact on the progression of this project, including temporary shutdown of the lab and delays in routine orders due to supply chain issues. We requested and have been approved a 12-month no cost extension. During the extension period, we will complete all the remaining work, in particular, the Major Task 4--Determine whether brown adipocytes have an impact on pro-tumorigenic microenvironment in the postpartum mammary gland.

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Not applicable.

Significant changes in use or care of vertebrate animals

No significant changes in use or care of vertebrate animals.

Significant changes in use of biohazards and/or select agents

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

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  A manuscript has been prepared for submission to *Development*:
  Chung W-C, Egan SE, Xu K. A tumor-suppressive function of Notch3 in mammary gland via restricting parity-identified mammary epithelial cells.
  Acknowledgement of federal support (yes).

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each*

one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to Report.

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to Report.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to Report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to Report.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to Report.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance,

or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other*

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name: Keli Xu (PI)	No change
Name: Wen-Cheng Chung (Scientist I)	No change

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to Report.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*

(Manuscript to be submitted)

A tumor-suppressive function of Notch3 in mammary gland via restricting parity-identified mammary epithelial cells

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Abstract

Notch3 promotes mammary luminal cell specification. Forced Notch3 activation induced mammary tumors, however recent studies suggested a tumor-suppressive role for Notch3. Here we report the expression and functional analysis of Notch3 in the mouse mammary gland. Notch3 is expressed in the luminal compartment throughout mammary gland development, but switches to the basal cells at the initiation of post-lactational involution. Deletion of Notch3 caused a decrease of Notch activation in the luminal cells and diminished luminal progenitors at puberty, and reduced alveolar progenitors during pregnancy. Parous *Notch3*$^{-/-}$ mammary glands developed hyperplasia with accumulation of CD24^{hi}CD49f^{lo} cells, some of which progressed to invasive tumors with luminal feature. We found that deletion of Notch3 abolished Notch activation in the basal cells during involution, accompanied by decreased apoptosis and reduced brown adipocytes, leading to the expansion of parity-identified mammary epithelial cells (PI-MECs). Interestingly, postpartum microenvironment is required for stem cell activity of *Notch3*$^{-/-}$ PI-MECs. High expressions of NOTCH3 and UCP1 are associated with prolonged survival in patients with luminal breast cancer. These results highlight an unexpected tumor-suppressive function of Notch3 in parous mammary gland through the restriction of PI-MECs.