AWARD NUMBER: W81XWH-17-1-0027

TITLE: Prevention of Breast Cancer Skeletal Metastases with Parathyroid Hormone

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REPORT DATE: FEB 2019

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	2. REPORT TYPE	3. DATES COVERED
Feb 2019	Annual	1 Feb 2018 – 31 Jan 2019

4. TITLE AND SUBTITLE

Prevention of Breast Cancer Skeletal Metastases with Parathyroid Hormone

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-17-1-0027

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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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

During this research period we have demonstrated that parathyroid hormone (PTH) started at the same time as intramammary implantation of murine 4T1-BGL breast cancer cells can decrease metastases to the skeleton but not to lung, liver or spleen. This is associated with changes in expression of metastasis genes in bone. We have further demonstrated that 4 weeks of PTH can decrease engraftment and growth of murine or human breast cancer cells injected into the intratibial bone marrow cavity. In preliminary studies we have demonstrated that knockdown of the PTH receptor PTH1R in 4T1 breast cancer cells abrogates the effect of PTH on reducing bone metastases, as does removal of PTH1R signaling in osteoblasts.

15. SUBJECT TERMS

parathyroid hormone, breast cancer, metastases, osteoblasts, bone marrow

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

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INTRODUCTION

Breast cancer is the most common cancer among women, and by the time breast cancer has spread to distant organs it is invariably incurable. The skeleton is one of the more frequent sites affected by the spread of breast cancer because the skeleton produces many factors that attract cancer cells. Osteoporosis, a disease of fragile bones, is common among breast cancer patients due to bone loss from chemotherapy, premature menopause, and estrogen blockade. Parathyroid hormone (PTH, teriparatide) is the only treatment for osteoporosis that increases bone formation, and therefore could be useful for breast cancer patients. **However, the safety of PTH in breast cancer patients is unknown**. We have found that PTH decreases the spread of cancer cells to bones in mice. In this application, we will evaluate whether PTH started after breast cancer has been established can still reduce the spread of cancer to bone. We also propose experiments to understand how PTH works to cause these beneficial effects. If we can successfully demonstrate that PTH treatment of mice with breast cancer reduces the frequency of skeletal metastases, then trials could quickly be started in breast cancer patients since teriparatide is already an FDA-approved medication. The potential benefit to patients is great – by decreasing the frequency of skeletal metastases, which occur in over 70% of patients with advanced breast cancer, breast cancer patients would collectively experience less pain and fewer fractures.

KEYWORDS – parathyroid hormone, breast cancer, metastases, osteoblasts, bone marrow

ACCOMPLISHMENTS

What were the major goals of the project?

1. Specific Aim 1 – To determine whether PTH can reduce skeletal metastases from established breast cancer
    - **i.** Treatment of PTH concurrent with establishment of murine and human primary tumors
    - **ii.** Treatment of PTH concurrent with intratibial injection of murine and human breast cancer cells
    - **iii.** Treatment of PTH concurrent with establishment of murine primary tumor lacking PTH1R
2. Specific Aim 2 – To determine whether loss of PTH receptor signaling in osteoblasts promotes breast cancer skeletal metastases
    - **i.** Treatment of PTH concurrent with establishment of primary or intratibial tumor in PTH1R-OsxKO mice
    - **ii.** Treatment of PTH receptor antagonist concurrent with establishment of human primary or intratibial tumor
3. Specific Aim 3 – To examine the effects of PTH signaling on molecular interactions between tumor cells and osteoblasts
    - **i.** Co-culture 4T1-BGL cells and primary osteoblasts treated with PTH

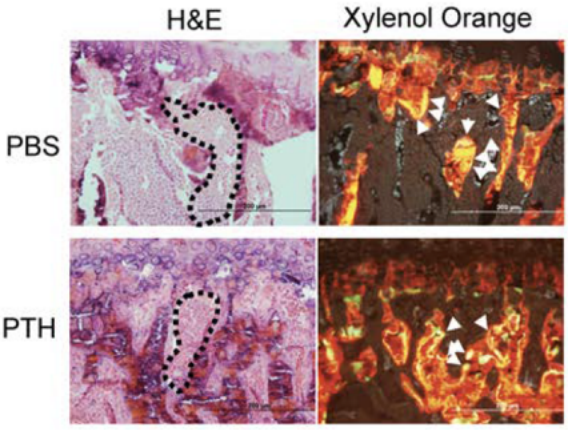
What was accomplished under these goals?

1. Specific Aim 1 – To determine whether PTH can reduce skeletal metastases from established breast cancer

i. Treatment of PTH concurrent with establishment of murine primary tumors

a. Intramammary injection of mice with 4T1-BGL cells and PTH or PBS

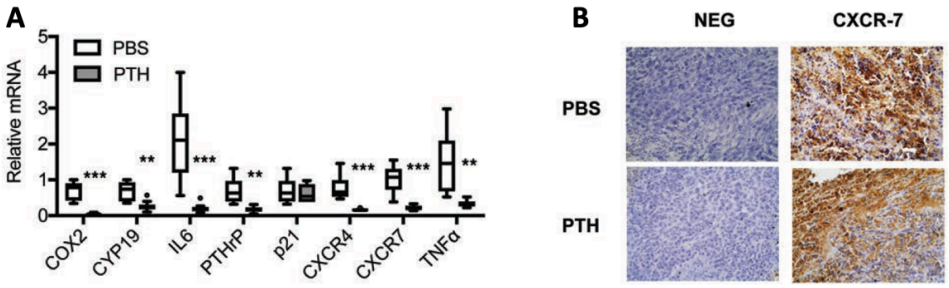
- 40 female Balb/c mice at 10 weeks of age received mammary fat pad injections with 4T1-BGL cells. ~150,000 cells, suspended in 50 µl of culture medium-Matrigel mixture, were injected directly into the 4th mammary fat pad on the left side. The following day mice were randomized to receive either PTH(1-34) 80 mcg/kg/day (Bachem) or phosphate-buffered saline (PBS) (n = 20 each) for 4 weeks. In the previous reporting period we reported on the bioluminescence imaging. We also performed histological analyses to demonstrate that tumor cells were found in areas of active bone formation (highlighted by double calcein labeling) (Figure 1).



- Figure 1. Representative sections of double calcein labeling in hind limbs with metastases from mice treated with PBS/PTH. Sections are stained with xylene orange to visualize calcein labeling (indicated with white arrowheads) or H&E to visualize corresponding histology (areas of tumor are indicated with dotted lines). Scale bar: 200 µm.

b. Identification of gene targets of PTH in hind limbs

- We isolated RNA from the primary tumor, skeletal tumors, and bones using for validation of candidate genes identified in Aim 3. Gene expression was determined by real-time PCR using the CFX96 real-time PCR detection system (BioRad, Hercules, CA), with gene-specific primers and SYBR green qPCR kit (Biorad, Hercules CA). Target gene expression were normalized to β-actin and the relative changes in mRNA levels were assessed by the comparative CT method. In the primary tumor we found that intermittent PTH attenuated the expression of several key genes involved in tumor growth and metastasis (Pthrp, Cxcr4, Cxcr7, Tnfα, and Il6) (Figure 2A). While expression of Cxcr7 mRNA was decreased with PTH treatment, no differences could be seen at the protein level (Figure 2B).

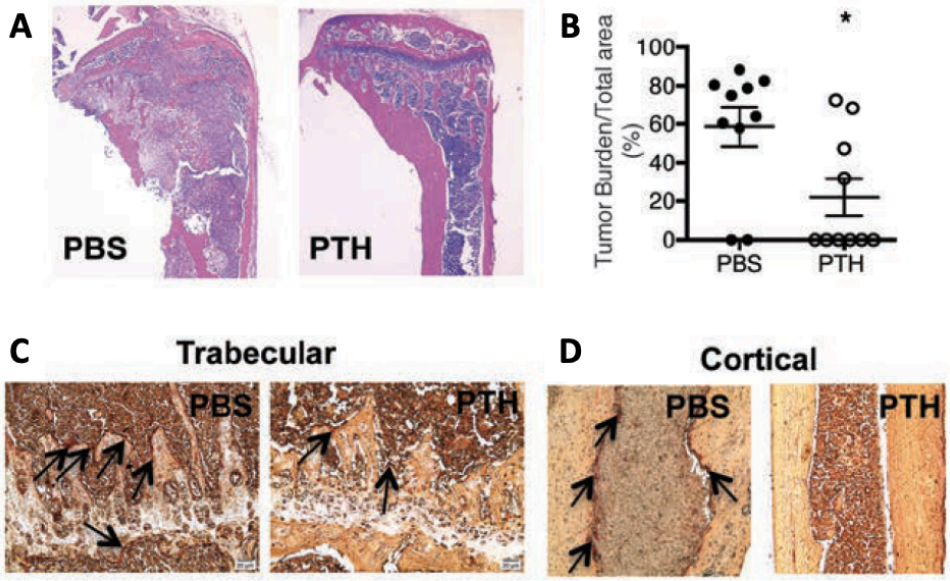


- Figure 2. (A) Expression of select target genes in primary tumors dissected from mice pretreated with PTH, as described in Figure 1A. (B) Representative CXCR7 staining in primary tumors treated with PTH, as described in Figure 1A (original magnification, ×40). All values represent mean ± SD (n = 10) for each group. **P < 0.01, ***P < 0.001 when compared with PBS group, by 1-way ANOVA with Bonferroni's test as post-hoc analysis.

ii. Treatment of PTH concurrent with intratibial injection of murine breast cancer cells

a. Intratibial injection of mice with 4T1-BGL cells and PTH or PBS (months 12-18)

- In the previous reporting period 4T1 murine breast cancer cells were injected into syngeneic Balb/c mice. Six-week-old mice were treated with either PTH (PTH 1-34; 80μg/kg/day) or PBS for 4 weeks prior to intratibial injection. 4T1 grown to 70% confluence were harvested during the log phase of growth and 1 x 10^3 cells were re-suspended in 20μl of sterile PBS. The tumor cell suspensions were then injected into the right tibiae of the mice using a 27-gauge needle under isoflurane anesthesia. The left tibia was injected with the same volume of PBS (sham-injected) to control for tumor cell injections. Treatments were continued for the next 4 weeks or until a 10% difference in measurement between the two hind limbs was observed. Hind limb thickness was measured weekly using digital calipers. Hind limbs were fixed in 10% formalin and processed for microCT analyses and immunohistochemistry, which were reported in the previous reporting period.
- We performed histological analyses which revealed marked osteolysis of bone microarchitecture in all 4T1-PBS mice and a significant reduction in tumor burden in 4T1-PTH mice (Figure 3A,B). Additionally, a greater number of tartrate-resistant acid phosphatase–positive (TRAP-positive) osteoclasts was found on the surfaces of both trabecular and cortical bone in the control mice when compared with PTH-treated mice (Figure 3C,D).



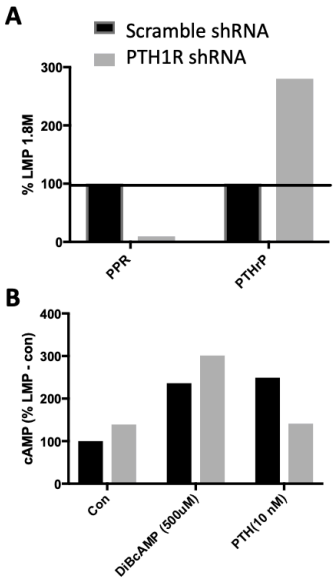
- Figure 3 (A) Representative H&E-stained images of tibia from PBS- and PTH-treated mice with intratibial injections of 4T1 cells (original magnification, ×4). (B) Quantitation of tumor burden. Representative TRAP staining of (C) trabecular region (original magnification, ×20) and (D) cortical region (original magnification, ×10) from tibiae of mice treated with PBS/PTH with intratibial injections of 4T1 cells. TRAP-positive osteoclasts are indicated by arrows. All values represent mean ± SD of n = 10 for each group. *P < 0.05, **P < 0.01 when compared with PBS group, by 1-way ANOVA with Bonferroni's test as post-hoc analysis.

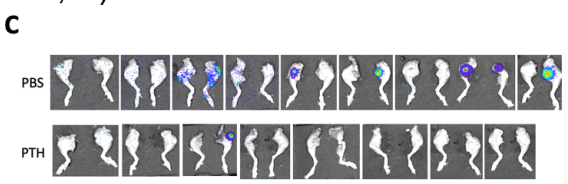
iii. Treatment of PTH concurrent with establishment of murine primary tumor lacking PTH1R

a. Intramammary injection of mice with 4T1-BGL cells lacking PTH1R and PTH or PBS

- In the previous reporting period we reported that series of MiR30-based shRNA's for murine Pthr1 were designed, synthesized by GeneArt and cloned into LMP vector and tested for their abilities to reduce the cAMP response to PTH treatment in osteosarcoma OS80 cells. The most effective shRNAs were Pthr1.358 and Pthr1.2112, which reduced cAMP production induced by PTH(1–34) by ~ 90 and ~ 60%, respectively (Ho, Oncogene 2015). These shRNA plasmids were kindly provided by Dr. Carl Walkley. We prepared retrovirus and infected 4T1-BGL cells then selected with puromycin. Once drug resistance was achieved, the expression of the shRNA was confirmed by flow cytometry for green fluorescent protein. Cells expressing the shRNA were then tested for Pthr1 expression by quantitative real-time PCR.
- We have transduced 4T1-BGL cells with shRNA targeting PTH1R to generate 4T1-PTH1RKD cells with knockdown of Pth1r mRNA (Figure 4A). We have confirmed functional loss of PTH1R signaling by examining cyclic AMP (cAMP) generation above basal is attenuated in Pth1r shRNA 4T1 cells (Figure 4B). ~150,000 cells, suspended in 50 µl of culture medium-Matrigel mixture, were injected directly into the 4th mammary fat

pad on the left side. The following day mice were randomized to receive either PTH(1-34) 80 mcg/kg/day (Bachem) or phosphate-buffered saline (PBS) (n = 20 each) for 4 weeks. Preliminary analyses of bioluminescence imaging studies reveal that in the absence of Pth1r expression in 4T1-BGL cells, PTH can no longer decrease the frequency of breast cancer metastases to bone (Figure 4C, D)





D

	Scramble shRNA +PBS	Scramble shRNA +PTH	PTH1R shRNA +PBS	PTH1R shRNA +PTH
Lungs	12/20	10/20	15/20	13/20
Liver	5/20	4/20	1/20	3/20
Spleen	4/20	4/20	1/20	4/20
Hind limbs	**11/20**	**3/20***	9/20	9/20

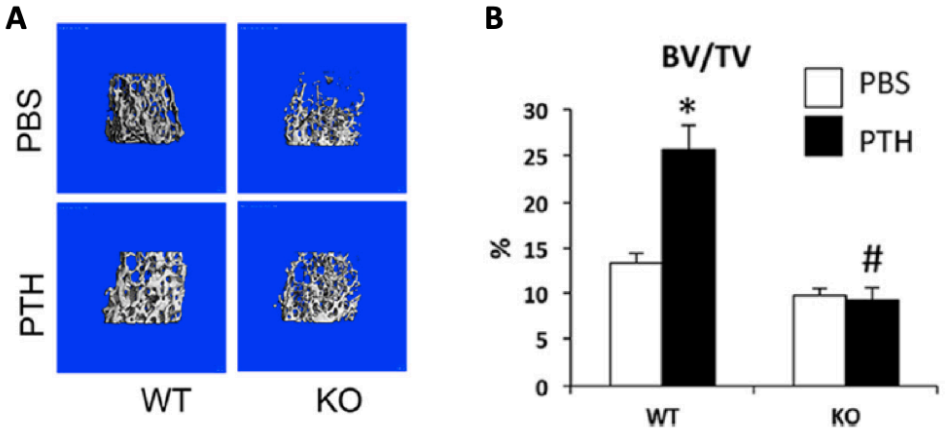
*$P = 0.004$

- Figure 4 shRNA knockdown of PTH1R in 4T1 breast cancer cells. (A) Relative mRNA levels for Pth1r (PPR) and Pthrp in cells transduced with scramble (black) or Pth1r (gray) shRNA. Knockdown of Pth1R decreases Pth1r but not Pthrp mRNA levels. (B) Basal cyclic AMP (cAMP) levels are unchanged by Pth1r mRNA. Di-butyryl cAMP increases cAMP levels in both control and Pth1r shRNA breast cancer cells. However PTH treatment increases cAMP only in control breast cancer cells. (C) Representative BLI images of metastases to the hind limbs in. (D) Numbers of metastases to lungs, liver, spleen and hind limbs.

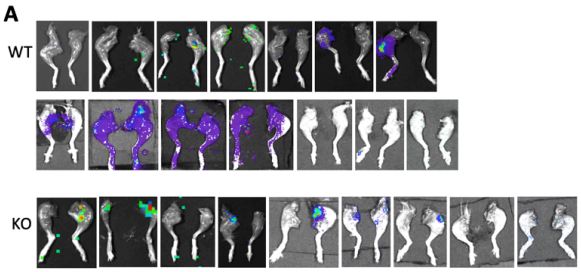
2. Specific Aim 2 – To determine whether loss of PTH receptor signaling in osteoblasts promotes breast cancer skeletal metastases
   - **i.** Treatment of PTH concurrent with establishment of primary tumor in PTH1R-OsxKO mice
     - **a.** Intramammary injection of $G_s\alpha$-OsxKO and control mice with 4T1-BGL and PTH or PBS
       - We backcrossed PTH1R-OsxKO mice to a Balb/c background for a minimum of 5 generations, and confirmed that 4T1-BGL cells can engraft in PTH1R-OsxKO control littermates. Unfortunately for reasons as yet unclear, PTH1R-OsxKO mice do not survive into adulthood on a Balb/c background. We therefore switched to using a model of impaired PTH1R signaling. The G stimulatory protein subunit $G_s\alpha$ mediates the anabolic actions of PTH1R. We have demonstrated that mice lacking $G_s\alpha$ in osteoprogenitors ($G_s\alpha$-OsxKO mice) cannot respond to anabolic PTH with

an increase in bone volume (Figure 5), and recapitulate many of the skeletal and hematopoietic phenotypes of PTH1R-OsxKO mice.

- We have generated $G_s\alpha$-OsxKO mice by mating $G_s\alpha$(fl/fl) mice with transgenic mice in which Cre recombinase is driven by the Osterix promoter. Doxycycline (100 μg/ml in drinking water) is administered until weaning to delay expression of Cre recombinase and allow for survival of $G_s\alpha$-OsxKO mice until adulthood. $G_s\alpha$(fl/fl) littermates are used as controls for all the experiments. We have backcrossed $G_s\alpha$-OsxKO mice to a Balb/c background for a minimum of 5 generations, and confirmed that 4T1-BGL cells can engraft in $G_s\alpha$-OsxKO control littermates. ~150,000 cells, suspended in 50 μl of culture medium-Matrigel mixture, were injected directly into the 4th mammary fat pad of $G_s\alpha$-OsxKO and control on the left side. A total of 20 mice of each genotype will be treated with PTH(1-34) 80 mcg/kg/day or PBS for 4 weeks. We have completed PBS treatment on 14 control and 10 $G_s\alpha$-OsxKO mice (Figure 6). Preliminary analyses demonstrate that in the absence of PTH1R signaling, PTH no longer reduces breast cancer metastases to bone.



- Figure 5 Intermittent PTH does not increase trabecular bone mass in $G_s\alpha$-OsxKO female mice. (A) Micro-CT representative images of distal femur trabecular bone in female control (WT) and $G_s\alpha$-OsxKO (KO) mice treated with 10 μg/ml doxycycline from plug until delivery, then injected with 80 μg/kg/day PTH(1–34) or PBS 5 days/week (wk) starting at 8 weeks of age for 4 weeks. (B) Micro-CT analysis of bone volume (BV/TV).



B

	WT	KO
Lungs	12/14	8/10
Liver	6/14	4/10
Spleen	6/14	2/10
Hind limbs	8/14	7/10

- Figure 6 (A) Representative BLI images of metastases to the hind limbs of control (WT) and $G_s\alpha$-OsxKO (KO) mice in the treatment model. (B) Numbers of metastases in lungs, liver, spleen and hind limbs.

3. Specific Aim 3 – To examine the effects of PTH signaling on molecular interactions between tumor cells and osteoblasts
   - **i.** Co-culture 4T1-BGL cells and primary osteoblasts treated with PTH
     - **a.** Examine the expression of tumor metastasis genes induced by PTH
       - Total RNA was isolated from MC3T3-E1 cells using the Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. 5 μg RNA was subjected to reverse transcription using the SuperScript III first strand synthesis kit (Invitrogen) and gene expression determined by real-time PCR using the CFX96 real-time PCR detection system (BioRad, Hercules, CA), with gene-specific primers and SYBR green qPCR kit (Biorad, Hercules CA). Target gene expressions were normalized to β-actin and the relative changes in mRNA levels were assessed by the comparative CT method. To determine the effects of intermittent PTH on the genes involved in tumor metastasis, RNA was isolated from MC3T3-E1 cells treated with intermittent PTH and co-cultured with 4T1 cells, and expression levels of 84 tumor metastasis genes were determined using the RT2 Profiler Tumor metastasis PCR Array (Qiagen) according to the manufacturer's instructions (Figure 7).

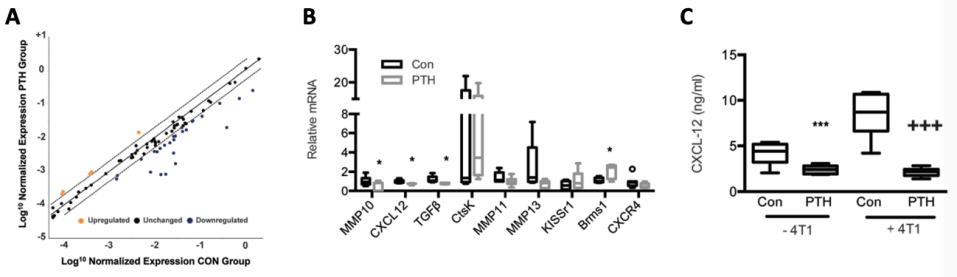


Figure 7 (A) RT2 Profiler Tumor PCR Metastasis PCR Array analysis of MC3T3-E1 gene expression. (B) Genes with >2 fold changes were further validated with real-time quantitative PCR. (C) CXCL12 protein levels in MC3T3-E1 cells treated with control or PTH in the absence or presence of 4T1 cells. All values represent mean ± SEM of at least 3 individual experiments conducted in triplicate. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001 when compared with control (con) group. +++P < 0.001 when compared with control (+4T1), by 1-way ANOVA with Bonferroni's test as post-hoc analysis.

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

- Nothing to report

How were the results disseminated to communities of interest?

- Our initial results have been published in JCI Insight.

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

1. Specific Aim 1 – To determine whether PTH can reduce skeletal metastases from established breast cancer
   i. Treatment of PTH concurrent with intratibial injection of murine and human breast cancer cells

a. Identification of gene targets of PTH in hind limbs
    - We will isolate RNA from the primary tumor, skeletal tumors, and bones using for validation of candidate genes identified in Aim 3. Total RNA will be isolated using the Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturers instructions. 5 μg RNA will be subjected to reverse transcription using the SuperScript III first strand synthesis kit (Invitrogen) and gene expression determined by real-time PCR using the CFX96 real-time PCR detection system (BioRad, Hercules, CA), with gene-specific primers and SYBR green qPCR kit (Biorad, Hercules CA). Target gene expressions will be normalized to β-actin and the relative changes in mRNA levels were assessed by the comparative CT method.

ii. Treatment of PTH concurrent with establishment of murine primary tumor lacking PTH1R
    a. Intramammary injection of mice with 4T1-BGL cells lacking PTH1R and PTH or PBS
        - We have transduced 4T1-BGL cells with shRNA targeting PTH1R to generate 4T1-PTH1RKD cells with knockdown of Pth1r mRNA. ~150,000 4T1-PTH1RKD or 4T1-control cells, suspended in 50 µl of culture medium-Matrigel mixture, will be injected directly into the 4th mammary fat pad on the left side. The following day mice were randomized to receive either PTH(1-34) 80 mcg/kg/day (Bachem) or phosphate-buffered saline (PBS) (n = 20 each) for 4 weeks.
    b. Bioluminescence and micro-CT imaging of tumor and metastasis growth
        - Bioluminescence imaging (BLI) will be performed weekly on Balb/c female mice with intramammary implantation of 4T1-PTH1RKD or 4T1-control cells and treated with PBS or PTH under isoflurane anesthesia using an IVIS 200 Imaging platform (Caliper Life Sciences, Inc., Hopkinton, MA) following injection of luciferin substrate (3.33 mg/mouse). Luciferin substrate will be injected into the mice 10 minutes prior to euthanasia. Average radiance will be measured and quantified for all organs using the Living Image Software version 4.3.1.
        - Tibiae dissected from nude mice with intramammary injection of 4T1-PTH1RKD cells will be scanned with a high resolution micro tomographic system, Scanco VicaCT 40 (Scanco Medical AG, Switzerland). Tibiae will be aligned perpendicular to the scanning axis and the entire tibia scanned. The scanning will be conducted using the following settings: 55 kVp and 145 µA intensity, 200ms integration time, 1000 projections, with a 0.5mm Al filter at a resolution of 10 µm/voxel. Each region of interest consists of approximately 100 micro-CT sections beginning 5% distal to the tibial growth plate and including the tibial metaphysis. 3D structural analyses will be completed using the accompanying software.
    c. Intratibial injection of mice with 4T1-BGL cells lacking PTH1R and PTH or PBS
        - We have transduced 4T1-BGL cells with shRNA targeting PTH1R to generate 4T1-PTH1RKD cells with knockdown of Pth1r mRNA. 1 x 10^3 4T1-PTH1RKD or 4T1-control breast cancer cells will be re-suspended in 20µl of sterile PBS and injected into the right tibiae of the mice using a 27-

gauge needle under isoflurane anesthesia. A slow drilling motion will be used to advance the needle to avoid fractures. The left tibia will be injected with the same volume of PBS (sham-injected) to control for tumor cell injections. Mice will be randomized to either PTH (PTH 1-34; 80μg/kg/day) or PBS following intratibial injection and treatments will be continued for the next 4 weeks or until a 10% difference in measurement between the two hind limbs is observed. Hind limb thickness will be measured weekly using digital calipers. Hind limbs will be fixed in 10% formalin and processed for microCT analyses and immunohistochemisry.

d. Bioluminescence and micro-CT imaging of tumor and metastasis growth
   - Bioluminescence and micro-CT imaging will be performed as described above.

2. Specific Aim 2 – To determine whether loss of PTH receptor signaling in osteoblasts promotes breast cancer skeletal metastases
   i. Treatment of PTH concurrent with establishment of intratibial tumor in PTH1R-OsxKO mice
      a. Intratibial injection of $G_s\alpha$-OsxKO and control mice with 4T1-BGL and PTH or PBS
         - We have generated $G_s\alpha$-OsxKO mice by mating $G_s\alpha$(fl/fl) mice with transgenic mice in which Cre recombinase is driven by the Osterix promoter. Doxycycline (100 μg/ml in drinking water) is administered until weaning to delay expression of Cre recombinase and allow for survival of $G_s\alpha$-OsxKO mice until adulthood. $G_s\alpha$(fl/fl) littermates are used as controls for all the experiments. 1 x 10^3 4T1 breast cancer cells will be re-suspended in 20μl of sterile PBS and injected into the right tibiae of the mice using a 27-gauge needle under isoflurane anesthesia. A slow drilling motion will be used to advance the needle to avoid fractures. The left tibia will be injected with the same volume of PBS (sham-injected) to control for tumor cell injections. Mice will be randomized to either PTH (PTH 1-34; 80μg/kg/day) or PBS following intratibial injection and treatments will be continued for the next 4 weeks or until a 10% difference in measurement between the two hind limbs is observed. Hind limb thickness will be measured weekly using digital calipers. Hind limbs will be fixed in 10% formalin and processed for microCT analyses and immunohistochemisry.
      b. Bioluminescence and micro-CT imaging of tumor and metastasis growth in $G_s\alpha$-OsxKO mice
         - Bioluminescence imaging (BLI) will be performed weekly on control or KO female mice with intratibial injection of 4T1 cells and treated with PBS or PTH under isoflurane anesthesia using an IVIS 200 Imaging platform (Caliper Life Sciences, Inc., Hopkinton, MA) following injection of luciferin substrate (3.33 mg/mouse). Luciferin substrate will be injected into the mice 10 minutes prior to euthanasia. Average radiance will be measured and quantified for all organs using the Living Image Software version 4.3.1.
         - Tibiae dissected from nude mice with intratibial injection of 4T1 cells will be scanned with a high resolution micro tomographic system, Scanco

VicaCT 40 (Scanco Medical AG, Switzerland). Tibiae will be aligned perpendicular to the scanning axis and the entire tibia scanned. The scanning will be conducted using the following settings: 55 kVp and 145 μA intensity, 200ms integration time, 1000 projections, with a 0.5mm Al filter at a resolution of 10 μm/voxel. Each region of interest consists of approximately 100 micro-CT sections beginning 5% distal to the tibial growth plate and including the tibial metaphysis. 3D structural analyses will be completed using the accompanying software.

c. Identification of gene targets of PTH in hind limbs of $G_s\alpha$-OsxKO mice
    - We will isolate RNA from the skeletal tumors and bones using for validation of candidate genes identified in Aim 3. Total RNA will be isolated using the Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturers instructions. 5 μg RNA will be subjected to reverse transcription using the SuperScript III first strand synthesis kit (Invitrogen) and gene expression determined by real-time PCR using the CFX96 real-time PCR detection system (BioRad, Hercules, CA), with gene-specific primers and SYBR green qPCR kit (Biorad, Hercules CA). Target gene expressions will be normalized to β-actin and the relative changes in mRNA levels were assessed by the comparative CT method.

ii. Treatment of PTH receptor antagonist concurrent with establishment of human primary or intratibial tumor

a. Intramammary injection of Nude mice with MDA-MB-231-fLuc cells and PTH(7-34) or PBS
    - Female nude mice (Charles River Laboratories) at 10 weeks of age will receive mammary fat pad injections with MDA-MB-231-fLuc cells. ~150,000 cells, suspended in 50 μl of culture medium-Matrigel mixture, will be injected directly into the 4th mammary fat pad on the left side. The following day mice will be randomized to receive either PTH(7-34) 80 mcg/kg/day (Bachem) or phosphate-buffered saline (PBS) (n = 30 each) for 4 weeks.

b. Bioluminescence imaging and micro-CT of tumor and metastasis growth in Nude mice
    - Bioluminescence and micro-CT imaging will be performed as described above.

c. Identification of gene targets of PTH in hind limbs of nude mice
    - We will isolate RNA from the primary tumor, skeletal tumors, and bones using for validation of candidate genes identified in Aim 3. Total RNA will be isolated using the Trizol reagent (Invitrogen, Carlsbad, CA) according to the manufacturers instructions. 5 μg RNA will be subjected to reverse transcription using the SuperScript III first strand synthesis kit (Invitrogen) and gene expression determined by real-time PCR using the CFX96 real-time PCR detection system (BioRad, Hercules, CA), with gene-specific primers and SYBR green qPCR kit (Biorad, Hercules CA). Target gene expressions will be normalized to β-actin and the relative changes in mRNA levels were assessed by the comparative CT method.

3. Specific Aim 3 – To examine the effects of PTH signaling on molecular interactions between tumor cells and osteoblasts
    i. Co-culture 4T1-BGL cells and primary osteoblasts treated with PTH
        a. Examine the expression of tumor metastasis genes induced by PTH
            - In addition to VCAM-1, we have identified CXCL12, CD44, TGFb2, and EphB2 as other candidate genes downregulated in MC3T3 osteoblasts by PTH treatment. For each of these genes, we will retrovirally overexpress the gene in breast cancer cells and osteoblasts. We will then perform co-culture assays to determine the effects of PTH treatment when CXCL12, CD44, TGFβ1, or EphB2 are overexpressed. For those factors that blunt the inhibition of PTH on breast cancer cell migration, we will examine expression of pathway components by immunostaining of skeletal metastases in mice treated with PTH compared to PBS control, to examine whether expression is regulated by PTH treatment.

IMPACT

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

- We have demonstrated that in mice, recombinant parathyroid hormone can decrease breast cancer metastases to bone.

What was the impact on other disciplines?

- Nothing to report.

What was the impact on technology transfer?

- Nothing to report.

What was the impact on society beyond science and technology?

- Since recombinant PTH (teriparatide) is already FDA-approved, clinical trials could be initiated once pre-clinical testing demonstrates safety. This could have significant clinical impact, by reducing the occurrence of skeletal metastases and its attendant complications, including fracture and pain.

CHANGES/PROBLEMS

Changes in approach and reasons for change

- Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

- Nothing to report.

Changes that had significant impact on expenditures

- Nothing to report.

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

- Nothing to report.

Significant changes in use or care of human subjects

- Nothing to report.

Significant changes in use or care of vertebrate animals

- Nothing to report.

Significant changes in use of biohazards and/or select agents

- Nothing to report.

PRODUCTS

Publications, conference papers, and presentations

- Journal publications
    i. Swami, JCI Insight 2017
- Books or other non-periodical, one-time publications
- Other publications, conference papers, and presentations
    i. Lectures at MD Anderson (Houston, TX), Massachusetts General Hospital (Boston, MA), University of Miami (Miami, FL), Vanderbilt University (Nashville, TN), University of Michigan (Ann Arbor, MI), Baylor College of Medicine (Houston, TX)
    ii. Invited speaker at ENDO 2019 annual meeting of the Endocrine Society (New Orleans, LA)

Website(s) or other Internet site(s)

- Nothing to report

Technologies or techniques

- Nothing to report

Inventions, patent applications, and/or licenses

- Nothing to report

Other products

- PTH1R-deficient 4T1 breast cancer cells

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name	Joy Wu
Project Role	PI
Research Identifier	0000-0002-1897-4503
Nearest person month worked	3
Contribution to project	Dr. Wu has designed the experiments, supervised Drs. Swami and Kimura, and analyzed the data and conclusions.
Funding support	Nothing to report

Name	Srilatha Swami
Project Role	Research Associate
Research Identifier	
Nearest person month worked	6
Contribution to project	Dr. Swami has performed the mouse experiments and the osteoblast migration assays.
Funding support	Nothing to report

Name	Takaharu Kimura
Project Role	Postdoctoral fellow
Research Identifier	
Nearest person month worked	6
Contribution to project	Dr. Kimura has performed work with the mouse, immunostaining and flow cytometry experiments.
Funding support	Nothing to report

Name	Hui Zhu
Project Role	Research Associate
Research Identifier	
Nearest person month worked	2
Contribution to project	Dr. Zhu has performed histology and immunostaining experiments.
Funding support	Nothing to report

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

- Dr. Joy Wu
    i. Previously pending grant now active since the last reporting period
        a. NIH 2 R01 AR063717-06 - Enhanced Bone Healing Around Implants by Transplanted NF-kB Driven Immunomodulating MSCs (PI – Goodman)
            - 09/14/2012 – 07/31/2022
            - 0.6 calendar months
            - $220,000
            - The major goal of this project is to study the role of NF-kB and inflammation in implant-associated bone loss.
            - Overlap: none
    ii. Previously pending grant now active since the last reporting period
        a. Radius Health, Inc. - G Stimulatory Protein-Mediated Signaling Downstream of PTH1R (PI – Wu)
            - 05/01/2018 – 04/30/2020
            - 0.6 calendar months
            - $62,241
            - This project will investigate the role of G stimulatory signaling in the bone anabolic effects of abaloparatide.
            - Overlap: none
    iii. Previously pending grant now active since the last reporting period
        a. NIH 1 R56 DK112869 - Role of the Parathyroid Hormone Receptor in Osteoblast Support of Hematopoiesis (PI – Wu)
            - 07/01/2018 – 06/30/2019
            - 0.36 calendar months
            - $61,173
            - This project will examine the mechanisms by which parathyroid hormone signaling in osteoblasts regulates bone marrow and spleen erythropoiesis.
            - Overlap: none
- Dr. Mark Pegram
    i. Previously active grant closed since the last reporting period
        a. National Institutes of Health - HER2-targeted exosomal delivery of therapeutic mRNA for enzyme pro-drug therapy (PI – Matin)
            - 08/01/2013 - 07/31/2018
            - 0.36 calendar
            - $616,062 (Subs: $0)
            - Overlap: none
    ii. Previously active grant closed since the last reporting period
        a. The Breast Cancer Research Foundation - Overcoming Resistance to Antibody-Dependent Cell-Mediated Cytotoxicity in Breast Cancer (PI – Pegram)
            - 10/01/2014 - 09/30/2018
            - 1.2 calendar
            - $211,996 (Subs: $0)
            - Overlap: none
    iii. Previously active grant closed since the last reporting period

a. Susan G. Komen Breast Cancer Foundation - Patient-specific profiles and biomarkers of resistance in HER2+ breast cancer (PI – Curtis)
    - 05/19/2015 - 09/24/2018
    - 0.24 calendar
    - $171,958 (Subs: $34,541)
    - Overlap: none

iv. Previously active grant closed since the last reporting period
    - MACRO - A Phase 3, Randomized Study of Margetuximab Plus Chemotherapy vs Trastuzumab Plus Chemotherapy in the Treatment of Patients with HER2+ Metastatic Breast Cancer Who Have Received Two Prior Anti-HER2 Therapies and Require Systemic Treatment (PI – Pegram)
    - 11/25/2015 - 11/24/2018
    - 0.14 calendar
    - $388,990 (Subs: $0)
    - Overlap: none

v. Previously active grant closed since the last reporting period
    - MedImmune LLC / Covance, Inc. - A Phase 1 Multicenter, Open-label, Dose-escalation, and Dose-expansion Study to Evaluate the Safety, Pharmacokinetics, Immunogenicity, and Antitumor Activity of MEDI4276 in Subjects with Select HER2-expressing Advanced Solid Tumors (PI – Pegram)
    - 12/01/2015 - 11/30/2018
    - 0.51 calendar
    - $556,474 (Subs: $0)
    - Overlap: none

vi. Previously pending grant now active since last reporting period:
    a. Breast Cancer Research Foundation - Overcoming Resistance to Antibody-Dependent Cell-Mediated Cytoxicity in Breast Cancer (PI – Pegram)
        - 10/01/18 – 09/30/19
        - 1.2 calendar
        - $209,523
        - Overlap: none

vii. Previously pending grant now active since last reporting period:
    a. Odonate - A multinational, multicenter, randomized , Phase 3 Study of Tesetaxel plus a reduced dose of Capecitabine versus Capecitabine along in patients with HER2 Negative, Hormonal Receptor Positive, Locally Advanced or Metastatic Breast Cancer previously treated with Taxane (PI – Pegram)
        - 07/02/2018-07/31/2024
        - 0.81 calendar
        - $884,074
        - Overlap: none

viii. Previously pending grant now active since last reporting period:
    a. G1 Therapeutics - A Phase 1, Open-Label, Multicenter Study to Assess the Safety, Tolerability, Pharmacokinetics, and Preliminary Antitumor Activity of

Ascending Doses of G1T48 in Women with Estrogen Receptor-Positive, HER2-Negative Advanced Breast Cancer (PI – Pegram)

- 12/01/2018-12/31/2020
- 0.1 calendar
- $276,264
- Overlap: none

What other organizations were involved as partners?

- Nothing to report

SPECIAL REPORTING REQUIREMENTS

Collaborative awards

- Nothing to report

Quad charts

- Nothing to report

APPENDICES

None.