

AD _____

Award Number: DAMD17-99-1-9401

TITLE: The Role of Estrogen Receptor- α in Breast Cancer
Metastases to Bone

PRINCIPAL INVESTIGATOR: Theresa A. Guise, M.D.

CONTRACTING ORGANIZATION: The University of Texas Health
Science Center at San Antonio
San Antonio, Texas 78284

REPORT DATE: September 2000

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

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

OMB No. 074-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE September 2000	3. REPORT TYPE AND DATES COVERED Annual (1 Sep 99 - 31 Aug 00)	
4. TITLE AND SUBTITLE The Role of Estrogen Receptor- α in Breast Cancer Metastases to Bone			5. FUNDING NUMBERS DAMD17-99-1-9401	
6. AUTHOR(S) Theresa A. Guise, M.D.				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) The University of Texas Health Science Center at San Antonio San Antonio, Texas 78284 E-MAIL: guise@uthscsa.edu			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. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES			20010926 135	
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 Words) Breast cancer commonly metastasizes to the skeleton in patients with advanced disease to cause either bone destruction or new bone formation. Since patients with breast cancer may survive several years with their bone metastases, it is important to understand the pathophysiology of this process in order to improve therapy and prevention. The proposed work seeks to investigate tumor cell-bone interactions in breast cancer metastases to bone with specific attention to the role of 1) estrogen receptor- α (ER- α in mediating tumor production of bone-active factors to cause osteolytic and osteoblastic metastases using a mouse model of bone metastases and 2) bone-derived transforming growth factor β (TGF β) in modulating the effects of ER- α on tumor cell growth in bone. Defining the mechanisms responsible for breast cancer metastases to bone will provide insight into future therapy and prevention. <i>The following hypotheses will be tested:</i> 1. Estrogen stimulates breast cancer cell production of factors which disrupt normal bone remodeling to result in osteolytic or osteoblastic metastases. 2. Estrogen stimulates PTHrP production by TGF β -responsive breast cancer cells to result in osteolytic metastases. TGF β enhances ER- α -mediated transcriptional activity in breast cancer cells to stimulate growth. 3. Estrogen stimulates production of osteoblastic factors, such as ET-1, by breast cancer cells which are TGF β unresponsive. Restoration of TGF β responsiveness should result in PTHrP production and osteolytic metastases. Three specific aims were proposed to test the hypotheses and we report here the progress for Specific aim 1 in year 1 of this Academic Award: 1. To determine the role of ER- α in osteolytic or osteoblastic breast cancer metastases to bone using an in vivo model. Stable MDA-MB-231 cell lines were constructed which express wild-type ER- α and mutants Ser47Thr, Lys531Glu, and Tyr537Asn. MDA-MB-231 clones which express ER- α (Tyr537Asn) mutant demonstrated increased ER-mediated transcriptional activity in the absence of estradiol, as assessed by transient transfection with the ERE-luciferase compared with empty vector control. Transcriptional activity of the stable clones was not affected by estradiol treatment, but exogenous TGF β 1 increased ERE-luciferase activity in all stable clones. Basal as well as TGF β -stimulated PTHrP secretion by the Tyr537Asn ER α mutant clones was increased compared with the empty vector controls. These data suggest that ER- α -mediated transcription is associated with increased tumor production of PTHrP. This, in combination with the effect of TGF β to enhance ER- α -mediated transcription, and potentially growth, may be a mechanism for the propensity of breast cancer to metastasize to the skeleton.				
14. SUBJECT TERMS Breast Cancer, bone metastases, PTHrP, TGF β , ER- α			15. NUMBER OF PAGES 11	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited	

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

Table of Contents

Cover.....	1
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	4-7
Key Research Accomplishments.....	8
Reportable Outcomes.....	8-9
Conclusions.....	9
References.....	10
Appendices.....	11

INTRODUCTION

Breast cancer commonly metastasizes to the skeleton in patients with advanced disease to cause either bone destruction (osteolytic metastases) or new bone formation (osteoblastic metastases) and significant morbidity^{3,5}. Since patients with breast cancer may survive several years with their bone metastases, it is important to understand the pathophysiology of this process in order to improve therapy and prevention. The proposed work seeks to investigate tumor cell-bone interactions in breast cancer metastases to bone with specific attention to the role of 1) estrogen receptor- α (ER- α) in mediating tumor production of bone-active factors to cause osteolytic and osteoblastic metastases using a mouse model of bone metastases and 2) bone-derived transforming growth factor β (TGF β) in modulating the effects of ER- α on tumor cell growth in bone. A constitutively active ER- α (Tyr537Asn), identified from a bone metastases¹⁰, when expressed in human breast cancer cells is associated with increased production of parathyroid hormone-related protein (PTHrP), a stimulator of osteolytic metastases. Furthermore, TGF β enhances the ER- α -mediated transcriptional activity induced by the Tyr537Asn in human breast cancer cells. Defining the mechanisms responsible for breast cancer metastases to bone will provide insight into future therapy and prevention. *The following hypotheses will be tested:*

- 1. Estrogen stimulates breast cancer cell production of factors which disrupt normal bone remodeling to result in osteolytic or osteoblastic metastases.**
- 2. Estrogen stimulates PTHrP production by TGF β -responsive breast cancer cells to result in osteolytic metastases. TGF β enhances ER- α -mediated transcriptional activity in breast cancer cells to stimulate growth.**
- 3. Estrogen stimulates production of osteoblastic factors, such as ET-1, by breast cancer cells which are TGF β unresponsive. Restoration of TGF β responsiveness should result in PTHrP production and osteolytic metastases.**

The following specific aims are proposed to test the hypotheses:

- 1. To determine the role of ER- α in osteolytic or osteoblastic breast cancer metastases to bone using an in vivo model.** Wild-type ER- α and various mutants (Ser47Thr, Lys531Glu, and Tyr537Asn) will be stably transfected into breast cancer cell lines which are known to cause either osteolytic or osteoblastic metastases in a mouse model (MDA-MB-231, ZR-75-1, MCF-7, T47D, MDA-MB-468) as well as into cell lines which are tumorigenic in nude mice but do not cause bone metastases and clonal lines isolated. In vitro growth, PTHrP production, ET-1 production, TGF β -responsiveness, ER- α -mediated transcriptional activity and effect of exogenous estrogens and antiestrogens will be tested in stable cell lines. In vivo, the effect of expression of wt ER- α or mutants on bone metastases will be studied in a mouse model.
- 2. To determine if the effect of TGF β to increase ER- α -mediated transcriptional activity is specific for the constitutively active ER- α Tyr537Asn mutant compared with wt ER- α or is cell-specific.**

Stable MDA-MB-231 cell lines expressing ER- α mutants or wt will be treated with TGF β , with or without estrogens or antiestrogens. ER- α -transcriptional activity will be assessed by transient transfection with ERE-luc and PTHrP secretion into conditioned media will be assessed by immunoradiometric assay. ER- α mutants (Ser47Thr, Lys531Glu, and Tyr537Asn) or wt will be stably expressed in ER-positive cell lines (ZR-75-1, MCF-7 (both lines), and T47D) and assessed as in the MDA-MB-231 stable constructs.

- 3. To determine the relationship between TGF β signaling and ER- α -mediated transcription.** Specific molecular aspects to be addressed include 1) whether these effects are mediated through the known TGF β serine-threonine kinase-Smad signaling pathway and 2) whether TGF β enhances production of nuclear receptor coactivators of ER response, such as AIB1 or SRC-1 to enhance ER-dependent transcription. ER- α mutants (Ser47Thr, Lys531Glu, and Tyr537Asn) or wt will be transiently transfected into stable MDA-MB-231 clones which stably express one the following components of the

TGF β receptor-signaling pathway: truncated (dominant-negative) type II receptor, constitutively active type I receptor, Smad2 dominant-negative along with ERE-luciferase reporter construct. Cells will be treated with TGF β and ER- α -mediated transcriptional activity will be assessed. Western blots will be performed on cell lysates for nuclear coactivators of ER response, AIB1 and SRC-1. In each specific aim, ER- α -positive (MCF-7) and ER- α -negative (MDA-MB-468) cell lines in which both TGF β -responsive and -unresponsive sublines exist will be used to assess the interaction of TGF β -ER- α within the same cell line.

BODY

The research accomplishments completed during year 1 are described according to the approved statement of work. Tasks 1-3 were originally scheduled for completion by month 18.

STATEMENT OF WORK

1. To determine the role of ER- α in breast cancer cells which cause osteolytic or osteoblastic metastases. (Months 1-18). Rationale: Women with ER-positive primary tumors are more likely to develop bone metastases^{3,7}. Although scant data suggest that estrogen may regulate PTHrP expression in the uterus⁹, and in a breast cancer cell line, there is no clear relationship between PTHrP and ER in primary breast cancer. The sparse clinical data available on ER expression in breast cancer bone metastases indicate that 60-75% are ER-negative⁴ despite the fact that women with ER-positive primary tumors are more likely to develop bone metastases. Furthermore, bone metastases were frequently ER-negative in those patients in whom the primary tumors were ER-positive⁴. Recently, 3 missense mutations were identified in the ER- α gene from metastatic breast cancer: Ser47Thr, Lys531Glu, and Tyr537Asn¹⁰. The first 2 ER mutants had similar activity to wild-type (wt) ER while the Tyr537Asn ER mutant demonstrated a potent, estradiol-independent transcriptional activity as compared to wt ER. This constitutive activity of Tyr537Asn was unaffected by estradiol, tamoxifen or the pure antiestrogen ICI 164,384. This Tyr537Asn mutant was derived from a bone metastases which was ER-negative by ligand binding analysis. The mutation is located in exon 8 of the carboxy-terminal portion of the hormone-binding domain of the ER- α , a potential phosphorylation site² implicated in hormone binding, dimerization, and hormone-dependent transcriptional activity. Such a mutation may be responsible for the development and progression of breast cancer metastases to bone, and since it does not bind ligand, may be classified as an ER-negative tumor. Since bone metastases are infrequently sampled, the prevalence of this ER mutation is unknown. However, the exact mutation has also been identified in an endometrial carcinoma⁸. To determine the role of ER- α in the development and progression of osteolytic metastases, we proposed to express these wild-type ER- α or mutants, Ser47Thr, Lys531Glu, and Tyr537Asn, into the ER- α -negative breast cancer cell line, MDA-MB-231 which causes osteolytic bone metastases in a mouse model¹.

Task 1: Wild-type ER- α and various mutants (Ser47Thr, Lys531Glu, and Tyr537Asn) will be stably transfected into breast cancer cell lines which are known to cause either osteolytic or osteoblastic metastases in a mouse model (MDA-MB-231, ZR-75-1, MCF-7, T47D, MDA-MB-468) as well as into cell lines which are tumorigenic in nude mice but do not cause bone metastases and clonal lines isolated.

Stable MDA-MB-231 cell lines were constructed which express wild-type ER- α and mutants Ser47Thr, Lys531Glu, and Tyr537Asn. Over 50 clones of each different ER- α transfectants were screened by measuring luciferase activity in the presence or absence of 17 β -estradiol after transient transfection with the estrogen response element linked to luciferase (ERE-luc). Among the wild-type ER- α and ER- α mutants Ser47Thr and Lys531Glu transfectants, at least 5 clones responded to 17 β -estradiol with a significant increase in ERE-luciferase activity. Six clones were identified from the ER- α mutant

Tyr537Asn group which had increased ERE-luciferase activity in the absence of 17 β -estradiol. These six clones did not respond further to 17 β -estradiol or the antiestrogen tamoxifen.

We have been unsuccessful in constructing stable cell lines of the other human breast cancer lines, ZR-75-1, MCF-7, T47D and MDA-MB-468 which express wild-type ER- α or ER- α mutants Ser47Thr, Lys531Glu, and Tyr537Asn. In fact, we have been unable to stably express any cDNA in ZR-75-1 and T47D, despite using multiple conditions and transfection methods. MCF-7 cells initially expressed the transfected ER constructs, however, clones did not remain stable. Finally, we have successfully constructed clones of MDA-MB-468 which express Smad 4 and we are in the process of testing stability of these clones.

Task 2: In vitro growth, PTHrP production, ET-1 production, TGF β -responsiveness, ER- α -mediated transcriptional activity and effect of exogenous estrogens and antiestrogens will be tested in stable cell lines.

Figure 1 shows that stable MDA-MB-231 clones which express ER- α (Tyr537Asn) mutant demonstrates increased ER-mediated transcriptional activity in the absence of estradiol, as assessed by transient transfection with the ERE-luciferase compared with empty vector control. Transcriptional activity of the stable clones was not affected by estradiol treatment, but exogenous TGF β 1 increased ERE-luciferase activity in all stable clones (FIGURE 1). Furthermore, basal as well as TGF β -stimulated PTHrP secretion by the Tyr537Asn ER mutant clones was increased compared with the empty vector controls (FIGURE 2). These data suggest that ER- α -mediated transcription is associated with increased tumor production of PTHrP. This, in combination with the effect of TGF β to enhance ER- α -mediated transcription, and potentially growth, may be a mechanism for the propensity of breast cancer to metastasize to the skeleton.

FIGURE 1: TGF β enhances ER- α -mediated transcriptional activity in stable MDA-MB-231 clones which express the constitutively active ER- α [MDA/ER(Tyr537Asn)]. MDA/ER(Tyr537Asn) and empty vector control clones (MDA/pcDNA3) were transiently transfected with ERE-luciferase reporter, switched to phenol red-free media with charcoal-stripped serum at 8 hr and incubated for 24 hrs more and treated with TGF β , estradiol or both for 24 hrs. Values represent the mean \pm SEM of triplicate measurements. Statistical analysis by ANOVA.

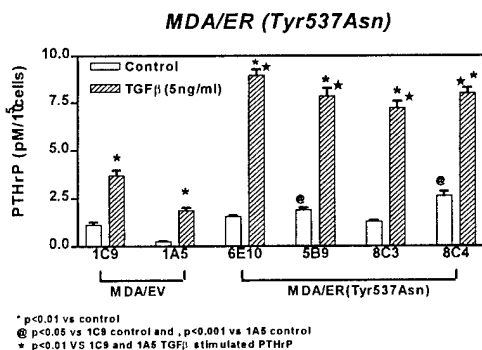
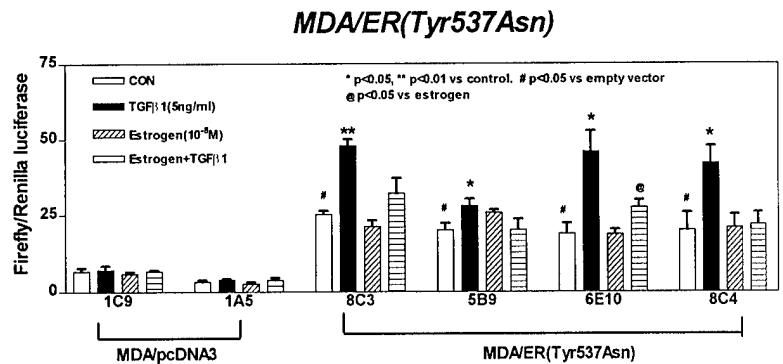
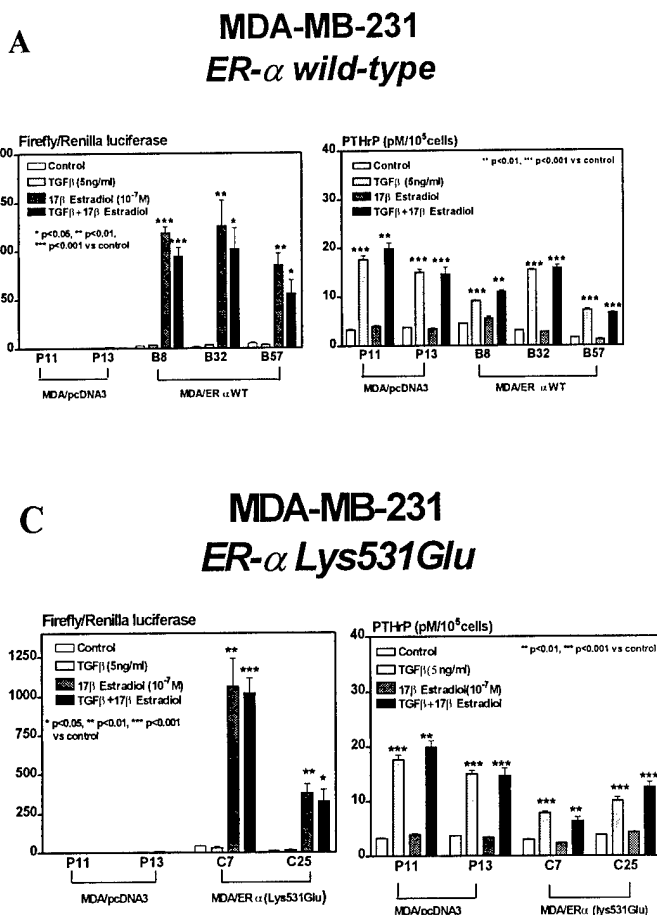


FIGURE 2: TGF β enhances PTHrP production by MDA-MB-231 which express the constitutively active ER- α [MDA/ER(Tyr537Asn)] PTHrP secretion into 24 hour conditioned media obtained from samples illustrated in figure 1. PTHrP was measured by immunoradiometric assay and corrected for cell number. Values represent the mean \pm SEM of triplicate measurements. Statistical analysis by ANOVA. MDA/EV=empty vector pcDNA3 clones.

To determine if the effects of TGF β on ER-mediated transcription were specific to the ER- α (Tyr537Asn) mutant, we constructed stable MDA-MB-231 cell lines which expressed wild-type ER- α , or ER- α mutants which were identified in soft tissue metastases, Ser47Thr and Lys531Glu. These data, (FIGURES 3a-c) illustrate that although ER-mediated transcription was increased in response to 17 β -estradiol in clones which expressed wild-type or Ser47Thr and Lys531Glu mutants, there was no additional effect of TGF β . Furthermore, the combination of 17 β -estradiol and TGF β did not increase PTHrP production over TGF β alone. There was no significant difference between wild-type ER- α and the Ser47Thr or Lys531Glu mutants. These data suggest that the ER- α (Tyr537Asn) mutant, isolated from a bone metastasis, may confer specific properties to the breast cancer cells which facilitate osteolytic bone metastases.

FIGURE 3: Estradiol, but not TGF β , increase ER- α -mediated transcriptional activity (left panel) and PTHrP production (right panel) in stable MDA-MB-231 clones expressing the wild-type ER- α (a) or mutants Ser47Thr (b) and Lys531Glu (c) compared with empty vector control clones (MDA/pcDNA3). Clones were treated with TGF β , estradiol or both. ERE luciferase activity and PTHrP measurements were assessed as in figure 7 & 8 Values represent the mean \pm SEM of triplicate measurements. Statistical analysis by ANOVA.



Task 3: In vivo, the effect of expression of wt ER- α or mutants on bone metastases will be studied in a mouse model. Stable MDA-MB-231 clones from figures 1-3 have been inoculated into nude mice to determine the effect of wild-type ER- α or ER- α mutants Ser47Thr, Lys531Glu, and Tyr537Asn on bone and soft tissue metastases. These experiments are in progress.

KEY RESEARCH ACCOMPLISHMENTS

- Establishment of stable MDA-MB-231 cell lines which express wild-type ER- α and mutants Ser47Thr, Lys531Glu, and Tyr537Asn.
- Determination that TGF β increased PTHrP production and ER-mediated transcription in stable MDA-MB-231 clones which expressed the constitutively active ER- α mutant Tyr537Asn.

REPORTABLE OUTCOMES

Manuscripts, abstracts, presentations:

The following were supported by this Academic Award:

Presentations

1. Molecular mechanisms of osteolytic bone metastases. 2nd North American Conference on "Skeletal Complications of Malignancy." The Paget Foundation-sponsored symposium, Montreal, Canada, October, 1999.
2. Molecular mechanisms of bone metastases. Shriner's Hospital, McGill University, Montreal Canada, October, 1999.
3. Molecular mechanisms of bone metastases: osteolytic and osteoblastic. Endocrine Scholars Lecture Series. University of Connecticut, Farmington, CT, November, 1999.
4. Mechanisms of bone metastases. Department of Cancer Biology Cancer Metastasis Research Program Seminar Series, The University of Texas MD Anderson Cancer Center, Houston, TX, January, 2000.
5. Molecular mechanisms of osteolytic metastases: implications for therapy. Endocrine Grand Rounds. Johns Hopkins University, Baltimore, MD. February, 2000.
6. PTHrP in bone metastases: regulation by TGF β . Advances in Mineral Metabolism, Snowmass, CO, March, 2000.
7. Role of PTHrP in malignancy. Medicine Grand Rounds, Henry Ford Hospital, Detroit, MI, May, 2000
8. Osteoblastic bone metastases: new insight into mechanisms responsible for bone formation. European Calcified Tissue Society Meeting. Tampere, Finland, May, 2000.
9. Molecular mechanisms of osteolytic bone metastases. Oncology Grand Rounds, University of Michigan, Ann Arbor, MI, July 2000.
10. Cancer and Hypercalcemia. Ashland Endocrine Conference. Ashland, OR, August, 2000.
11. PTHrP as a local mediator of breast cancer osteolysis. Molecular biology of bone working group. American Society of Bone and Mineral Research Meeting, Toronto, Canada, September, 2000.
12. Molecular mechanisms of osteolytic metastases: implications for therapy. Endocrine Grand Rounds, Case Western Reserve University, Cleveland, OH. October, 2000.
13. Breast cancer metastases to bone: role of PTHrP and TGF β . Oncology Grand Rounds, University of Pittsburgh, Pittsburgh, PA. October, 2000.
14. Osteoblastic bone metastases: Role of ET-1. Drug Discovery Lecture Series, University of Pittsburgh, Pittsburgh, PA, October, 2000.
15. Cancer and Bone. Plenary Lecture, First Joint Meeting of the International Bone and Mineral Society and the European Calcified Tissue Society. Madrid, Spain, June 2001.
16. Clinical Insights into biology of the osteoblast. Symposium on novel anabolic approaches to osteoporosis. Endocrine Society Meeting, Denver, CO, June 2001.

Abstracts

JJ Yin, JM Chirgwin, SAW Fuqua, TA Guise. Expression of a constitutively active estrogen receptor (ER)- α increases PTHrP production and TGF β -responsiveness by human breast cancer cells. American Society for Bone and Mineral Research Meeting, September, 1999, St. Louis, MO

Patents and licenses applied for or issued: None

Degrees obtained that are supported by this award: None

Development of cell lines, tissue or serum repositories: Stable cell MDA-MB-231 cell lines which express wild-type ER- α and mutants Ser47Thr, Lys531Glu, and Tyr537Asn.

Informatics such as data bases and animal models: None

Funding applied for based on work supported by this award:

1. National Institutes of Health (NCI), "Breast cancer osteolysis: PTHrP regulation by TGF β ". (R01-CA69158; Guise, PI, 25% effort).
2. Burroughs Wellcome Fund Clinical Scientist Award in Translational Research, "Endothelin A Receptor Blockade: Consequences for Bone Physiology in Normal & Pathologic Conditions" (1002256; Guise, PI, 25% effort).

Employment or research opportunities applied for and/or received on training supported by this award: None

CONCLUSIONS

Breast cancer osteolysis is common and the morbidity is devastating. Not only are the consequences of intractable bone pain, fracture, hypercalcemia and nerve compression syndromes debilitating, but the tumor is incurable once it has metastasized to bone. The fact remains that women with breast cancer and bone metastases live many years with this incurable complication and, thus, are at high risk for such morbidity. A more aggressive approach to prevent the development of bone metastases as well as to treat established lesions is a necessary addition to the standard armamentarium for breast cancer therapy in order to impact on this morbidity. Although bisphosphonates are now FDA-approved for treatment of established bone metastases and have had significant impact on bone pain and fracture⁶, considerable advances are necessary for the eventual prevention or reversal of bone metastases. These data indicate a central role for TGF β to potentiate ER- α -mediated transcription induced by a constitutively active ER- α . The above studies provide rationale for targeting the downstream effects of TGF β on breast cancer cells to treat and eventually prevent osteolysis.

REFERENCES

1. Arguello F, Baggs RB, Frantz CN 1988 A murine model of experimental metastasis to bone and bone marrow. *Cancer Res* 48:6876-6881
2. Arnold SF, Obourn JD, Jaffe H, Notides AC 1995 Phosphorylation of the human estrogen receptor on tyrosine 537 in vivo by src family tyrosine kinases in vitro. *Mol Endocrinol* 9:24-33
3. Coleman RE, Rubens RD 1987 The clinical course of bone metastases from breast cancer. *British Journal of Cancer*. 55(1):61-66
4. Frenay M, Milano G, Formento JL, Francoual M, Moll JL, Namer M 1991 Oestrogen and progesterone receptor status in bone biopsy specimens from patients with breast cancer *European Journal of Cancer* 27(2):115-118
5. Guise TA, Mundy GR 1998 Cancer and bone. *Endocrine Reviews* 19(1):18-55.
6. Hortobagyi GN, Theriault RL, Porter L, Blayney D, Lipton A, Sinoff C, Wheeler H, Simeone JF, Seaman J, Knight RD 1996 Efficacy of pamidronate in reducing skeletal complications in patients with breast cancer and lytic bone metastases. Protocol 19 Aredia Breast Cancer Study Group. *N Engl J Med* 335:1785-1791
7. Koenders PG, Beex LV, Langens R, Kloppenborg PW, Smals AG, Benraad TJ 1991 Steroid hormone receptor activity of primary human breast cancer and pattern of first metastasis. The Breast Cancer Study Group. *Breast Cancer Research & Treatment*. 18(1):27-32
8. Kohler MF, Berkholz A, Risinger JI, Elbendary A, Boyd J, Berchuck A 1995 Mutational analysis of the estrogen-receptor gene in endometrial carcinoma. *Obstetrics & Gynecology*. 86(1):33-37
9. Thiede MA, Harm SC, Hasson DM, Gardner RM 1991 In vivo regulation of parathyroid hormone-related peptide messenger ribonucleic acid in the rat uterus by 17 β -estradiol. *Endocrinology* 128:2317-2323
10. Zhang Q-X, Borg A, Wolf DM, Oesterreich S, Fuqua SAW 1997 An estrogen receptor mutant with strong hormone-independent activity from a metastatic breast cancer. *Cancer Research* 57:1244-1249

Expression of a Constitutively Active Estrogen Receptor (ER)- α Increases Parathyroid Hormone-Related Protein (PTHrP) Production And Transforming Growth Factor (TGF)- β -Responsiveness by Human Breast Cancer Cells. JJ Yin, JM Chirgwin, SAW Fuqua, TA Guise. University of Texas Health Science Center, San Antonio

Breast cancer patients with ER-positive primary breast cancers are more likely to develop bone metastases, but the molecular mechanisms responsible for these clinical observations are unknown. There has been an increase in the recent understanding of mediators involved in breast cancer osteolysis. PTHrP mediates breast cancer osteolysis and its expression is increased in breast cancer metastases to bone, likely a result of bone-derived TGF β . Although estrogen has been shown to increase PTHrP expression in the uterus as well as in a breast cancer cell line, no clear relationship between PTHrP and ER in primary breast cancer has been demonstrated. To understand the relationship between estrogen receptor and osteolysis, we determined if a missense mutation in ER- α , which was detected in a human bone metastases, had the capacity to alter tumor cell production of PTHrP, in the basal state and in response to TGF β . The mutant, in which tyrosine at amino acid 537 is replaced with asparagine, Tyr537Asn, demonstrated a potent, estradiol-independent transcriptional activity as compared to wild-type (wt) ER. The point mutation is located in exon 8 of the carboxy-terminal portion of the hormone-binding domain of the ER, to which dimerization and transcriptional functions have been ascribed. It is also a potential phosphorylation site implicated in hormone binding, dimerization, and hormone-dependent transcriptional activity. This constitutive activity of Tyr537Asn was unaffected by estradiol, tamoxifen or the pure antiestrogen ICI 164,384. The cDNA for this Tyr537Asn ER mutant was transfected into MDA-MB-231 human breast cancer cells, which lack ER- α , and 4 single clones were isolated which demonstrated increased ER-mediated transcriptional activity in the absence of estradiol, as assessed by transient transfection with the estrogen response element linked to luciferase (ERE-luc) compared with 2 empty vector control clones. Receptor expression in stable clones was demonstrated by western blot. The transcriptional activity of the stable clones was not affected by estradiol treatment, but treatment with exogenous TGF β 1 resulted in a significant increase in ERE-luciferase activity in all stable clones compared with empty vector controls. Furthermore, basal as well as TGF β -stimulated PTHrP secretion by the Tyr537Asn ER mutant clones was significantly increased compared with the empty vector controls. Growth rates in vitro did not differ significantly between the Tyr537Asn mutant clones and the empty vector in the basal state or in response to exogenous 17 β -estradiol. However, Tyr537Asn mutant clones were slightly, but significantly growth inhibited by TGF β . These data suggest that ER- α -mediated transcription is associated with increased tumor production of PTHrP, basally and in response to TGF β . Furthermore, the data suggest convergence between the estrogen and TGF β signaling pathways. The combined effect of the Tyr537Asn mutant to enhance tumor-produced PTHrP and TGF β to enhance ER- α -mediated transcription may be a mechanism for the propensity of breast cancer to metastasize to the skeleton.