

AD \_\_\_\_\_

Award Number: W81XWH-04-1-0693

TITLE: Dietary Fish Oil in Reducing Bone Metastasis of Breast Cancer

PRINCIPAL INVESTIGATOR: Nandini Ghosh-Choudhury, Ph.D.

CONTRACTING ORGANIZATION: University of Texas Health Science Center  
Houston, TX 77225

REPORT DATE: September 2005

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.

**20060503006**

**REPORT DOCUMENTATION PAGE**Form Approved  
OMB No. 0704-0188

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 Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

<b>1. REPORT DATE (DD-MM-YYYY)</b> 01-09-2005		<b>2. REPORT TYPE</b> Annual		<b>3. DATES COVERED (From - To)</b> 1 Sep 2004 – 31 Aug 2005	
<b>4. TITLE AND SUBTITLE</b> Dietary Fish Oil in Reducing Bone Metastasis of Breast Cancer				<b>5a. CONTRACT NUMBER</b>	
				<b>5b. GRANT NUMBER</b> W81XWH-04-1-0693	
				<b>5c. PROGRAM ELEMENT NUMBER</b>	
<b>6. AUTHOR(S)</b> Nandini Ghosh-Choudhury, Ph.D.  E-mail: choudhury@uthscsa.edu				<b>5d. PROJECT NUMBER</b>	
				<b>5e. TASK NUMBER</b>	
				<b>5f. WORK UNIT NUMBER</b>	
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b>  University of Texas Health Science Center Houston, TX 77225				<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>	
<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b> U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				<b>10. SPONSOR/MONITOR'S ACRONYM(S)</b>	
				<b>11. SPONSOR/MONITOR'S REPORT NUMBER(S)</b>	
<b>12. DISTRIBUTION / AVAILABILITY STATEMENT</b> Approved for Public Release; Distribution Unlimited					
<b>13. SUPPLEMENTARY NOTES</b>					
<b>14. ABSTRACT</b> We hypothesized that fish oil will be inhibitory for bone metastasis of breast cancer cells. For the successful formulation of a novel therapeutic supplement using fish oil diet, the molecular mechanism of fish oil action needs to be determined. We report three novel findings in our annual report for this proposal towards the mechanism of action of fish oil: fish oil or $\omega$ -3 polyunsaturated fatty acids (PUFAs) (1) increase the level of tumor suppressor protein PTEN, (2) inhibit the activity of PI 3 kinase, thus blocking a potent growth promoting signaling pathway and (3) increase gene expression of BMP-2. We are currently investigating each of these observations in details to further elucidate the underlying mechanism of fish oil induced breast cancer growth inhibition. The role of BMP-2 in skeletal metastasis of breast cancer cells is not known. We are also currently studying the effect of BMP-2 overexpression on breast tumor metastasis to bone using a well established nude mouse model for bone metastasis using cardiac injection. The same approach is also being used to study the effect of fish oil diet on bone metastasis.					
<b>15. SUBJECT TERMS</b> Omega 3-fatty acids, bone morphogenetic protein-2 (BMP-2), breast cancer bone metastasis					
<b>16. SECURITY CLASSIFICATION OF:</b>			<b>17. LIMITATION OF ABSTRACT</b>  UU	<b>18. NUMBER OF PAGES</b>  7	<b>19a. NAME OF RESPONSIBLE PERSON</b> USAMRMC
<b>a. REPORT</b> U	<b>b. ABSTRACT</b> U	<b>c. THIS PAGE</b> U			<b>19b. TELEPHONE NUMBER (include area code)</b>

## Table of Contents

Cover.....	1
SF 298.....	2
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	6
Reportable Outcomes.....	6
Conclusions.....	6
References.....	7

## Introduction:

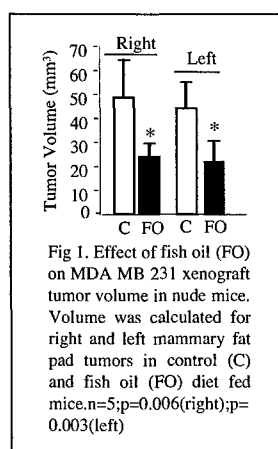
Bone metastases in breast cancer patients are not only a sign of incurable nature of the underlying disease but are associated with specific complications. Bone pain and pathological fractures impair the quality of life of those affected. Thus an effort directed towards preventing or reducing skeletal complications must be of highest priority in formulating breast cancer therapy. Fish oil, rich in  $\omega$ -3 polyunsaturated fatty acids (PUFAs) such as DHA and EPA, will be tested for its effectiveness in preventing bone metastasis of breast cancer cells. Our initial experiments show that in MDA MB 231 cells, EPA increases expression of BMP-2, a member of bone morphogenetic proteins (BMP) family. We also reported that recombinant BMP-2 decreased breast cancer cell proliferation in culture(1,2) and our preliminary data suggest that BMP-2 can inhibit tumor growth in a breast cancer xenograft animal model. Thus if BMP-2 can act as a downstream target for fish oil, it will help in reducing breast cancer cell growth. The role of BMP-2 in preventing bone metastasis of breast cancer cells has not been studied yet. Since our long term goal is to prevent bone metastasis in breast cancer patients, we will test whether BMP-2 can inhibit bone metastasis of the breast cancer cells using an animal model of bone metastasis. Our aim for this proposal is to elucidate the molecular mechanism of fish oil-induced inhibition of breast cancer cell growth so that it could be used as a dietary supplemental therapy for the betterment of the life of the breast cancer patients.

## Body:

**Task 1.** To develop a new therapeutic approach using dietary fish oil for treating breast cancer cell growth and metastasis: (Months 1-12)

### (A) Effect of Fish Oil on tumor growth:

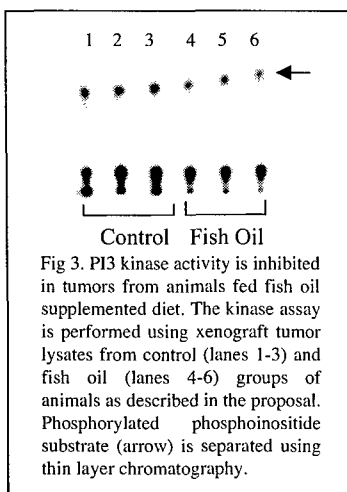
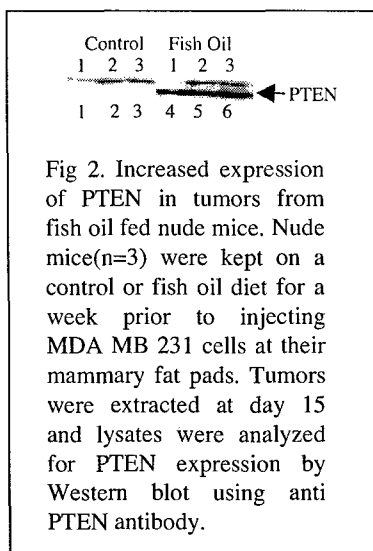
We tested the effect of fish oil diet on xenograft tumor growth in animals. We maintained the nude mice on a 10% fish oil diet before injecting MDA MB 231 human breast cancer cells in the mammary fat pads. The tumor volumes were scored in these mice and were compared with the control diet group. Fish oil diet reduced the tumor volume significantly ( $p = 0.006$  and  $p = 0.003$  for right and left side tumors, respectively; Fig 1).



(B) Determine the effect of fish oil on bone metastasis of breast cancer cells: We have injected the breast cancer cells into the left cardiac ventricle of nude mice and are currently in the process of monitoring the metastatic progression of the cells.

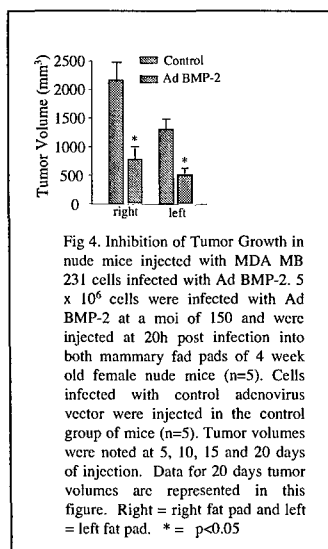
**Task 2:** Study the underlying mechanism of fish oil-induced growth inhibition of breast cancer cells in animal and tissue culture models: (Months 1-12)

In order to determine the molecular mechanism of fish oil mediated inhibition of tumor growth, we examined by immunoblot analysis the expression of tumor suppressor proteins in lysates prepared from these tumors. Our initial analysis showed significant elevation of PTEN (phosphatase and tensin homolog deleted from chromosome 10) tumor suppressor protein (3) in tumor extracts originating from nude mice with diet containing 10% fish oil (Fig 2). This is the first demonstration of regulation of a tumor suppressor protein by fish oil. PTEN dephosphorylates the antiapoptotic D3-phosphoinositides produced by phosphatidylinositol (PI) 3 kinase and induces apoptosis. Since PI 3 kinase is anti-apoptotic, we also examined the activity of this lipid kinase in tumor lysates. Immune complex kinase assay of PI 3 kinase immunoprecipitates from tumor lysates of fish oil fed mice showed significantly reduced PI 3 kinase activity (Fig. 3). These data, again for the first time, demonstrate that in breast tumor growth, fish oil regulates PI 3 kinase, which plays important role in carcinogenesis.



**Task 3:** Test therapeutic efficacy of BMP-2 to inhibit breast cancer cell growth and bone metastasis in a mouse model using adenovirus vector: (Months 1-12)

To assess the effect of BMP-2 *in vivo* in tumor cell growth, we injected MDA MB 231 cells infected with either Ad-BMP-2 (an adenovirus vector expressing BMP-2) or a control Ad- $\beta$ -Gal into nude mouse mammary fat pad. Expression of BMP-2 significantly inhibited tumor cell growth (Fig. 4).



We are currently studying the effect of BMP-2 expression using a bone metastatic model of nude mice.

#### **Key Research Accomplishments:**

- We demonstrated that fish oil diet significantly reduced tumor size in a xenograft model of MDA MB 231 cells.
- As a molecular mechanism of this reduction we show that fish oil diet can induce the PTEN tumor suppressor protein expression in xenograft tumors.
- In addition we showed that fish oil diet can reduce the PI 3 kinase signaling pathway thus blocking a major cell survival pathway in this xenograft model.
- Finally we showed that BMP-2 expression can also significantly reduce tumor size in the MDA MB 231 breast cancer xenograft model.

#### **Reportable Outcomes:**

Part of this work has been presented as an Abstract in the Era of Hope Conference (June, 2005).

Molecular mechanism of beneficial effect of fish oil in breast tumor growth

Nandini Ghosh-Choudhury, Triparna Ghosh-Choudhury, Gabriel Fernandes and Goutam Ghosh-Choudhury

#### **Conclusions:**

We provide data to prove the role of fish oil diet in inhibiting breast tumor growth in mice. We also demonstrated that fish oil diet is activating expression of a tumor suppressor protein, PTEN in breast tumors fed 10% fish oil supplements. This finding is supported by the subsequent loss of activity of PI 3 kinase, a key member of cell survival pathway. Additionally we showed that BMP-2 expression in breast cancer cells can also significantly inhibit breast tumor growth in a xenograft model. We are currently extending these findings to test the role of fish oil diet and BMP-2 in preventing bone metastasis of breast cancer cells. We are also testing to identify the downstream target molecules that are being modified by fish oil diet and BMP-2 that are helping to keep the growth of the breast cancer cells arrested.

## References:

1. Ghosh-Choudhury N, Ghosh-Choudhury G, Celeste A, Ghosh PM, Moyer M, Abboud SL, Kreisberg J 2000 Bone morphogenetic protein-2 induces cyclin kinase inhibitor p21 and hypophosphorylation of retinoblastoma protein in estradiol-treated MCF- 7 human breast cancer cells. *Biochim Biophys Acta* 1497(2):186-96.
2. Ghosh-Choudhury N, Woodruff K, Qi W, Celeste A, Abboud SL, Ghosh Choudhury G 2000 Bone morphogenetic protein-2 blocks MDA MB 231 human breast cancer cell proliferation by inhibiting cyclin-dependent kinase-mediated retinoblastoma protein phosphorylation. *Biochem Biophys Res Commun* 272(3):705-11.
3. Waite KA, Eng C 2002 Protean PTEN: form and function. *Am J Hum Genet* 70(4):829-44.