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

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Introduction:

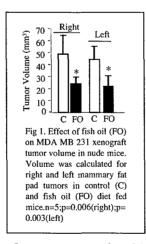
Bone metastases in breast cancer patients are not only a sign of incurable nature of the underlying disease but are associated with specific complications. Bone pain and pathological fractures impair the quality of life of those affected. Thus an effort directed towards preventing or reducing skeletal complications must be of highest priority in formulating breast cancer therapy. Fish oil, rich in ω -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).

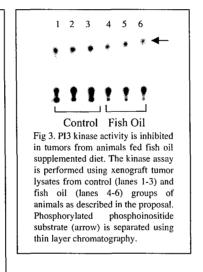


(B) <u>Determine the effect of fish oil on **bone metastasis** of breast cancer cells: We have injected the breast cancer cells into the left cardiac ventricle of nude mice and are currently in the process of monitoring the metastatic progression of the cells.</u>

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. Immunecomplex 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.

Fig 2. Increased expression of PTEN in tumors from fish oil fed nude mice. Nude mice(n=3) were kept on a control or fish oil diet for a week prior to injecting MDA MB 231 cells at their mammary fat pads. Tumors were extracted at day 15 and lysates were analyzed for PTEN expression by Western blot using anti PTEN antibody.



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- β -Gal into nude mouse mammary fat pad. Expression of BMP-2 significantly inhibited tumor cell

growth (Fig. 4).

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Fig 4. Inhibition of Tumor Growth in nude mice injected with MDA MB
231 cells infected with Ad BMP-2. 5
x 10 ⁶ cells were infected with Ad
BMP-2 at a moi of 150 and were
injected at 20h post infection into both mammary fad pads of 4 week
old female nude mice (n=5). Cells
infected with control adenovirus
vector were injected in the control
group of mice (n=5). Tumor volumes were noted at 5, 10, 15 and 20 days
of injection. Data for 20 days tumor

volumes are represented in this figure. Right = right fat pad and left = left fat pad. * = p < 0.05

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 xenopgraft 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.

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