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PRINCIPAL INVESTIGATOR: **Selvarangan Ponnazhagan, Ph.D.**

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14. ABSTRACT Bone is the most common site of metastasis for human breast cancer (BCa), which results in significant morbidity and mortality in patients with advanced disease. A vicious cycle, arising due to the interaction of BCa cells and cells in the bone microenvironment results in the activation of osteoclasts and increased osteolytic bone destruction. The major treatment to reduce the burden of bone metastasis in BCa patients is bisphosphonate therapy. Despite significant efforts to improve the potency of bisphosphonates, the complications are only retarded but not prevented. Thus, development of newer therapies that can both ameliorate the threshold of bone destruction and increase survival of patients with metastatic breast disease will be highly beneficial. The central hypothesis of the proposed work is bone-targeted delivery of genetically-engineered MSC, over-expressing OPG, will prevent osteolytic bone damage and restore skeletal remodeling. Further, based on the requirement of angiogenesis for tumor growth in primary and metastatic sites, in combination with a systemically stable anti-angiogenic therapy, long-term survival will significantly increase. These hypotheses will be tested in this proposal using an immunocompetent, preclinical mouse model of BCa dissemination to all major bones as in human patients.					
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INTRODUCTION

Bone is the most common site of metastasis for human breast cancer (BCa), which results in significant morbidity and mortality in patients with advanced disease. A vicious cycle, arising due to the interaction of BCa cells and cells in the bone microenvironment results in the activation of osteoclasts and increased osteolytic bone destruction. The major treatment to reduce the burden of bone metastasis in BCa patients is bisphosphonate therapy. Despite significant efforts to improve the potency of bisphosphonates, the complications are only retarded but not prevented. Thus, development of newer therapies that can both ameliorate the threshold of bone destruction and increase survival of patients with metastatic breast disease will be highly beneficial. The central hypothesis of the proposed work is bone-targeted delivery of genetically-engineered MSC, over-expressing OPG, will prevent osteolytic bone damage and restore skeletal remodeling. Further, based on the requirement of angiogenesis for tumor growth in primary and metastatic sites, in combination with a systemically stable anti-angiogenic therapy, long-term survival will significantly increase. These hypotheses will be tested in this proposal using an immunocompetent, preclinical mouse model of BCa dissemination to all major bones as in human patients.

Specific Aims:

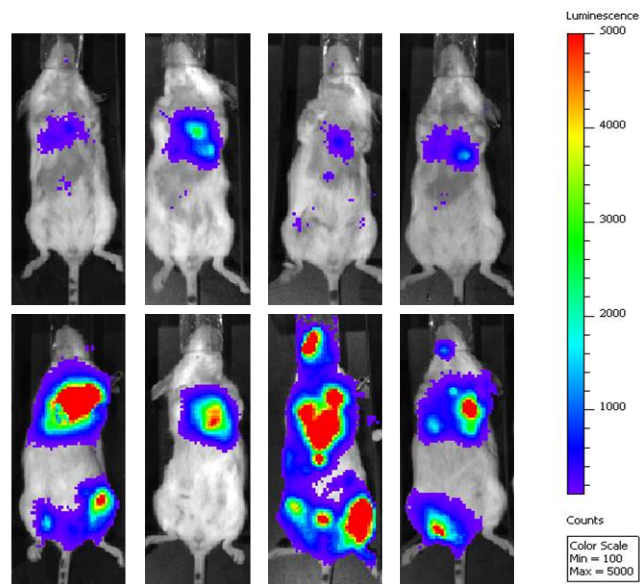
- 1) To determine therapeutic effects of genetically-modified MSC, overexpressing OPG, for osteolytic bone damage using a bone-targeted delivery, in an immunocompetent mouse model of BCa dissemination to the bone
- 2) To determine the combined effect of MSC-OPG therapy with systemically-stable anti-angiogenic therapy for long-term survival.

BODY

During the last year, we have studied synergistic therapeutic effects of osteoprotegerin and endostatin in breast cancer progression and bone lesions in a mouse model.

Breast cancer has the highest rate of incidence among women in the United States and is the second leading of cause of death behind lung cancer. Breast cancer frequently metastasizes to the skeleton producing osteolytic lesions and result in significant morbidity and mortality in these patients. The malignant cells upregulate the expression of RANKL within the skeleton. RANKL upon binding to RANK on the surface of the pre-osteoclasts leads to enhanced maturation and activity of the osteoclasts which is responsible for active bone resorption. OPG is a decoy receptor for RANKL and prevents RANKL-RANK interaction. In our laboratory a recombinant adeno-associated virus (rAAV) vector expressing ligand binding domain of human OPG fused to Fc region of human IgG1 under the control of hybrid CMV chicken beta actin promoter was shown to prohibit growth of MDA-MB-435 breast tumor cells within the skeleton via inhibition of osteoclast mediated bone resorption. Formation of new blood vessels that supply nutrient to the growing tumor is one of the hallmarks of cancer and inhibition of angiogenesis has been shown to be highly effective in slowing down cancer progression in many studies and clinical trials. rAAV expressing anti-angiogenic human endostatin was able to significantly delay prostate cancer progression in a spontaneous transgenic mouse model of prostate adenocarcinoma (Isayeva et al., 2009). In the present study we aimed to test the combined therapeutic potential of OPG and endostatin in a murine model of highly aggressive metastatic breast cancer.

Recombinant adeno-associated virus (rAAV) containing either OPG or endostatin were generated and purified using standard protocols. Viral titers were determined by slot-blot analysis. 10^{11} viral particles of either rAAV-OPG.Fc or rAAV-endostatin or rAAV-OPG.Fc and rAAV-endostatin combined were delivered intra-muscular in the gastrocnemius muscle of right and left leg respectively in 4-6 week-old immunocompetent BALB/c female mice. Control animals were injected with rAAV expressing GFP. Highly aggressive murine mammary cancer cell line 4T1 expressing firefly luciferase (10^5 cells in 100 μ l saline) was injected via intra-cardiac route one week following the viral gene transfer. Breast cancer progression was monitored via bioluminescence imaging for 3 weeks. The tumor growth is measured as ratio-increase in bioluminescence counts following tumor cell implantation until the end of the experiment.



The mice treated with only rAAV-OPG.Fc showed significant inhibition of metastatic tumor growth in the visceral organs and skeleton, whereas enhanced tumor growth was observed in mice treated with rAAV-endostatin as well as rAAV-OPG.Fc and rAAV-endostatin combined (Figure 1, 2 & 3).

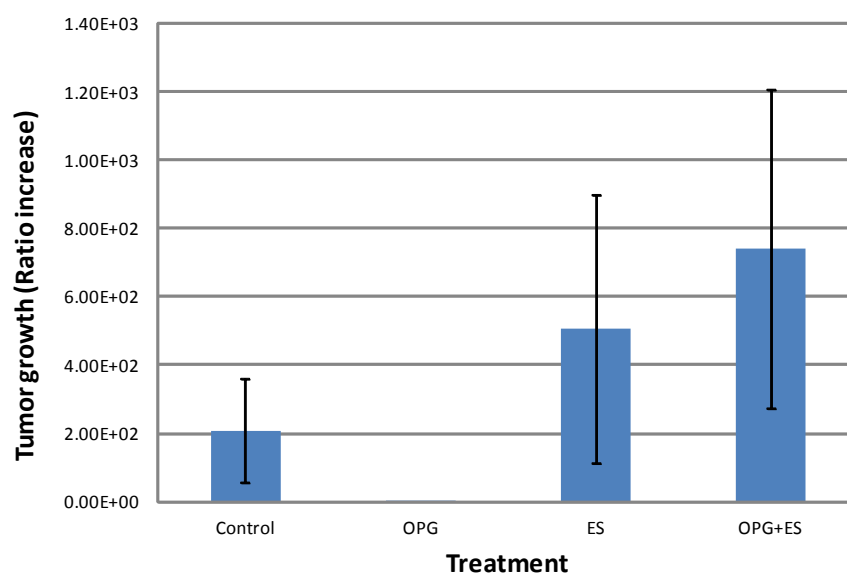


Figure 2 : Tumor growth in the tibia

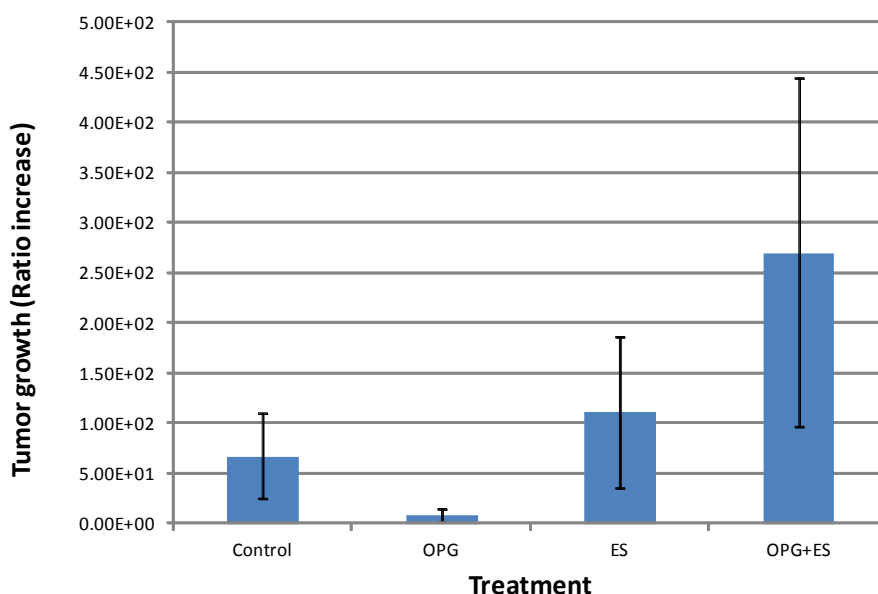


Figure 3: Overall Tumor Growth

KEY RESEARCH ACCOMPLISHMENTS

AAV-OPG.Fc treatment was highly effective in inhibiting metastatic progression of 4T1 mammary carcinoma cells in the mouse model. On the contrary, rAAV-endostatin promoted metastatic progression of 4T1 cells when given either alone or in combination with rAAV-OPG.Fc and warrants further investigation.

REPORTABLE OUTCOMES

None

CONCLUSIONS

rAAV-OPG.Fc treatment was highly effective in inhibiting metastatic progression of 4T1 mammary carcinoma cells in this mouse model. On the contrary, rAAV-endostatin promoted metastatic progression of 4T1 cells when given either alone or in combination with rAAV-OPG.Fc and warrants further investigation.

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