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TITLE: Systemic and Gene Modified Mesenchymal Stem Cell Therapy for Metastatic Prostate Cancer

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14. ABSTRACT Bone is the frequent metastatic site for human prostate cancer resulting in significant morbidity and mortality in patients with advanced disease. The type of bone defect encountered in prostate cancer bone metastasis is osteoblast lesions resulting in excess bone. However, initiation of osteoclastogenesis is first aided by osteolysis, mediated by osteoclasts. The areas provided as source for osteoblast accumulation later leads to thickening of the bone. In this proposal, we planned to address arresting both the events of osteolysis and osteoblastogenesis by biological inhibitors of these two events. Osteoprotegerin (OPG) is a "decoy" receptor that competes with RANK for RANKL, thus, modulating the effects of RANKL. Thus, OPG remains an effective molecule for future therapies for bone metastasis. We sought to achieve sustained effects of OPG combining cell therapy and gene therapy approaches. Similarly, for inhibiting osteoblast activity we chose noggin, capable of arresting osteoblast formation. The aims were to determine therapeutic effects of OPG and noggin expression by rAAV gene therapy in a murine model of prostate cancer bone metastasis. So far, we produced high-titer recombinant AAV vectors encoding osteoprotegerin, and noggin and currently testing the feasibility of MSC therapy for reducing bone burden initiated by cancer growth. Continuation of the ongoing studies in to next year will provide valuable information on therapeutic effects of this therapy for prostate cancer bone metastasis.					
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Annual Report: April 01, 2007 – May 31, 2008

INTRODUCTION

Bone is the frequent metastatic site for human prostate cancer resulting in significant morbidity and mortality in patients with advanced disease. A vicious cycle, arising due to the interaction of cancer cells and the bone microenvironment results in the upregulation of factors promoting osteoblastogenesis. Presently, it is clear that the event of osteoblastogenesis in prostate cancer bone metastasis is preceded by osteoclastogenesis. Thus, osteolysis and osteoblastogenesis can be inhibited by interrupting one or more of the steps involved in the cycle.

A better understanding of bone remodeling and molecular events in osteolytic and osteoblastic bone lesions identified the role of key activators and inhibitors of both these events. The receptor activator of nuclear factor κ B ligand (RANKL), produced by osteoblasts, activated T cells and marrow stromal cells stimulates the recruitment, differentiation, and activation of osteoclasts by binding to RANK. Osteoprotegerin (OPG) is a “decoy” receptor that competes with RANK for RANKL thereby modulating the effects of RANKL. Thus, OPG is a promising molecule for inhibiting osteoclastogenesis. On the other hand, noggin, a secreted glycoprotein with proven antagonistic activity on bone morphogenetic proteins (BMP) and osteoblast differentiation will inhibit osteoblastic lesions. To achieve sustained effects of these two molecules, gene therapy is more powerful than pharmacological therapies. Since bone metastasis is a secondary event that occurs in late-stage disease or during recurrence, genetic therapies aimed at controlling this process should be sustained and localized. Thus, for sustained expression of therapeutic levels of OPG or noggin, a vector capable of stable expression of the transgene without vector-associated toxicity and immunity is ideal. The adeno-associated virus vectors (AAV) are more promising to this end. With recombinant AAV, it is possible to obtain significant therapeutic gains by either systemic or bone-targeted transduction using mesenchymal stem cells with bone homing signals.

The central hypothesis of the proposed work is that systemic or bone targeted gene therapy using recombinant AAV and gene-modified mesenchymal stem cell vehicles capable of bone homing and inhibiting osteoclastic and osteoblastic bone lesions in prostate cancer by stable expression of OPG and noggin respectively will be effective treatment modalities for prostate cancer bone metastasis. The proposed studies will seek to identify the effects of OPG and noggin therapy also as a means to delineate the intricate role of osteoclastogenesis and osteoblastogenesis in the progression of prostate cancer bone metastasis. This hypothesis will be evaluated in the present study by using a novel bone-targeted mesenchymal stem cell vehicle, and non-invasive bioluminescent imaging of the implanted prostate cancer cell growth and metastases in SCID mouse.

Specific Aims: 1) To develop and characterize rAAV encoding human OPG and noggin, and clones of PC-3, LAPC-9 and LNCaP cell lines stably expressing luciferase for non-invasive imaging, 2) To determine preventive and therapeutic effects of systemic and bone-targeted OPG expression by rAAV gene therapy in SCID mice with osteolytic, osteoblastic and mixed lesions of prostate cancer bone metastasis, and 3) To determine preventive and therapeutic effects of BMP antagonist noggin by rAAV gene therapy in metastatic prostate cancer mice models *in vivo*.

BODY

We have made excellent progress in the last one year. In the previous year (report covering 2006-2007) we reported our initial finding that treatment of mice bearing osteolytic PC-3, a prostate cancer cell line, lesion with mesenchymal stem cells (MSC) expressing osteoprotegerin (OPG) using a recombinant adeno-associated virus (rAAV) prevented osteolytic lesions. We confirmed this finding by repeating the experiment in larger groups of mice. Representative data is shown in Figure 1. Micro-CT and trichrome staining (Figure 2) and bone mineral density and bone mineral content, based on Dual-X ray

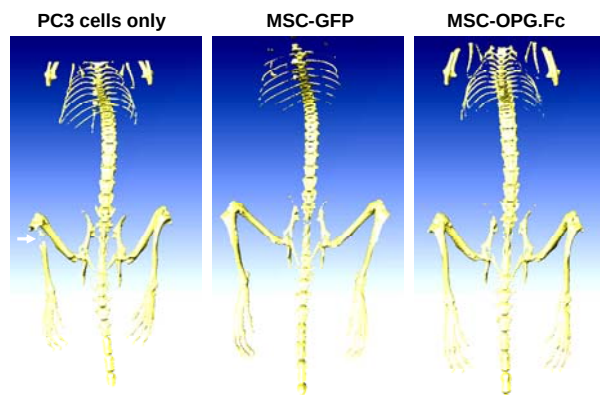


Figure-1

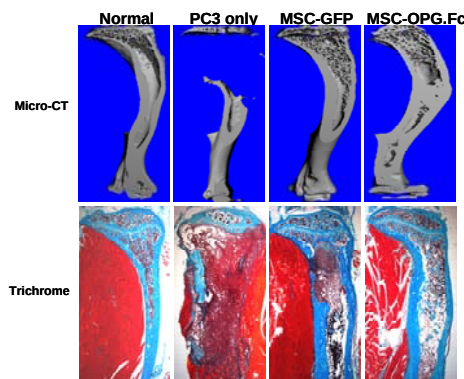


Figure - 2

(DXA) (Figure 3) also confirmed the increase in bone density and new bone formation.

Interestingly, when highly pure MSC were used without rAAV-OPG vector, there was still significant protection against osteolysis (please see MSC-GFP group in Figures 1-3) although not as efficient as in the group with rAAV-OPG.

This prompted to us to further investigate and understand the mechanistic insights of this therapy for possible clinical application in future.

To this end, we developed a 3-dimensional culture system for the growth of PC-3 cells mimicking the *in vivo* situation and co-cultured MSC that were mock-transduced or transduced with rAAV-GFP or rAAV-OPG. First, the PC-3 cells were analyzed for proliferation index by MTT assay. Results, shown in Figure 4 indicated that in the presence of MSC modified to express OPG, there was a significant increase in the rate of proliferation. Next, we isolated RNA from the PC-3 cells grown in 3-D culture with modified and unmodified MSC and performed RNA

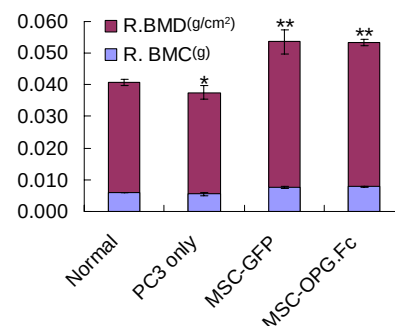


Figure - 3

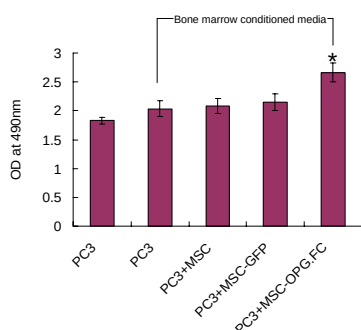
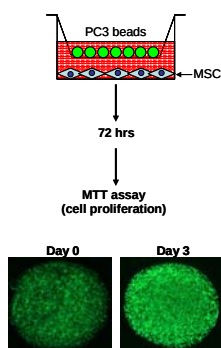
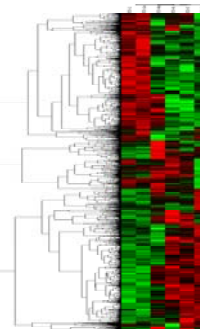


Figure 4



- UPREGULATED GENES**
- Homo sapiens cadherin 15, M-cadherin (myotubule) (CDH15), mRNA.
 - Homo sapiens anterior gradient homolog 2 (Xenopus laevis) (AGR2), mRNA.
 - Homo sapiens hypothetical protein MGC14376 (MGC14376), transcript variant 2, mRNA.
 - CR736094 NCI_CGAP_GCB1 Homo sapiens cDNA clone IMAGp971A1355; IMAGE:824783 5, mRNA sequence
 - Homo sapiens cysteine-rich with EGF-like domains 2 (CRELD2), mRNA.
 - DKFZp686N1332_r1 686 (synonym: hicc3) Homo sapiens cDNA clone DKFZp686N1332 5, mRNA sequence

Figure - 5

microarray analysis to identify potential genes that are upregulated in the association. Interestingly, there were six genes, which showed upregulated expression in the presence of MSC-OPG (Figure 5).

Presently, we are performing mechanistic studies to delineate the role of each one in the pathology and growth of prostate tumor in bone and to determine how OPG therapy or MSC therapy can help in bone restoration.

For the third specific aim, so far, we have successfully produced rAAV encoding noggin, which is an antagonist for BMP-2. This vector was first tested *in vitro* to confirm high-level expression of the transgene. Results, shown in Figure 6, demonstrated high-level expression of noggin in 293 cells following rAAV-noggin transduction. Next, we conducted a study to determine the potential of rAAV-noggin in preventing osteoblast lesions in bone, which occur in end-stage prostate cancer patients.

In the *in vivo* study, we used 22Rv cells, which make osteoblast lesions as xenograft in athymic mice. Results of the first experiment (shown in Figures 7 & 8) indicated that following rAAV-noggin therapy by intramuscular administration of the vector, the amounts of tumor burden in the bone has decreased. We are currently determining the treatment effects by histomorphometry, immunohistochemistry and micro-CT of the bone.

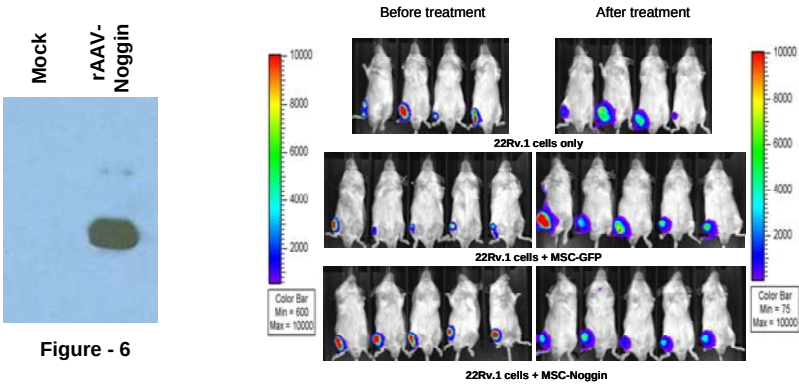


Figure - 6

Figure 7

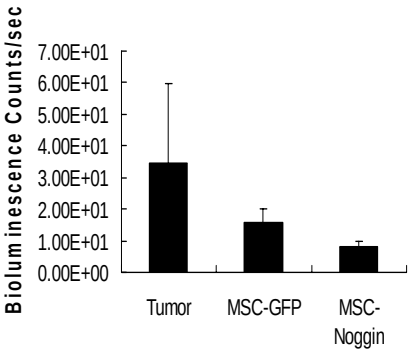


Figure - 8

In the coming year, we are also interested in testing the effects of rAAV-noggin by both *i.m.* and modified MSC therapy the human prostate cancer cell line C4-2, which also make osteoblast lesions in the bone. To this end, we conducted pilot studies to validate and confirm the growth of C4-2 cells in the bone of athymic mice and an increase in osteoblast lesions. Results of these studies are provided in Figure 9.

Thus, we hope that the project is in a very exciting phase for the third year.

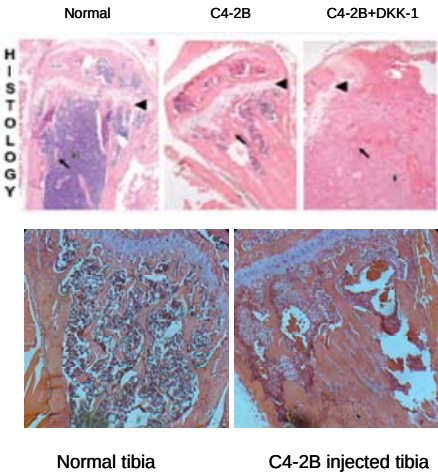


Figure - 9

KEY RESEARCH ACCOMPLISHMENTS

- Demonstrated that MSC expressing OPG prevents osteolytic bone damage produced by the prostate cancer cell line PC-3
- Identified potential genes that are upregulated to mediate this effect.
- Developed and completed initial validation of rAAV-noggin both *in vitro* and *in vivo* for preventing osteoblast lesions in bone, commonly encountered in advanced stage prostate cancer patients.

REPORTABLE OUTCOMES

(Papers published or communicated)

1. Isayeva, I., Chanda, D., Eltoum, I-E., and Ponnazhagan, S. Effects of sustained anti-angiogenic therapy in multi-stage prostate cancer in TRAMP mice. *Cancer Res.* 2007, 67:5789-5797.
2. Kumar, S., and Ponnazhagan, S. Bone homing of pluripotent mesenchymal stem cells by ectopic $\alpha 4$ integrin expression. *FASEB J.* 2007, 14:3917-3927.
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4. Ren, C., Kumar S., Diptiman C., Kallman, L., Chen, J., Mountz, J and Ponnazhagan, S. Anti-tumor activity of mesenchymal stem cells expressing interferon- β in a mouse prostate cancer lung metastasis model. *Gene Ther.* 2008 (in press).

CONCLUSIONS

In the last year of funding we made significant progress in all specific aims. We have demonstrated the potential of MSC expressing OPG in preventing osteolytic lesions in the bone, which occur as a prelude to osteoblastic bone lesions in prostate cancer bone metastasis. Further, we developed and validated rAAV encoding noggin in preventing osteoblastic bone defect in athymic nude mice with human prostate cancer cells in the bone, capable of producing osteoblastic bone phenotype. We will continue to study these two areas further in the coming year.

PERSONNEL RECEIVING PAY FROM THIS GRANT

Selvarangan Ponnazhagan, Ph.D.

Diptiman Chanda, Ph.D.

REFERENCES

N/A

APPENDICES

N/A