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<b>13. ABSTRACT (Maximum 200 Words)</b> Prostate cancer (PC) is the most commonly diagnosed solid malignancy and second leading cause of cancer death in US men. PC has a specific propensity to metastasize to bone. Mechanisms underlying organ-specific metastasis to bone likely include both chemoattractive homing phenomena and selective proliferative advantages of cancer cells in the marrow. Recent evidence implicates the chemokine SDF-1 $\alpha$ and its receptor CXCR4 in organ-specific metastasis of breast cancer. Using several methods, we determined the expression of CXCR4 in prostate cancer bone tumor cells and cultured PC cells, and SDF-1 $\alpha$ expression in human bone marrow stromal cells and human fetal bone tissue. <i>In vitro</i> treatment of PC cells with SDF-1 $\alpha$ showed an increase in MMP-9 gene expression and protein secretion. Both PI3 kinase and MAP kinase inhibitors abrogated SDF-1 $\alpha$ induced MMP-9 secretion in PC-3 cells, but PI3 kinase inhibition is more potent abrogation of MMP-9 expression in PC-3 cells. These data suggest that chemoattractive mechanisms mediated by SDF-1 $\alpha$ and CXCR4 activate PI3 kinase signaling pathway to release MMPs into local environment. Improved understanding of chemokine-receptor interactions and subsequent intracellular signaling pathways involved proteinase gene expression may lead to new therapeutic strategies aimed at interrupting the interactions between PC cells and bone.			
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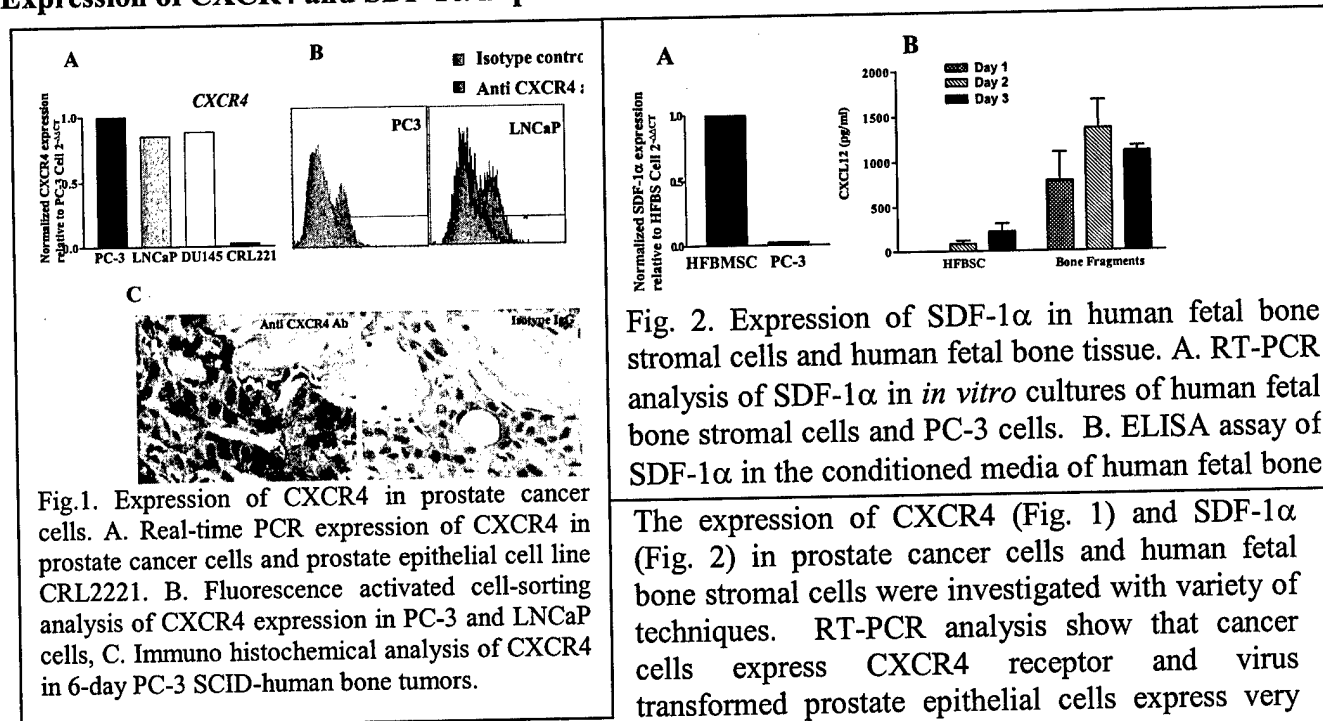
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## INTRODUCTION:

Prostate cancer cells frequently metastasize to bone. The bone-associated chemokines may play a role in the chemoattraction of prostate cancer cells to bone. The interaction of bone associated chemokines with the chemokine receptors on prostate cancer cells leads to the activation of signaling pathways and subsequent expression of proteases including MMP-9. The literature suggests the chemokine, SDF-1 $\alpha$  and its cognate receptor CXCR4 are involved in the metastasis of breast cancer cell to lymph node and lung(1), further several recent studies show the existence these chemokine and chemokine receptor interactions in several types of cancers including, prostate(2,3), ovarian(4), melanoma(5), colon(6), pancreatic(7,8) and glioblastoma (9,10) tumors. The purpose of current proposal is to demonstrate the presence of functional chemokine receptor; CXCR4 in PC cells and clinical PC bone metastasis and to test the hypothesis that interaction of bone associated SDF-1 $\alpha$  with CXCR4 expressed on prostate cancer cells elicits intra cellular signaling events leading to the MMP-9 gene expression. The experiments in the current proposal were designed to provide link between the chemoattraction of cancer towards target environment mediated by the SDF-1 $\alpha$  and CXCR4, and expression of proteases by activation of cell signaling pathways in the bone target environment.

## BODY

### Expression of CXCR4 and SDF-1 $\alpha$ in prostate cancer cells and human fetal bone stromal cells.



The expression of CXCR4 (Fig. 1) and SDF-1 $\alpha$  (Fig. 2) in prostate cancer cells and human fetal bone stromal cells were investigated with variety of techniques. RT-PCR analysis show that cancer cells express CXCR4 receptor and virus transformed prostate epithelial cells express very low levels of CXCR4 receptor (Fig.1 A). The chemokine SDF-1 $\alpha$  is expressed on the primary cultures of human fetal bone stromal cells and PC-3 cells express very low levels (Fig. 2A). Fluorescence activated cell-sorting analysis of PC-3 and LNCaP cells show the cell surface expression of CXCR4 protein in both cell lines (Fig1 B). SCID-human PC-3 bone tumor tissue stained positive for CXCR4 expression (Fig1C). Human fetal bone stromal cell and human fetal bone tissue secreted SDF-1 $\alpha$  protein were determined using SDF-1 $\alpha$  specific ELISA, the results show that both cells and bone tissue secreted the chemokine SDF-1 $\alpha$  and bone tissue secreted higher levels of SDF-1 $\alpha$  (Fig2B).

**SDF-1 $\alpha$  induces MMP-9 expression in prostate cancer cells:** MMP-9 gene expression in PC-3, LNCaP and DU145 cells was measured using the MMP-9 gene specific primers in RT-PCR experiment. Both PC-3 and LNCaP cells induced the MMP-9 gene expression. DU145 cells are not

responsive to SDF-1 $\alpha$  in MMP-9 gene expression (Fig. 2A). SDF-1 $\alpha$  induced MMP-9 gene expression is further confirmed by MMP-9 promoter activation studies using the full length MMP-9 promoter element cloned in luciferase reporter vectors. PC-3 cells have a basal promoter activation and upon SDF-1 $\alpha$  treatment there is a 10-12 fold more increase in MMP-9 promoter activity (Fig.2B). Further SDF-1 $\alpha$  induced MMP-9 protein release from the PC-3 cells were evaluated by analyzing the conditioned media obtained from the PC-3 cells treated with different concentrations of SDF-1 $\alpha$ . The results show that there is dose dependent increase in the proMMP-9 secretion from the cancer cells (Fig.2C).

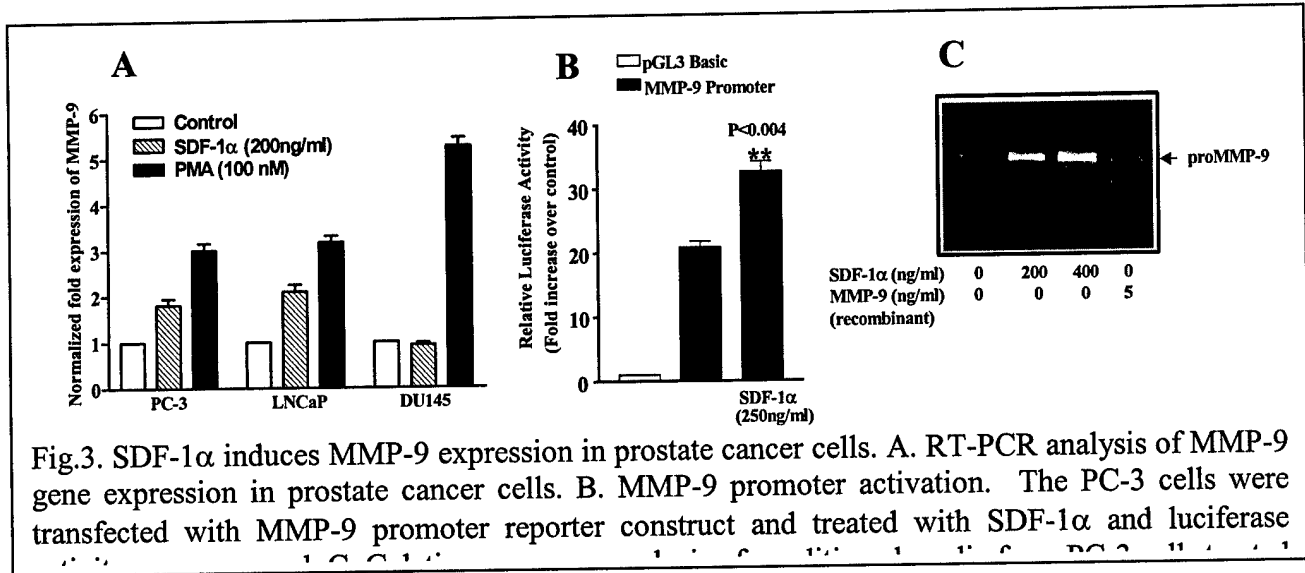


Fig.3. SDF-1 $\alpha$  induces MMP-9 expression in prostate cancer cells. A. RT-PCR analysis of MMP-9 gene expression in prostate cancer cells. B. MMP-9 promoter activation. The PC-3 cells were transfected with MMP-9 promoter reporter construct and treated with SDF-1 $\alpha$  and luciferase

### SDF-1 $\alpha$ induced signaling pathways participate in MMP-9 gene expression in PC-3 cells:

SDF-1 $\alpha$  induces PI3 kinase activation and subsequent phosphorylation of Akt in PC-3 cells. PC-3 cells were treated with SDF-1 $\alpha$  and PI3 kinase inhibitor LY294002 and total cellular proteins were analyzed for Akt and activated form of Akt (pAkt). SDF-1 $\alpha$  induced the phosphorylation of Akt at serine 473 position and PI3 kinase inhibitor LY294002 inhibited the SDF-1 $\alpha$  induced Akt activation (Fig. 4). Further we evaluated the significance of Akt activation in MMP-9 gene expression PC-3 cells.

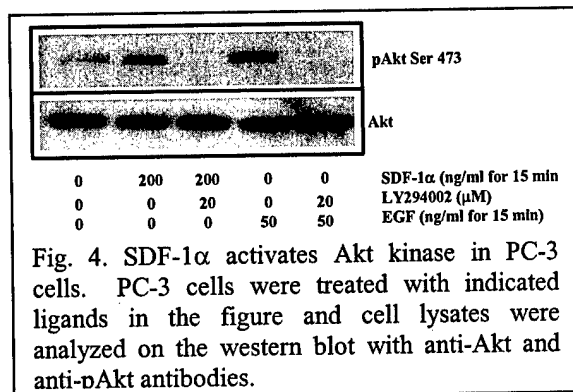
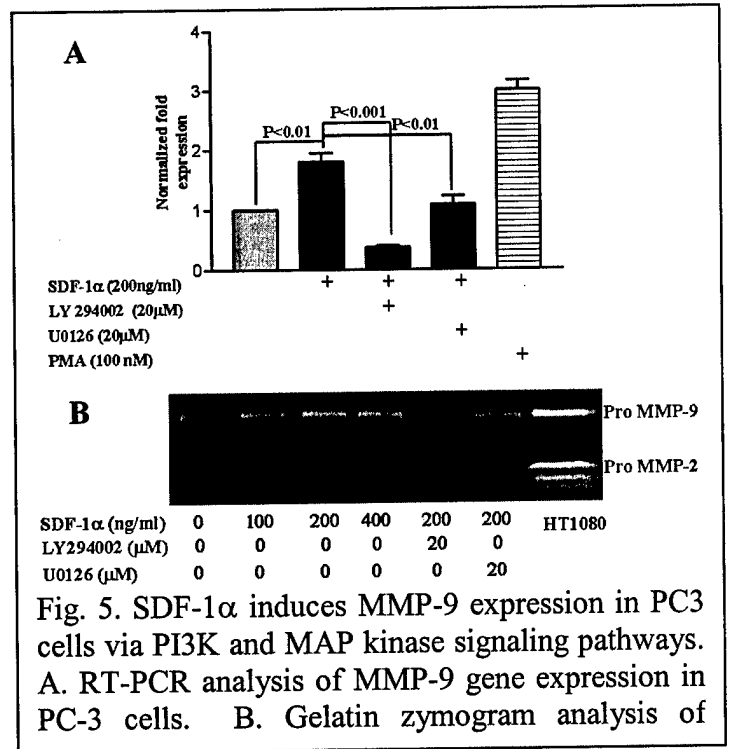


Fig. 4. SDF-1 $\alpha$  activates Akt kinase in PC-3 cells. PC-3 cells were treated with indicated ligands in the figure and cell lysates were analyzed on the western blot with anti-Akt and anti-pAkt antibodies.

PC-3 cells were treated with SDF-1 $\alpha$ , PI3 kinase inhibitor LY294002 and MAP kinase inhibitor U0126 and PMA (positive control for MMP-9 gene expression in cancer cells) for 24 hours. MMP-9 gene expression is evaluated by RT-PCR (Fig. 5 A) and proMMP-9 protein secretion were measured by gelatin zymography (Fig. 5B). Both RT-PCR and zymography data suggest that SDF-1 $\alpha$  induced MMP-9 gene expression in PC-3 cells and SDF-1 $\alpha$  induced MMP-9 gene expression in PC-3 cells were inhibited by pretreatment of cells with both PI-3 kinase and MAP kinase inhibitors. PI3 kinase inhibition is more potent in inhibiting the MMP-9 gene expression and secretion from the PC-3 cells.

## KEY RESEARCH ACCOMPLISHMENTS

- *In vitro* and *in vivo* prostate cancer cells express CXCR4 receptor and bone associated cells express the CXCR4 ligand SDF-1 $\alpha$ .
- SDF-1 $\alpha$  and CXCR4 interaction in prostate cancer cells induce MMP-9 gene expression. In PC-3 cells SDF-1 $\alpha$  can induce the expression and secretion of pro MMP-9
- SDF-1 $\alpha$  can activate the PI3 kinase/Akt pathway in PC-3 cells.
- Relative role of PI3K/MAPK pathway studies with well accepted pharmacological inhibitors suggest that SDF-1 $\alpha$  induced MMP-9 gene expression in PC-3 cells are sensitive to both pathways and assessing relative sensitivity of these two inhibitors suggest that PI3K/Akt pathway is more potent in inducing MMP-9 gene expression.



## REPORTABLE OUTCOMES

1. Podium presentation: Chinni SR., "CXCR4 and MMP-9 in prostate cancer bone metastasis" at IV th International conference on cancer induced bone disease, San Antonio, TX, December 2003
2. Abstract: Chinni, SR; Dong, Z; Sivalogan S; Trindade Filho, JC; Bhagat, S; Bonfil RD and Cher, ML. CXCR4 and MMP-9 in prostate cancer bone metastasis. IV th International conference on cancer induced bone disease, San Antonio, TX., December 2003.

## CONCLUSIONS

These results suggest that chemokine, SDF-1 $\alpha$  and its cognate receptor CXCR4 are expressed in the cellular phenotypes of prostate cancer bone metastasis. Particularly, cellular elements of bone microenvironment have gene and protein expression for chemokine SDF-1 $\alpha$  and several prostate cancer cells, which metastasize to bone microenvironment express the CXCR4. SDF-1 $\alpha$  induced MMP-9 gene expression and secretion in cancer cells provides further functional connection between the chemoattraction and tumor cell growth in bone microenvironment by activation cancer cell associated signaling pathways.

## REFERENCES

1. Muller, A., Homey, B., Soto, H., Ge, N., Catron, D., Buchanan, M. E., McClanahan, T., Murphy, E., Yuan, W., Wagner, S. N., Barrera, J. L., Mohar, A., Verastegui, E., and Zlotnik, A. (2001) *Nature* **410**, 50-56
2. Taichman, R. S., Cooper, C., Keller, E. T., Pienta, K. J., Taichman, N. S., and McCauley, L. K. (2002) *Cancer Res* **62**, 1832-1837
3. Sun, Y. X., Wang, J., Shelburne, C. E., Lopatin, D. E., Chinnaiyan, A. M., Rubin, M. A., Pienta, K. J., and Taichman, R. S. (2003) *J Cell Biochem* **89**, 462-473
4. Scotton, C. J., Wilson, J. L., Scott, K., Stamp, G., Wilbanks, G. D., Fricker, S., Bridger, G., and Balkwill, F. R. (2002) *Cancer Res* **62**, 5930-5938
5. Murakami, T., Maki, W., Cardones, A. R., Fang, H., Tun Kyi, A., Nestle, F. O., and Hwang, S. T. (2002) *Cancer Res* **62**, 7328-7334
6. Zeelenberg, I. S., Ruuls-Van Stalle, L., and Roos, E. (2003) *Cancer Res* **63**, 3833-3839
7. Koshiba, T., Hosotani, R., Miyamoto, Y., Ida, J., Tsuji, S., Nakajima, S., Kawaguchi, M., Kobayashi, H., Doi, R., Hori, T., Fujii, N., and Imamura, M. (2000) *Clin Cancer Res* **6**, 3530-3535
8. Mori, T., Doi, R., Koizumi, M., Toyoda, E., Ito, D., Kami, K., Masui, T., Fujimoto, K., Tamamura, H., Hiramatsu, K., Fujii, N., and Imamura, M. (2004) *Mol Cancer Ther* **3**, 29-37
9. Barbero, S., Bajetto, A., Bonavia, R., Porcile, C., Piccioli, P., Pirani, P., Ravetti, J. L., Zona, G., Spaziante, R., Florio, T., and Schettini, G. (2002) *Ann NY Acad Sci* **973**, 60-69
10. Rubin, J. B., Kung, A. L., Klein, R. S., Chan, J. A., Sun, Y., Schmidt, K., Kieran, M. W., Luster, A. D., and Segal, R. A. (2003) *Proc Natl Acad Sci U S A* **100**, 13513-13518

## APPENDICES

### Appendix 1

#### **CXCR4 and MMP-9 in prostate cancer bone metastasis**

Chinni, SR; Dong, Z; Sivalogan S; Trindade Filho, JC; Bhagat, S; Bonfil RD and Cher, ML

*Wayne State University*

Mechanisms underlying organ-specific metastasis to bone likely include both chemoattractive homing phenomena and selective proliferative advantages of cancer cells after their arrival in the marrow. Recent evidence implicates the chemokine SDF-1 $\alpha$  and its receptor CXCR4 in organ-specific metastasis of breast cancer. Previously, we demonstrated that circulating prostate cancer (PC) cells selectively colonize human fetal bones implanted in SCID mice and that matrix metalloproteinases, particularly MMP-9, play a role in the proliferation of PC cells in bone and subsequent bone matrix remodeling. Herein, we injected PC3 cells into fetal human bone implants, and harvested the tissues at 1, 3 days for their microscopic location and 14 days for MMP-9 activity measurements. Using several methods, we determined the expression of CXCR4 in prostate cancer bone tumor cells and cultured PC cells, and the effect of both exogenous SDF-1 $\alpha$  and co-cultures of prostate cancer cells and bone stromal cells on the secretion of proMMP-9. PC cells injected directly into implanted bones at early time periods migrated toward endosteal surfaces of trabeculae suggesting a chemoattractive-migratory mechanism. *In vitro* treatment of PC-3 cells with SDF-1 $\alpha$  and co-cultures of cancer cells and stromal cells showed a dose-dependent effect on the secretion of proMMP-9. PI3 Kinase inhibitors abrogated MMP-9 secretion. These data suggest that chemoattractive mechanisms stimulate the migration of cancer cells towards mineralized trabeculae, and that PI3 kinase signaling induced by SDF-1 $\alpha$  and CXCR4 releases MMPs into local environment.