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TITLE: Superoxide Dismutase and Transcription Factor SOX9 as Mediators of Tumor Suppression by MAC25 (IGFBP-RP1) in Prostate Cancer Cells

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13. ABSTRACT (Maximum 200 Words)

The hypothesis of this proposal is that: The mac25/IGFBP-rp1 gene functions as a tumor suppressor for prostate epithelium by induction of additional genes regulating response to oxidative stress, senescence, and differentiation. One of these, SOD2, impairs tumor cell growth by detoxifying superoxides which may initiate transformation, or by interaction with growth promoting signal pathways via modulation of protein phosphorylation. Another, SOX9, induces a subset of genes required for differentiation of normal cells with reduced rates of proliferation and higher susceptibility to apoptosis. We propose to adress this hypothesis by the following four Specific aims: 1) Determine if either SOD2 or SOX9 is necessary and/or sufficient for effective tumor suppression by mac25; 2) Analyze the effects of both SOD2 and SOX9 on specific proteins and activities of cell cycle and apoptotic pathways known to be regulated by mac25; 3) Analyze the interaction between mac25 and androgen-responsive pathways. Production of specific proteins associated with differentiation, including cytokeratins, cadherins, CD44 and CD57, and PSA, will also be determined; 4) Assess the extent to which either SOD2 or SOX9 can account for the gene expression pattern associated with tumor suppression by mac25. Herein, we report on studies that address specific aims 2 and 3.

14. SUBJECT TERMS
SOX9; SOD-2; Cell Signaling; Apoptosis; Cell Cycle Proteins; Androgens; Androgen Receptor; Transcription Factor; Antisense RNA

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Introduction: We have previously determined that the senescence –associated gene m ac25 is a tumor suppressor for prostate cancer. We have also determ ined, using cDNA microarrays that manganese superdioxide dismutase (SOD-2) and the transcription factor SOX-9, are two genes that have their expression increased by mac35 in prostate epithelial cells. We have shown that the expression of SOD-2 decreases in prostate cancer and re-expression of SOD-2 results in cell senescence and decreased tumor growth. Recent data have shown that a polymorphisms in the SOD-2 gene that results in a decrease in function is associated with higher rates of prostate cancer. The purpose of this study was to determine the effects of SOX9 on development of prostate cancer and the interaction with SOD-2. In the first year of this study we reported that :

- Sox-9 and SOD2 expression is decreased in prostate cancer and SOX9 decreases in models of prostate cancer metastasis.
- SOD-2 and SOX9 both contribute to the tumor suppressing action of mac25 but each by its unique mechanism.
- Antisense and sense adenoviral constructs have been developed for SOD-2 and SOX9.
- Confocal microscopy demonstrates nuclear localization of mac25.

In the second year of this study we have concentrated our efforts on Task 2 of the proposal in which we said that we would construct adenoviral vectors for SOD-2 and SOX9 and determine the effects of expression of these genes on downstream proteins and signaling pathways.

Body: Approved work statement for year 2

Task 2: Analyze the effects of both SOD2 and SOX9 on specific proteins and activities of cell cycle and apoptotic pathways known to be regulated by mac25.

a) Determine expression of specified cell cycle proteins, and perform flow cytometry, in M12/SOD2 and M12/SOX9. Repeat in presence of antisense oligos in cases where both SOD2 and SOX9 are upregulated. (Months 12-18)

UNIGENE	NAME	LNCaP_SOX9	M12_SOX9	PEC_SOX9
Hs.283722	START_DOMAIN_CONTAINING_7_(STARD7)	1.89	3.2	2.08
	UNKNOWN_162	2.04	1.82	1.51
	HUMAN_DNA_SEQUENCE_FROM_CLONE_RP11-503C24_ON_CHROMOSOME_6_COMPLETE_SEQUENCE	1.37	1.38	1.35
Hs.150477	WERNER_SYNDROME_(WRN)	1.4	1.58	1.65
Hs.78672	LAMININ_ALPHA_4_(LAMA4)	1.46	1.57	1.83
Hs.183805	ANKYRIN_1_ERYTHROCYTIC_(ANK1)	1.43	1.74	1.48
Hs.75659	MPV17_TRANSGENE_MURINE_HOMOLOG_GLOMERULOSCLEROSIS_(MPV17)	NA	1.52	1.29
		1.28	1.42	1.37

Hs.380843	RIBOSOMAL_PROTEIN_S6_(RPS6)	1.85	2.11	1.36
Hs.124854	CHROMOSOME_7_OPEN_READING_FRAME_13_(C7ORF13)	1.42	1.91	1.47
Hs.5920	UDP-N-ACETYLGLUCOSAMINE-2-EPIMERASE/N-ACETYLMANNOSAMINE_KINASE_(GNE)	1.67	1.68	1.3
Hs.132071	OVARIAN_CARCINOMA_IMMUNOREACTIVE_ANTIGEN_(OCIA)	1.62	1.47	1.3
Hs.21411	HOMO_SAPIENS_CDNA_FLJ30370_FIS_CLONE_BRACE2007832	1.35	1.43	1.27
Hs.9071	PROGESTERONE_RECEPTOR_MEMBRANE_COMPONENT_2_(PGRMC2)	1.45	1.62	1.29
Hs.93868	KIAA0662_GENE_PRODUCT_(KIAA0662)	1.37	1.35	1.24
Hs.87372	ESTS	1.3	1.53	1.33
Hs.301132	KIAA0195_GENE_PRODUCT_(KIAA0195)	1.42	NA	1.5
Hs.349961	RIBOSOMAL_PROTEIN_L6_(RPL6)	1.45	1.61	1.27
Hs.2007	TUMOR_NECROSIS_FACTOR_(LIGAND)_SUPERFAMILY,_MEMBER_6_(TNFSF6),_MRNA	1.39	1.26	1.53

Table 1. cDNA microarray of genes significantly upregulated after infection of the respective cell lines with an adenoviral construct containing the construct for SOX9 or lacZ control. , >0.67 = 2 =fold increase.

b) Clone SOD2 and SOX9 in adenoviral vector, optimize conditions, and test production. (Months 12-18) Figure 1

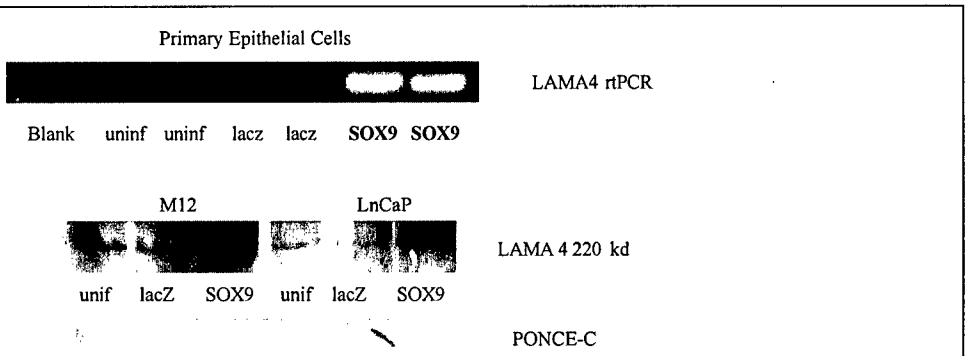

Figure. 1 SOX9 induces laminin subunit α4 in primary prostate epithelial cells and M12 and LNCaP prostate cancer cell lines. SOX9 expression was accomplished with an adenoviral SOX9 construct. The increase in laminin a4 was confirmed in each cell line by RT-PCR and Western blot analysis. lacZ is an adenoviral control vector.

c) Determine cell cycle protein expression and cell kinetics in cells infected with adenoviral constructs. (Months 18-24).

As we have reported (Drivdahl, Oncogene) , SOX9 induced the expression of the androgen receptor(AR) in M12 cells. During this past year we have further demonstrated that the induction of AR is cell cycle dependent with expression beginning in late S-phase and greatest in G2M.

Of the genes that were regulated by SOX9, we were particularily interested in laminin subunit α 4. This subunit has not been previously described to be expressed by the prostate epithelial cell. It is of particular interest because it is associate with the production of laminins 8 and 9. The laminins may significantly alter the invasive properties of tumors but little is known of their function in prostate cancer. We have transfected the α4 laminin subunit into our M12 cells, figure 2 and have confirmed that the cell now expresses laminin 8 in its extracellular matrix. When the cells are grown in culture *in vitro*, as seen in figure 3, they began to form prostate gland appearing organelles. Boyden chamber assays demonstrated that matrix prepared from M12mac25 and M12SOX9 expressing cells in which the matrix is enriched for laminins containing the α4 subunit markedly suppressed invasion, figure 4.

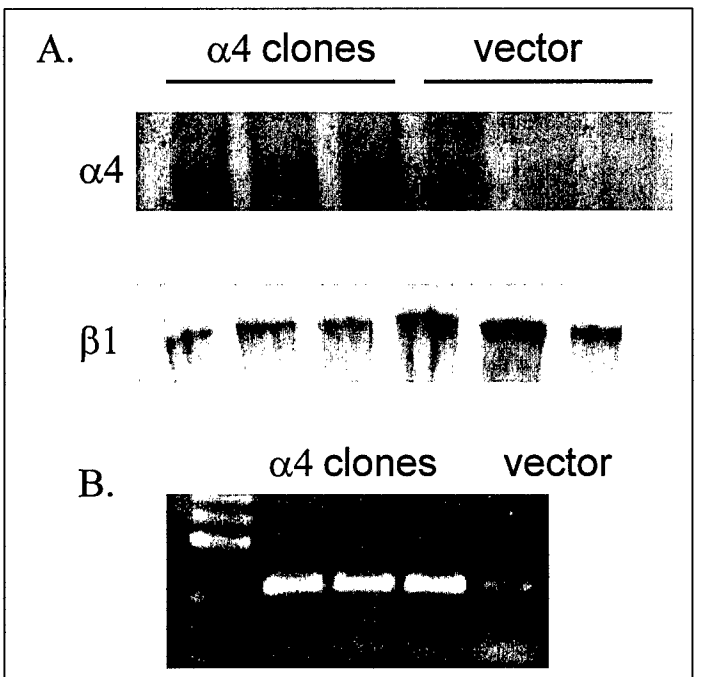

Figure 2. A. Western immunoblot of laminin α4 and β1 subunits from cell lysates of M12 cells transfected with an α4 cDNA plasmid or vector alone, 3 clones are presented. **B.** rtPCR for laminin α4 mRNA from 3 α4 transfected clones an a control vector transfected cell.

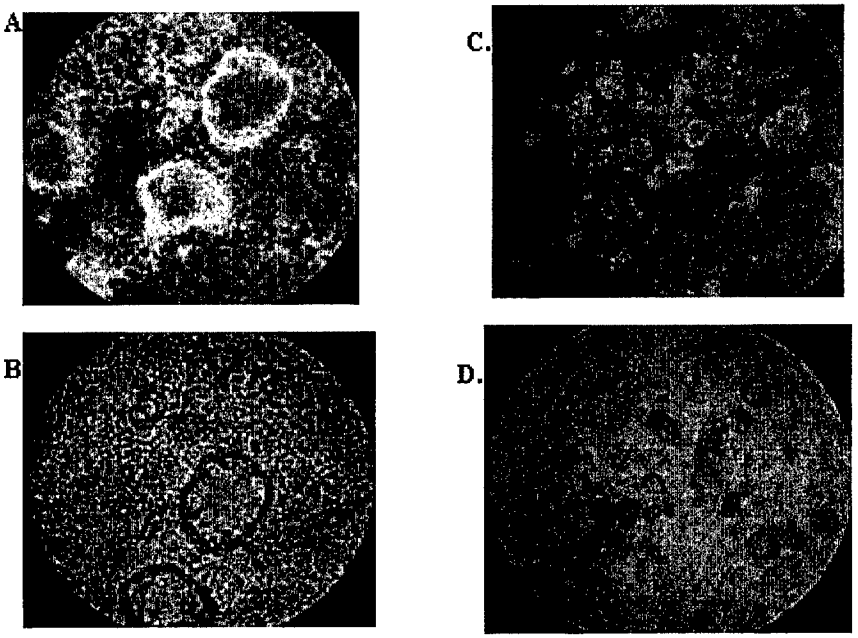

Figure 3. Phase contrast (A. and C.) and plain field (B. and D.) photomicrographs of M12 laminin α4 transfected cells and 100x (A. and B.) and 20x (C. and D.) magnification. Note the formation of organelles with a basal epithelium and beginning of polarized luminal cells. Control transfection results in no organelle formation (not shown). Cells were grown on plastic.

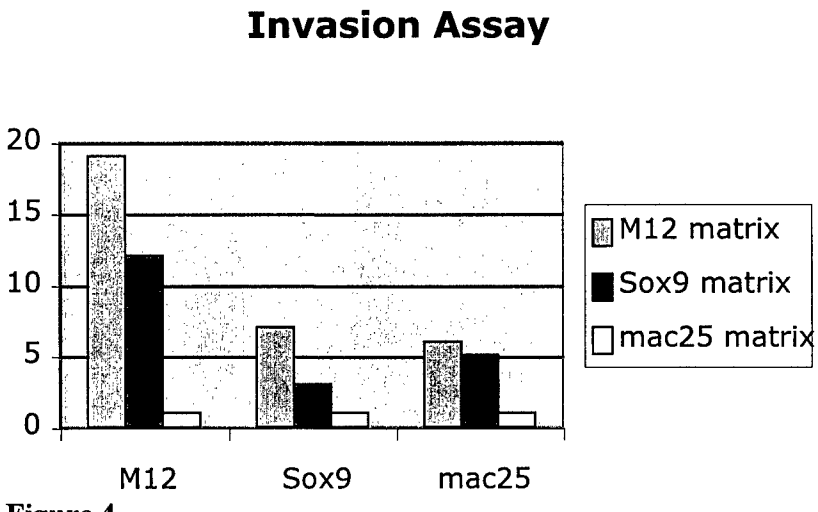

Figure 4.

C. Key Research Accomplishments

- SOX9 expression in M12 cells results in the incread express of 18 genes by 2 fold or more.
- Of the genes that are upregulated by SOX9, the α4 laminin subunit was of particular interest because of significant changes that occur to the ECM.
- Extracellular matrix enriched in α4 laminin subunit results in epithelial differentiation and significantly impairs *in vitro* assays of prostate cancer cell invasion.

D. Reportable outcomes:

Drivdahl R, Tennant MK, Sprenger CT, Nelson PS, Plymate SR 2004 Transcription Factor SOX9 Regulation of Prostate Cancer Growth. Oncogene. 3;23(26):4584-93

Plymate SR, Roberts CTJr, Tennant MK, Haugk K, Woodke L, Marcelli M, Ware JL, 2004 IGF-IR Regulation of Androgen Receptor Signaling in progression to Metastatic Prostate Cancer. Prostate. 61:276-284

York TP, Plymate SR, Nelson, PS Eaves LJ, Webb HM, Ware JL. 2005
cDNA Microarray Analysis Identifies Genes Induced in Common by Peptide Growth Factors and Androgen in Human Prostate Epithelial Cells. Revision Submitted to Mol Cancer Res.

E. Conclusions: The data generated during the past funding period (2004-2005) have demonstrated that SOX9 causes a significant decrease in prostate cancer progression, in part, by altering the structure of the extracellular matrix to promote differentiation and become a barrier to invasion.

F. References:

Drivdahl R, Tennant MK, Sprenger CT, Nelson PS, Plymate SR 2004 Transcription Factor SOX9 Regulation of Prostate Cancer Growth. Oncogene. 3;23(26):4584-93

Plymate SR, Roberts CTJr, Tennant MK, Haugk K, Woodke L, Marcelli M, Ware JL, 2004 IGF-IR Regulation of Androgen Receptor Signaling in progression to Metastatic Prostate Cancer. Prostate. 61:276-284

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G. Appendices: None