The Combination of Dynamic Alterations in Urinary VEGF Levels and Tissue ADAM9 Expression to Predict Lethal Phenotypic Progression of Prostate Cancer

**Chen-Chin Pen[1¶], Che-Ming Liu[2¶], Cho-Chin Lin[3, 4], Chia-Chen Lin[5], Teng-Fu Hsieh[6, 7], Sajni Josson[8], Yun-Chi He[9], Leland W. K. Chung[8], Keh-Liang Lin[3]*, and Shian-Ying Sung[2, 9, 10]*

[1]Urology Department, Tainan Hospital, Department of Health, Executive Yuan, Tainan,

[2]Program for Cancer Biology and Drug Discovery, China Medical University and Academia Sinica, Taichung

[3]Department of Laboratory Medicine, Tainan Hospital, Department of Health, Executive Yuan, Tainan

[4]Institute of Biochemistry and Biotechnology, Chung Shan Medical University, Taichung

[5]Pathology Department, Tainan Hospital, Department of Health, Executive Yuan, Tainan

[6]Division of Urology, Department of Surgery, Taichung Tzu Chi General Hospital, Taichung

[7]Graduate Institute of Clinical Medical Science, China Medical University, Taichung, Taiwan

[8]Department of Medicine, Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA;

[9]Graduate Institute of Cancer Biology, China Medical University, Taichung and

[10]Center for Molecule Medicine, China Medical University Hospital, Taichung, Taiwan.

Running title: VEGF plus ADAM9 as markers of lethal prostate cancer

Corresponding authors: Dr. Shian-Ying Sung, Center for Molecular Medicine and Graduate Institute of Cancer Biology, China Medical University & Hospital, 6 Hsueh-Shih Road, Room 907, Taichung, Taiwan 40454, R.O.C. Email: ssung@mail.cmu.edu.tw; Tel: +886-4-22052121 ext 7934; Fax: +886-4-22333496; Dr. Ke-Liang Lin, School of Medical Technology and Biotechnology, Chung Shan Medical University, 110, Sec. 1, Jianguo N. Road, Taichung, Taiwan 40201, R.O.C. Email: parker@csmu.edu.tw

¶These authors contributed equally to this paper.

Abstract

Recent evidence has demonstrated that the detection of urinary vascular endothelial growth factor (VEGF) and tissue a distintegrin and metalloproteinase 9 (ADAM9) levels is effective in determining prostate cancer progression. To evaluate the combination of VEGF and ADAM9 as early progression markers of lethal phenotypic cancer, quantification of urinary VEGF and tissue ADAM9 expression was studied in patients with late stage prostate cancer. Tissue biopsies were collected during palliative transurethral resection of prostate (TURP) surgery, and urine samples were collected before hormone therapy and 3, 6, and 12 months post-TURP. We observed a nearly 100% correlation between increasing urinary VEGF levels over time and prostate cancer progression, but no correlation was observed when comparing urinary VEGF concentrations at a single time point and cancer progression. In addition, we also observed the correlation of increasing ADAM9 nuclear positive staining and lethal phenotypic transition. Statistically analysis revealed that both the increase in urinary VEGF level and the present of the tissue ADAM9 nuclear staining were significant correlated with the risk of the patient with relapse prostate cancer ($P<0.05$). Thus, we suggest that the combination of changes in urinary VEGF and tissue staining of ADAM9 may be accurate for predicting the mortality of patients with prostate cancer during hormone therapy.

Keywords: ADAM9; urine VEGF; prostate cancer

Introduction

The ability to correctly predict patient outcome earlier than disease progression during therapy represents the most challenging assessment in clinical oncology. Although most patients with prostate cancer remain stable during hormone therapy, the unpredictability of poor prognosis in some patients represents the greatest challenge in determining the strategy and timing for advance therapy. Correctly calculating cancer progression before any detectable serum PSA relapse during hormone therapy enables better control and selection of advance therapies. Recent studies of prostate cancer have demonstrated a correlation between increased vascular endothelial growth factor (VEGF) (1) and a disintegrin and metalloproteinase 9 (ADAM9) (9, 23) relative to cancer progression. VEGF is one of the most critical angiogenic factors involved in vascular permeability, endothelial cell proliferation, and motility (18). Increased microvascular density in the cancer milieu, therefore, correlates with the metastasis potential of cancers (16). The level of VEGF can be easily detected in the serum (13) and urine (1) of cancer patients. In addition, urinary VEGF levels are a useful predictive marker for progression-free survival in patients with prostate cancer following radiotherapy (3).

ADAM9 expression increases during prostate cancer progression (9, 23), and clinical analyses demonstrate a correlation between increased ADAM9 and shortened prostate specific antigen (PSA) relapse-free survival rate in patients with prostate cancer (9). The expression of ADAM9 has been shown to be upregulated by stress and reactive oxygen species (ROS) in prostate cancer cells (23). It is also likely that the induction of ADAM9 is controlled by androgen in androgen-dependent prostate cancer cells but constitutively overexpressed in androgen-independent cancer cells (20). In addition, inhibition of ADAM9 expression has been shown to sensitize prostate cancer cells to the therapeutic effects of both radiotherapy and chemotherapies (15). Peduto further hypothesized that targeting ADAM9

could be a potential strategy for treating patients with cancer (19). Hence, it is conceivable that the level of ADAM9 in prostate cancer cells might be an indicator of malignant development.

Although serum and urine VEGF and tissue ADAM9 levels can be used as markers to predict mortality after radiotherapy (3, 15), evidence is still lacking as to whether VEGF levels can be used as a marker to predict prostate cancer progression during hormone therapy, especially for patients suffered by late-stage cancer with lower urinary tract symptoms (LUTS). Therefore, this study sought to determine the prognostic value of urinary VEGF levels in combination with immunohistochemical analysis of ADAM9 for patients with prostate cancer undergoing hormone therapy and transurethral resection of prostate (TURP) surgery. Our results indicated that dynamic VEGF alterations (before and after hormone therapy and TURP) in addition to ADAM9 nuclear staining together served as accurate early markers of lethal phenotypic progression.

Materials and methods

Patient Cohort

Patients with prostate cancer and normal control were collected for this study (Tainan Hospital, IRB2007–007). Informed consent was obtained from each patient. Total 8 prostate cancer patients and 9 normal health controls were collected during the same time. The patient cohort was carefully selected and included those exhibiting late-stage prostate cancer and severe LUTS that were ready for treatment with a combination of hormone therapy and TURP. Tissue samples were evaluated by a pathologist (C. C. L.), and demonstrated the Gleason Grade range between 5 and 8. In addition, the concentration of serum PSA was evaluated each time during the therapies. This indicated the initial serum PSA range before therapies was between 10 and 500 ng/ml (Table 1).

Sample Preparation

Urine was collected prior to the initiation of hormone and TURP therapies and at 1, 3, 6, and 12 months after TURP surgery. Specimens were collected and stored at -70°C until the time of analysis. Urine and tissue samples were anonymized before being released from the Tainan Hospital Pathology Department tissue bank for this study. Tissue biopsies were collected at the time of TURP surgery and evaluated by a pathologist before being released.

Cell Culture

The human prostate cancer epithelial cell line PC3 was purchased from the ATCC. All cells were cultured in T-medium (Invitrogen) with 5% FBS (Invitrogen) and Pen/Strep at 37°C.

Animal Study

Sis-week-old Balb/c nude mice were used. To produce metastatic tumors, mice were injected at the left ventricle with 5x105 luciferase expressing PC3 cells (PC3-luc). The cells were resuspended in PBS and injected with a 29-gauge needle in the volume of 50 μl. Mice

were monitored twice/weekly. The extravasation of PC3 tumors was determined by examination using bioluminescence imaging and collection of tumors.

Enzyme-Linked Immunosorbent Assay (ELISA)

Urinary VEGF was quantified by ELISA (R&D Systems) following standard procedures supplied by the manufacturer. The color development was read at 450 nm using an ELISA reader, and the results were expressed as the mean absorbance of triplicate samples ± standard error (SE) in pg/mL.

Immunohistochemical Staining

Five-micron thick paraffin-embedded tissue sections were deparaffinized and rehydrated. The tissue sections were incubated for 2 hours with primary antibodies: mouse monoclonal anti-human VEGF (1:50; Chemicon International) and mouse monoclonal anti-ADAM9 (1:100; R&D Systems). Specificity was verified by negative control reactions with the primary antibody replaced with isotype IgG control. Three different views per patient slide were analyzed. The + indicated positive staining observed in all the area that analyzed, +/- indicated at least one of three area revealed negative staining, - indicated no positive staining in all three views.

Western Blot Analysis

Cells cultured at 80–95% confluence were lysed in a lysis buffer, as previously described (21). The protein was then subjected to SDS-PAGE, and the blot was incubated with an anti-human ADAM9 monoclonal antibody (1 μg/ml; R&D Systems), anti-human Lamin B (nucleus marker; GeneTex), α-tubulin (cytosol marker; GeneTex), and then visualized using ECL (GE Healthcare Bio-Science).

Nuclear extraction

To separate the nuclear extraction fraction and cytosol fraction, tumors were collected and washed with ice-cold PBS. Cell lysis buffer (20 mM HEPES [pH 7.0], 10 mM potassium chloride, 2 mM magnesium chloride, 0.5% Nonidet P-40, 1 mM sodium vanadate [Na_3VO_4], 2 ug/ml aprotinin, 1 mM PMSF) were added to the cell pellet and lysed in a Dounce homogenizer for 30 strokes. Nuclei were collected by centrifugation for 5 min at 1500g and then resuspended in NETN buffer (0.5% NP-40, 20 mM Tris [pH 8.0], 50 mM NaCl, 50 mM NaF, 100 μM Na_3VO_4, 1 mM DTT, 1 mM PMSF). The nuclei mixture was sonicated and centrifuge at 12000g for 20 min and the nuclear lysate (supernatant) was collected.

Statistical Analysis

All data were subjected to One Way ANOVA analysis of variance and expressed as the mean ± SD, otherwise mentioned specifically in the text. A p value of <0.05 was considered statistically significant.

Results

Evaluation of VEGF as an Early Prediction Marker for Lethal Phenotypic Prostate Cancer

The patients were divided into stable and relapse groups according to the outcome of three-year survival following hormone therapy. No PSA elevation was observed during the first year of hormone therapy for any patient (Fig. 1A and 1B), indicating the limitation of using serum PSA to predict outcome in the early period of therapy. In addition, one patient remained stable with an initial PSA of ≥500 ng/ml (Fig. 1C) and Gleason score 3+2 (Table 1), indicating that better markers are required for patients during hormone therapy. To determine if there was any correlation between VEGF levels and cancer progression, urine VEGF levels were compared individually prior to the initiation of therapy (pre-OP) and at 3 months after initiation of hormone therapy and TURP (3 month post-OP). Although no significant correlation between urinary VEGF concentrations during the first year of hormone therapy was observed between the two groups (Fig. 1-A and B), dynamic studies of urine VEGF concentrations for each patient demonstrated a high correlation with cancer progression (Fig. 1D). Increasing VEGF tendencies indicated that relapse was likely to occur. By comparison, decreasing levels of VEGF over time were observed in the stable group. Hence, the dynamic alteration of urine VEGF (VEGF slope) between pre-OP and 3-month post-OP was a clear indication of lethal phenotypic prostate cancer transition before PSA relapse could be detected.

ADAM9 Staining as a Marker for Predicting the Development of Lethal Phenotypic Prostate Cancer

Previous reports have shown that the expression of ADAM9 increases in patients with malignant prostate cancer (9, 23). This raises the possibility of using ADAM9 as an independent prognostic marker of PSA relapse-free survival following hormone therapy. To

determine the potential of using urine VEGF expression and tissue ADAM9 levels as combined markers for lethal phenotypic prediction in patients treated with hormone therapy and TURP and to determine if addition of VEGF tissue staining could further enhance the prediction, tissue staining for ADAM9 and VEGF was performed in the same patient cohort and compared between the two patient groups was performed. Figure 2 shows tissues collected before TURP and stained for VEGF or ADAM9 in the refractory prostate cancer group. VEGF demonstrated an overall equal intensity of staining between low-grade region (black square) and high-grade tumor (red square) in the same tissue. By contrast, the majority of ADAM9 positive staining was observed in the high-grade cancer region of the prostate cancers (Fig. 2A and 2B). Furthermore, when we comparison of the hormone-responsive and hormone-refractory prostate cancer tissues demonstrated no difference in VEGF staining, and sometimes lighter staining was observed in the hormone refractory samples versus the hormone responsive patient tissue samples (Fig. 2C). In addition, we noticed the majority of the ADAM9 staining was located in the nuclear region in the patients with relapsed prostate cancer (Fig. 2D). To further elucidate ADAM9 localization in metastatic prostate cancers, mice underwent intracardiac injection of PC3 cells, and tumor was determined at the metastatic loci (Fig. 2E). Nuclear extraction from metastatic tumors showed positive of ADAM9 expression in nuclear fraction of xenograft tumor. In contrast, no ADAM9 expression was detectable in PC3 cells harvested from tissue culture (data not shown), indicating the increased ADAM9 localized to the nuclear region of metastatic prostate cancer cells.

Combination of Urinary VEGF Changes and ADAM9 nuclear/ER Positive Staining Enhances the Prediction of Lethal Phenotypic Prostate Cancer

The binary trends of urinary VEGF alterations and ADAM9 nuclear/ER staining tracked together 100% of the time during early hormone therapy (Table 1). Statistically analysis by Spearman's correlation coefficient demonstrated that both the increase in urinary VEGF level and the present of the tissue ADAM9 nuclear/ER staining were significant correlated with the risk of the patient with relapse prostate cancer (p=0.012). Logistically, a patient could have a biopsy before hormone therapy to evaluate possible risk based on ADAM9 tissue staining. This would be followed by the examination of kinetic variations in urinary VEGF before and during hormone therapy. This combination of VEGF and ADAM9 measurements might serve as a new predictor of early progression-free survival after hormone therapy and before any serum PSA rebound could be detected. In addition, combination of urinary VEGF concentration and ADAM9 nuclear positive staining is highly correlated with histopathologic grading of prostate cancer patient stage with only one patient indicating of Greason score of 2+3 but positive in ADAM9 nuclear staining and increased of urine VEGF concentration during therapy. Nevertheless, our data indicated ADAM9 staining and urine VEGF velocity before and during hormone therapy could enhance the prediction accuracy of Greason score.

Urinary proteins are highly correlated with personal health conditions and/or behaviors, such as uremia (2) or cigarette smoking (6, 10). To determine if smoking interfered with urine VEGF concentrations, we examined a group of healthy controls in regards to smoking, alcohol consumption, and the use of hair dye. The results showed that urine VEGF expression in this control group was lower compared to the patients with prostate cancer, suggesting that smoking, alcohol consumption, and the use of hair dye did not affect our data (Table 2).

Discussion

We report the initial quantification of urinary VEGF levels and tissue ADAM9 expression in patients with either stable or recurrent prostate cancer. One-year analysis of urine VEGF variation after palliative TURP and hormone therapy demonstrated that kinetic elevation of VEGF correlated highly with prostate cancer progression. Recent studies have indicated that overexpression of VEGF in prostate cancer cells and the cancer microenvironment enhances malignant progression (11, 14). The release of VEGF into the urine, therefore, can be used as a marker to predict cancer progression (1, 17). However, this method still lacks evidence showing that urine VEGF can serve as an accurate marker for predicting lethal phenotypic transition of patients with prostate cancer undergoing hormone therapy. In addition to the correlation between increasing VEGF levels and cancer progression, increases in ADAM9 have been shown to correlate with cancer progression and ROS production in cancer cells (20, 23). Furthermore, knockdown of ADAM9 enhances the therapeutic effects of radiation and chemotherapy (15). Our data demonstrated that the combination of kinetic analysis of urinary VEGF concentrations and ADAM9 expression in tissue biopsies could serve as markers for lethal phenotypic transition after hormone therapy and TURP. We also noticed that increased nuclear/ER staining of ADAM9 expression correlated with lethal phenotypic transition.

The study by Chan et al. indicates that the kinetics of VEGF expression during and after radiotherapy highly correlates with disease progression (3). Our study indicated that the dynamic alteration of urine VEGF was a good indication of lethal phenotypic prostate cancer transition before PSA relapse could be detected. These observations suggested that the levels of VEGF at a single time point in prostate cancer may not necessarily be related to malignancy; more exactly, the dynamic alterations of VEGF in a patient's urine could be a more favorable candidate marker. Although urine VEGF could be a marker of prostate cancer

risk (5, 12), our study demonstrated that the level of urine VEGF in a single measurement does not necessarily indicate a lethal phenotypic transition.

Our study further demonstrated that the combination of changes in VEGF and ADAM9 nuclear/ER staining could improve prognosis prediction during the early phase of hormone therapy. During cancer therapies, intracellular ROS in prostate cancer cells is induced in response to a variety of exogenous stressors such as radiation therapy, chemotherapeutic agents, androgen stimulation, overcrowding, and serum deprivation (15, 20, 23). However, ROS may also initiate a downstream signaling cascade that aids in tumor cell survival and progression (4, 22). Previously, we demonstrated an increased expression of ADAM9 in response to lethal concentrations of hydrogen peroxide, overcrowding, and serum deprivation (20, 23). In addition, studies of biopsy specimens from patients with prostate cancer have revealed elevated levels of ADAM9 expression in the cancerous portions of the gland; ADAM9 expression was essentially absent in the adjacent healthy prostate tissue (9, 23). Furthermore, we noticed complete nuclear staining in the metastasis group in the tissue-array studies, and this provided better clarification of the correlation between the chances of lethal phenotypic transition and ADAM9 nuclear/ER-positive staining. Although the increase in ADAM9 ER localization correlated with lethal phenotypic transition, the role of ADAM9 in the ER is still unclear. Current studies of ADAM9 indicate that it may be involved in the ER stress response (7, 15, 20, 23), though further studies are necessary to clarify the role of ADAM9 in this pathway. Nevertheless, increased ADAM9 ER staining could serve as an indicator of lethal phenotypic transition.

In summary, we demonstrated that assessment of changes in VEGF urinary levels in combination with ADAM9 nuclear/ER expression resulted in effective lethal phenotypic prediction for patients with prostate cancer undergoing hormone therapy. Furthermore, we suggest that the use of both markers together substantially increased the accuracy of this

prediction. As such, urinary VEGF changes and ADAM9 nuclear/ER expression might serve as vital tools for establishing disease recurrence and selecting patients that would benefit most from earlier and more advanced therapies.

Acknowledgements

We would like to thank Li-Chin Wu for the excellent technical support and Medcom Asia Inc. for help with English editing. We specially thank Dr. Yau-Huei Wei for kindly providing detail organelle isolation protocols. This work was supported in part by the National Science Council funds NSC99–2320-B-039–029-MY3, and NSC99–2632-B-039–001-MY3, China Medical University research fund CMU97–281, and National Health Research Institute IRG fund NHRI-EX-99–9902BI.

References

1. Bok R.A., Halabi S., Fei D.T., Rodriquez C.R., Hayes D.F., Vogelzang N.J., Kantoff P., Shuman M.A., and Small E.J. Vascular endothelial growth factor and basic fibroblast growth factor urine levels as predictors of outcome in hormone-refractory prostate cancer patients: a cancer and leukemia group B study. *Cancer Res.* 61: 2533-2536, 2001.
2. Boure T., and Vanholder R. Biochemical and clinical evidence for uremic toxicity. *Artif. Organs* 28: 248-253, 2004.
3. Chan L.W., Moses M.A., Goley E., Sproull M., Muanza T., Coleman C.N., Figg W.D., Albert P.S., Menard C., and Camphausen K. Urinary VEGF and MMP levels as predictive markers of 1-year progression-free survival in cancer patients treated with radiation therapy: a longitudinal study of protein kinetics throughout tumor progression and therapy. *J. Clin. Oncol.* 22: 499-506, 2004.
4. Chung L.W., Hsieh C.L., Law A., Sung S.Y., Gardner T.A., Egawa M., Matsubara S., and Zhau H.E. New targets for therapy in prostate cancer: modulation of stromal-epithelial interactions. *Urology* 62: 44-54, 2003.
5. Crew J.P. Vascular endothelial growth factor: an important angiogenic mediator in bladder cancer. *Eur. Urol.* 35: 2-8, 1999.
6. Edirisinghe I., and Rahman I. Cigarette smoke-mediated oxidative stress, shear stress, and endothelial dysfunction: role of VEGFR2. Ann N Y Acad Sci 1203: 66-72, 2010.
7. Fischer O.M., Hart S., Gschwind A., Prenzel N., and Ullrich A. Oxidative and osmotic stress signaling in tumor cells is mediated by ADAM proteases and heparin-binding epidermal growth factor. Mol Cell Biol 24: 5172-5183, 2004.
8. Frezza C., Cipolat S., and Scorrano L. Organelle isolation: functional mitochondria from mouse liver, muscle and cultured fibroblasts. *Nat. Protoc.* 2: 287-295, 2007.

9. Fritzsche F.R., Jung M., Tolle A., Wild P., Hartmann A., Wassermann K., Rabien A., Lein M., Dietel M., Pilarsky C., Calvano D., Grutzmann R., Jung K., and Kristiansen G. ADAM9 Expression is a Significant and Independent Prognostic Marker of PSA Relapse in Prostate Cancer. *Eur. Urol.* 54: 1097-1108, 2008.
10. Hill P., and Marquardt H. Plasma and urine changes after smoking different brands of cigarettes. *Clin. Pharmacol. Ther.* 27: 652-658, 1980.
11. Jackson M.W., Roberts J.S., Heckford S.E., Ricciardelli C., Stahl J., Choong C., Horsfall D.J., and Tilley W.D. A potential autocrine role for vascular endothelial growth factor in prostate cancer. *Cancer. Res.* 62: 854-859, 2002.
12. Jamaspishvili T., Kral M., Khomeriki I., Student V., Kolar Z., and Bouchal J. Urine markers in monitoring for prostate cancer. Prostate Cancer Prostatic Dis 13: 12-19, 2010.
13. Jones A., Fujiyama C., Turner K., Fuggle S., Cranston D., Bicknell R., and Harris A.L. Elevated serum vascular endothelial growth factor in patients with hormone-escaped prostate cancer. *BJU Int.* 85: 276-280, 2000.
14. Joseph I.B., Nelson J.B., Denmeade S.R., and Isaacs J.T. Androgens regulate vascular endothelial growth factor content in normal and malignant prostatic tissue. *Clin. Cancer Res.* 3: 2507-2511, 1997.
15. Josson S., Anderson C.S., Sung S.Y., Johnstone P.A., Kubo H., Hsieh C.L., Arnold R., Gururajan M., Yates C., and Chung L.W. Inhibition of ADAM9 expression induces epithelial phenotypic alterations and sensitizes human prostate cancer cells to radiation and chemotherapy. *Prostate* 71: 232-240, 2011.
16. Kaygusuz G., Tulunay O., Baltaci S., and Gogus O. Microvessel density and regulators of angiogenesis in malignant and nonmalignant prostate tissue. *Int. Urol. Nephrol.* 39: 841-850, 2007.

17. Miyake H., Muramaki M., Kurahashi T., Yamanaka K., and Hara I. Urinary levels of vascular endothelial growth factor in patients with prostate cancer as a predictor of disease progression. *Anticancer Res.* 25: 3645-3649, 2005.
18. Neufeld G., Cohen T., Gengrinovitch S., and Poltorak Z. Vascular endothelial growth factor (VEGF) and its receptors. *FASEB J.* 13: 9-22, 1999.
19. Peduto L. ADAM9 as a Potential Target Molecule in Cancer. *Curr. Pharm. Des.* 15: 2282-2287, 2009.
20. Shigemura K., Sung S.Y., Kubo H., Arnold R.S., Fujisawa M., Gotoh A., Zhau H.E., and Chung L.W. Reactive oxygen species mediate androgen receptor- and serum starvation-elicited downstream signaling of ADAM9 expression in human prostate cancer cells. *Prostate* 67: 722-731, 2007.
21. Sung S.Y., Hsieh C.L., Law A., Zhau H.E., Pathak S., Multani A.S., Lim S., Coleman I.M., Wu L.C., Figg W.D., Dahut W.L., Nelson P., Lee J.K., Amin M.B., Lyles R., Johnstone P.A., Marshall F.F., and Chung L.W. Coevolution of prostate cancer and bone stroma in three-dimensional coculture: implications for cancer growth and metastasis. *Cancer Res* 68: 9996-10003, 2008.
22. Sung S.Y., Hsieh C.L., Wu D., Chung L.W., and Johnstone P.A. Tumor Microenvironment Promotes Cancer Progression, Metastasis, and Therapeutic Resistance. *Curr. Probl. Cancer* 31: 36-100, 2007.
23. Sung S.Y., Kubo H., Shigemura K., Arnold R.S., Logani S., Wang R., Konaka H., Nakagawa M., Mousses S., Amin M., Anderson C., Johnstone P., Petros J.A., Marshall F.F., Zhau H.E., and Chung L.W. Oxidative Stress Induces ADAM9 Protein Expression in Human Prostate Cancer Cells. *Cancer. Res.* 66: 9519-9526, 2006.