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SHORT COMMUNICATION

# Glutathione and Glutathione S-Transferase in Benign and Malignant Prostate Cell Lines and Prostate Tissues

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**ABSTRACT.** Metastatic prostate adenocarcinoma is unresponsive to alkylator chemotherapy with virtually no prolonged remissions. Glutathione (GSH) and glutathione S-transferase (GST) have been reported to play a role in tumor resistance to alkylator therapy; however, there are no baseline studies that have investigated and compared GSH and GST in human prostate cell lines and tissues. Thus, we determined the GSH content and GST activity in benign prostate, in primary and metastatic prostate adenocarcinoma tissues, in immortal adenocarcinoma cell lines, and in primary cell cultures derived from both benign prostate and primary prostatic carcinoma tissue. The GSH content was higher in the immortal cell lines than in the fresh tissues and primary cultures. Conversely, the GST activity was significantly higher in the tissues and primary cultures than in the cell lines. The GSH content and GST activity of the primary cultured prostatic cells were similar to those of the prostate tissues. The differences between the immortal prostate cancer cell lines and prostate tissue are of sufficient magnitude to suggest that *in vitro* results with cell lines may not extrapolate to prostate cancer *in vivo*. The GSH content and GST activity in a prostate specific antigen-secreting human prostate tumor xenograft, LuCaP23, maintained in nude mice were similar to those of human prostate tissue and primary cultures. Both the xenograft and primary cultures from patients with prostate cancer may be more appropriate models than established cell lines for investigating techniques to increase the effectiveness of alkylators in prostate cancer. BIOCHEM PHARMACOL 51;1:87–90, 1996.

**KEY WORDS.** prostate cancer; glutathione; glutathione S-transferase

The failure of conventional chemotherapy and the development of resistance in tumors have led to a number of techniques to improve the effectiveness of current agents as well as to circumvent *de novo* and acquired resistance. An approach for alkylator resistance is the depletion of tumor GSH** content by BSO [1]. This is based on studies that reported a direct relationship between GSH content and drug resistance [2–4].

The role of GSH in modulating resistance to alkylating agents results from the GST-mediated conjugation of the electrophile to GSH, yielding the glutathionyl conjugate. Thus, both GSH depletion by BSO and GST inhibition have increased the tumoricidal activity of melphalan, a prototypic alkylating drug [5, 6]. However, there are no baseline data on the GSH content and total GST activity profiles of fresh prostate tissues as compared with those of established cell lines that would establish or suggest appropriate model systems for the study of GSH and GST modulation. Thus, we sought to identify the most appropriate model system that approximates the GSH content and total GST activity in fresh prostatic tissue.

## MATERIALS AND METHODS

The androgen-insensitive prostate cancer cells 1-LN, DU145, and PC3 [7–9] were studied during exponential growth in RMPI 1640 with 5% fetal bovine serum. LNCaP, an androgen-sensitive prostate cancer line [10], was maintained in the same medium with 1% ITS+ (Collaborative Research, Bedford, MA).

Primary cultures of human prostate epithelial cells were grown from pathologically confirmed benign or malignant primary cancers. Specimens were placed in tissue culture within 0.5 hr of removal from patients as previously described [11]. Immunohistochemical characterization of these cells as pros-

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** Abbreviations: GSH, glutathione; BSO, buthionine sulfoximine; GST, glutathione S-transferase; PSA, prostate specific antigen; and BPH, benign prostatic hypertrophy.

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tate-derived was performed using a panel of monoclonal antibodies to cytokeratins, PSA, and prostatic acid phosphatase. Tissue culture conditions were maximized to inhibit fibroblast proliferation and allow epithelial prostatic cell growth. The primary prostate cell cultures were tested for GSH content and total GST activity within 2–3 weeks of establishment in cell culture from the time they were removed surgically from patients.

Pathologically confirmed prostate tissue from patients with BPH, or primary or metastatic prostatic carcinoma was sectioned and snap-frozen in liquid nitrogen within 0.5 hr from surgical removal. Metastatic tissues from nine patients consisted of 4 samples from regional lymph nodes, 2 from bone metastases, 1 each from a pelvic mass, a subcutaneous nodule, and a lung metastasis. These samples were provided from tissue banks at Duke University Medical Center, Memorial Sloan-Kettering Institute, University of Vermont Comprehensive Cancer Center, and the NCI Repository Tissue Bank, Midwestern Division, Ohio State University.

The xenograft tumor was obtained from a patient with stage D2 adenocarcinoma of the prostate, who had not been treated with hormones, chemotherapy, or radiation therapy. A number of retroperitoneal lymph nodes and liver foci were implanted in male athymic (BALB/c nu/nu) mice. Three of twelve sublines producing high levels of PSA became the focus of additional studies [12]. Fluorescence *in situ* hybridization (FISH) analysis confirmed the fact that the tumors were human in origin. One of these sublines, LuCaP23, was passaged in nude mice; it was androgen sensitive and secreted PSA into the serum. When LuCaP23 was passed *in vitro*, it gradually became androgen insensitive and displayed decreased ability to secrete PSA. The xenograft tissue tested in this study was directly from the nude mice *in vivo* passage and displayed androgen sensitivity.*

For GSH and GST, cell pellets or tissues were suspended in 0.1 M phosphate buffer, pH 7.5, and disrupted by sonication or homogenization. The cell lysate or tissue homogenate was centrifuged, and the supernatant was used for GST, GSH, and protein. All determinations were performed in duplicate. GSH was assayed by HPLC using the monobromobimane derivatization method described by Anderson [12]. GST was determined by the change in absorbance at 340 nm of chlorodinitrobenzene incubated in the presence of GSH [13]. Protein concentrations were measured according to the method of Lowry *et al.* [14].

Statistical significance was determined by analysis of variance. The finding of significance, $P \leq 0.05$, resulted in determination of the source of the significance using the Fischer PLSD test. Data are reported as means ± SEM.

## RESULTS

### GSH

As shown in Table 1, the GSH content of the DU145 cell line was significantly greater than that of any of the other cell lines and tissues measured. The GSH contents of DU145 and PC3 were significantly higher than that found in the fresh surgical specimens. Interestingly, the GSH content of the PSA-secreting xenografts did not differ from that of freshly isolated prostate tissue or primary cultures. There was no statistically significant difference between the GSH contents of the xenograft tissues, primary cultures and the fresh tissue when compared with each other. However, these three groups were quite different statistically from the PC3 and DU145 cell lines ($P \leq 0.01$). There was a great deal of variability in GSH content both within and among samples from patient with BPH (N = 7), primary (N = 9), or metastatic carcinoma of the prostate (N = 7) (Fig. 1, top panel). However, there was no significant difference in mean GSH content between any of the benign, malignant, or metastatic tissues removed from patients.

**TABLE 1. Comparison of GSH and GST in Immortal Prostate Cancer Cell Lines, Primary Cultures, the PSA-Secreting Xenograft, and Prostate Tissues**

| Cell or tissue | GSH (nmol/mg protein) | GST (mU/mg protein) |
|---|---|---|
| Cell lines | | |
| PC3 | 30 ± 3* (8) | 4.5 ± 0.7 (8) |
| DU145 | 66 ± 5† (6) | 5.7 ± 1.0 (5) |
| 1-LN | 20 ± 2 (7) | 4.7 ± 0.9 (7) |
| LNCaP | 20 ± 3 (8) | 1.7 ± 0.4‡ (7) |
| Primary cultures | | |
| A. Benign | 18 ± 6 (4) | 15 ± 3§ (8) |
| B. Cancer | 22 ± 3 (5) | 22 ± 8§ (5) |
| PSA xenograft | | |
| (LuCaP23) | 16 ± 2 (4) | 10 ± 2‖ (4) |
| Tissue | | |
| Normal prostate | 12 ± 2 (7) | 20 ± 4§ (9) |
| Primary cancer | 16 ± 3 (9) | 23 ± 5§ (8) |
| Metastatic | 11 ± 4 (7) | 12 ± 2§ (9) |

Values are means ± SEM of the number of experiments given in parentheses.

* Significantly greater than benign ($P = 0.002$) and metastatic prostate ($P = 0.005$) tissue, and the xenograft ($P = 0.005$).

† Significantly greater than the other immortal cell lines ($P = 0.0002$–$0.01$), the xenograft ($P = 0.0001$), the primary cultures (normal $P = 0.0004$, cancer $P = 0.003$), and the prostate tissues ($P = 0.0001$ for all).

‡ Significantly less than the other immortal cell lines ($P = 0.008$–$0.03$).

§ Significantly greater than the immortal cell lines ($P = 0.002$–$0.02$).

‖ Significantly greater than the immortalized cell lines ($P = 0.001$–$0.05$).

### GST

Table 1 also shows that the GST activity of all of the immortalized cell lines was significantly less than that of the primary cultures, prostate tissues, or the xenograft tissues ($P \leq 0.01$). Despite the apparent difference between the metastatic tumors (N = 9), and the benign (N = 9) and primary prostate cancer tissue (N = 8) (Fig. 1, bottom panel), the large standard errors prevented statistical significance ($P = 0.14$). Similar to the results found for GSH, the GST activities in the primary cultures, fresh surgical tissues, and xenograft tissues were not statistically different from each other.

## DISCUSSION

The failure of conventional chemotherapy has led to recommendations that therapy for metastatic prostate cancer should

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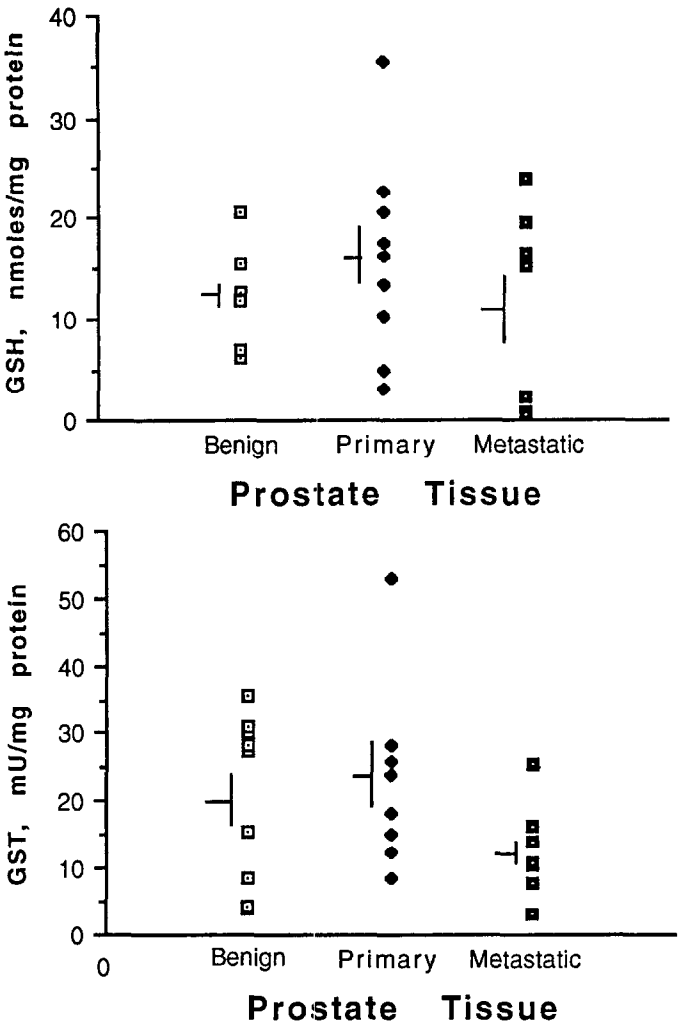


**FIG. 1. GSH (top panel) and GST (bottom panel) in individual samples from prostate tissue determined to be either benign, malignant limited to the prostate, or from metastases derived from a primary prostate adenocarcinoma. The number of data points differs from the N stated in the text due to a number of values that were either identical or too close in value to be shown in the figure.**

be limited to novel agents or old agents used in novel ways [15, 16]. One of these approaches was to deplete GSH to resensitize tumors that had become resistant to melphalan [1, 5, 6]. Thus, it is important to identify appropriate model systems that mimic fresh prostatic tissues in their GSH and total GST activity profiles.

We found that the GSH content of two androgen-insensitive cell lines (PC3 and DU145) growing at mid-log was significantly higher than that of fresh prostate and xenograft tissues. This may be because GSH content appears to be directly related to the state of proliferation of cells in culture [17]. Because the proportion of cells in mitosis in prostate tissue is small [18], prostate tissue would be equivalent to confluent cells in culture, which contain less GSH than during mid-log growth. In our study, the PSA-secreting prostate cancer xenograft and primary prostate cancer cultures, whose GSH content and GST activity were similar to those of fresh prostate tissue, may offer more potential for *in vivo* and *in vitro* studies, respectively, than the immortalized prostate cancer cell lines.

Modulation of GST activity also has been shown to affect the sensitivity of tumor cell lines to alkylating agents. Inhibition of GST by either ethacrynic acid or piriprost enhanced alkylator cytotoxicity to both rat and human cancer cell lines [19, 20]. In contrast to the GSH data, the GST activity of the immortal cell lines grown at mid-log was lower than either the fresh prostate tissues, the primary cell cultures, or the PSA-secreting xenograft tumor. Though this study did not examine GST isoenzyme patterns, the similar total GST activities of fresh tissues, primary cultures, and xenograft tissues may suggest that these two latter model systems may be more appropriate for study of the effects of GST modulation. This observation, as well as that for GSH content, suggests that the immortal prostate cancer lines are not an appropriate model to reflect the *in vivo* state.

Of additional importance to the *in vitro–in vivo* translation is the report that as much as a 5-fold difference in GSH content can exist in various cells comprising a single tumor [21]. This, in turn, would be reflected in the lack of a homogenous response to GSH depletion and to the ultimate success of subsequent alkylator therapy. The PSA-secreting xenograft maintained in the nude mouse or primary prostate cancer cultures, both with GSH content and GST activity similar to fresh prostatic adenocarcinoma tissue, may be a more useful model than immortalized prostate cancer cells for future studies evaluating the role of GSH and GST modulation in the treatment of human prostate cancer.

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## References

1. Green JA, Vistica DT, Young RC, Hamilton TC, Rogan AM and Ozols RF, Potentiation of melphalan cytotoxicity in human ovarian cancer cell lines by glutathione depletion. *Cancer Res* **44:** 5427–5431, 1984.
2. Alaoui-Jamali MA, Panasci L, Centurioni GM, Schecter R, Lehnert S and Batist G, Nitrogen mustard–DNA interaction in melphalan-resistant mammary carcinoma cells with elevated intracellular glutathione and glutathione-*S*-transferase activity. *Cancer Chemother Pharmacol* **30:** 341–347, 1992.
3. Yang WZ, Begleiter A, Johnston JB, Israels LG and Mowat MR, Role of glutathione and glutathione *S*-transferase in chlorambucil resistance. *Mol Pharmacol* **41:** 625–630, 1992.
4. Barranco SC, Townsend CM Jr, Weintraub B, Beasley EG, MacLean KK, Shaeffer J, Liu NH and Schellenberg K, Changes in glutathione content and resistance to anticancer agents in human stomach cancer cells induced by treatments with melphalan *in vitro*. *Cancer Res* **50:** 3614–3618, 1990.
5. Canada AT, Herman L, Kidd K, Robertson C and Trump D, Glutathione depletion increases the cytotoxicity of melphalan to the androgen-insensitive prostate cancer cell line, PC-3. *Cancer Chemother Pharmacol* **32:** 73–77, 1993.
6. Rosenberg MC, Colvin OM, Griffith OW, Bigner SH, Elion GB, Horton JK, Lilley E, Bigner DD and Friedman HS, Establishment of a melphalan-resistant rhabdomyosarcoma xenograft with cross-resistance to vincristine and enhanced sensitivity following

buthionine sulfoximine-mediated glutathione depletion. *Cancer Res* **49:** 6917–6922, 1989.
7. Ware JL, Paulson DF, Mickey GH and Webb KS, Spontaneous metastasis of cells of the human prostate carcinoma cel line PC-3 in athymic nude mice. *J Urol* **128:** 1064–1067, 1982.
8. Horoszewicz JS, Leong SS, Kawinski E, Karr JP, Rosenthal H, Chu TM, Mirand EA and Murphy GP, LNCaP model of human prostatic carcinoma. *Cancer Res* **43:** 1809–1818, 1983.
9. Kaighn ME, Narayan KS, Ohnuki Y, Lechner JF and Jones LW, Establishment and characterization of a human prostatic carcinoma cell line (PC-3). *Invest Urol* **17:** 16–23, 1979
10. Stone KR, Mickey DD, Wunderli H, Mickey GH and Paulson DF, Isolation of a human prostate carcinoma cell line (DU 145). *Int J Cancer* **21:** 274–281, 1978.
11. Roberson KM and Robertson CN, Isolation and growth of human primary prostate epithelial cultures. *Methods Cell Sci,* in press.
12. Anderson ME, Determination of glutathione and glutathione disulfide in biological samples. *Methods Enzymol* **113:** 548–564, 1985.
13. Habig WH, Pabst MJ and Jakoby WB, Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J Biol Chem* **249:** 7130–7139, 1974.
14. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem* **193:** 265–275, 1951.
15. Klein LA, Prostatic carcinoma. *N Engl J Med* **300:** 824–833, 1979.
16. Eisenberger MA, Bezerdjian L and Kalash S, A critical assessment of the role of chemotherapy for endocrine-resistant prostatic carcinoma. *Urol Clin North Am* **14:** 695–706, 1987.
17. Terradez P, Asensi M, Lasso de la Vega MC, Puertes IR, Viña J and Estrela JM, Depletion of tumor glutathione *in vivo* by buthionine sulphoximine: Modulation by the rate of cellular proliferation and inhibition of cancer growth. *Biochem J* **292:** 477–483, 1993.
18. Chiarodo A, Meeting report. National Cancer Institute Roundtable on Prostate Cancer: Future research directions. *Cancer Res* **51:** 2498–2505, 1991.
19. Tew KD, Bomber AM and Hoffman SJ, Ethacrynic acid and piriprost as enhancers of cytotoxicity in drug resistant and sensitive cell lines. *Cancer Res* **48:** 3622–3625, 1988.
20. Hansson J, Berhane K, Castro VM, Jungnelius U, Mannervik B and Ringborg U, Sensitization of human melanoma cells to the cytotoxic effect of melphalan by the glutathione transferase inhibitor ethacrynic acid. *Cancer Res* **51:** 94–98, 1991.
21. Lee FY, Vessey A, Rofstad E, Siemann DW and Sutherland RM, Heterogeneity of glutathione content in human ovarian cancer. *Cancer Res* **49:** 5244–5248, 1989.