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ORIGINAL PAPER

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A possible regulatory role of glyoxalase I in cell viability of human prostate cancer

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Abstract A role of glyoxalase I (Gly-I), a detoxifying enzyme, in cell viability of prostate cancer was investigated. Cell extracts obtained from 66 prostate tissue specimens and prostatic cancer PC-3 cells were assayed for Gly-I activity using the spectrophotometric method. Gly-I activity was consistently more than eightfold higher in prostate cancer (CAP) specimens ($n=37$) than in non-cancerous (NCP) specimens ($n=29$). To understand the importance of such a high Gly-I activity in CAP specimens, the effects of methylglyoxal (MG) on PC-3 cells were examined in vitro. MG, a putative toxic glycolytic metabolite, was capable of inducing severe (>99%) cell death in 24 h, along with a significant reduction in activities of Gly-I as well as glyceraldehyde 3-phosphate dehydrogenase (G3PDH), a key glycolytic enzyme. However, such severe cell death was effectively (~85%) prevented with N-acetylcysteine (NAC), a precursor of reduced glutathione (GSH) that is an essential cofactor for Gly-I, accompanied by the intact Gly-I and G3PDH activities. Therefore, Gly-I may play a critical detoxifying role in glycolysis to maintain cellular activity and viability of prostatic cancer cells.

Keywords Glyoxalase I · Cell viability · Prostate cancer · PC-3 cells

Introduction

The glyoxalase system is a vital enzymatic system in the detoxification of toxic electrophilic metabolites, such as methylglyoxal (MG) and other α-oxoaldehydes [18]. It is comprised of two enzymes, glyoxalase I (Gly-I) and glyoxalase II (Gly-II), requiring reduced glutathione (GSH) as a cofactor. Gly-I first catalyzes the conversion of MG to S-D-lactoylglutathione, which is then hydrolyzed to D-lactic acid with regeneration of GSH by Gly-II, as shown below:

$$\begin{aligned} &\mathrm{MG + GSH\ (\leftrightarrow Hemithioacetal)} \xrightarrow{\mathrm{Gly-I}} \\ &\quad \mathrm{S-D-Lactoylglutathione\ (+H_2O)} \\ &\qquad \downarrow \mathrm{Gly-II} \\ &\quad \mathrm{D-Lactate + GSH} \end{aligned}$$

This enzymatic system is active throughout embryogenesis, tissue maturation and continues until cell death [13]. Particularly, Gly-I has been studied in various biological/clinical aspects because of its wide distribution in human tissues [9]. For instance, altered activities of Gly-I in human malignancies have been documented in colon [16], lung [3], renal and bladder carcinomas [2]. These alterations are thus considered to be crucial for sustaining tumor viability/survival under an altering microenvironment with tumor growth.

Our recent study indicated that Gly-I activity might differ distinctly between prostate cancer (CAP) and non-malignant (normal and benign prostatic hyperplasia) specimens [1], although such differences had not been *quantitatively* demonstrated. Meanwhile, a biological role of Gly-I in CAP specimens also needs to be defined, because this may provide an insight into the growth/development of CAP. Accordingly, we performed a quantitative analysis of Gly-I activity in prostate specimens and explored a potential role of Gly-I in cancer cell viability using methylglyoxal (MG), a primary substrate for Gly-I [18].

Glycolysis is one of the crucial cellular processes required for cell homeostasis and viability. MG is a toxic metabolite supposedly produced during glycolysis [20] and a potent inhibitor of glyceraldehyde 3-phosphate dehydrogenase (G3PDH), an important regulatory

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enzyme of glycolysis [4]. An accumulation of this toxic metabolite is believed to interfere with a glycolytic pathway by inhibiting G3PDH, unless it is properly detoxified by Gly-I (Fig. 1). This would then lead to growth attenuation/cessation in the cells, due primarily to a subsequent cellular energy deficiency (depletion). MG has also been shown to cause DNA modification (formation of DNA adducts) resulting in an inhibition of DNA synthesis [20] as well as an inhibition of mitochondrial respiration [4]. For example, induction of G_1 growth arrest and apoptosis by MG has been reported in human leukemic HL-60 cells [8]. Thus, these findings led us to assume that the metabolic regulation (detoxification) of MG by Gly-I appears to be the substantial biochemical event required for homeostasis, growth and viability of the cancer cells.

To test such a possibility, MG was used as a cytotoxic agent to assess its in vitro effects on cell growth/viability and Gly-I/G3PDH activity in prostatic cancer PC-3 cells. A possible biological role of Gly-I and the importance of glycolysis in the CAP development will be also discussed.

Materials and methods

Prostate tissue specimens

Prostate tissue specimens were freshly obtained from 37 patients with prostate cancer (CAP) undergoing prostatectomy and from 15 patients with benign prostatic hyperplasia (BPH) at transurethral resection of the prostate. A selected portion of each specimen was excised and sent for histologic examination, while its adjacent section was stored at –80°C. Based on the pathology report, a total of 66 specimens were chosen for this study: 37 CAP and 29 non-cancerous (NCP) specimens including 14 uninvolved portions (negative for cancer) from CAP and 15 BPH specimens.

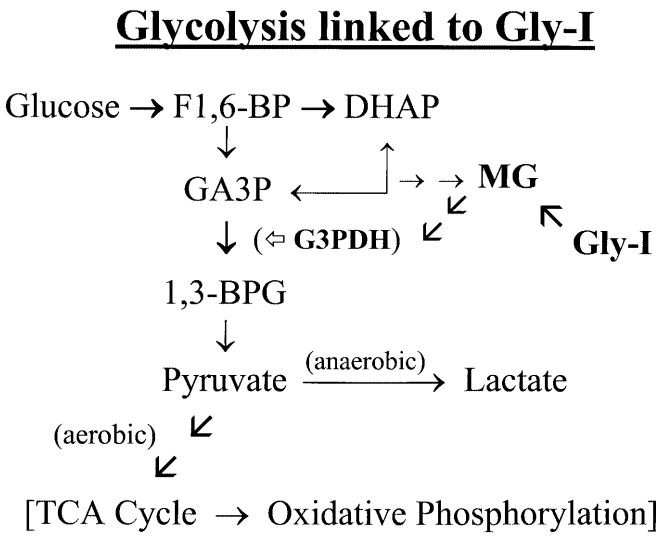

Fig. 1 Simplified diagram of Gly-I linked to glycolysis. *F1, 6-BP*, fructose 1, 6-bisphosphate; *DHAP*, dihydroxyacetone phosphate; *GA3P*, glyceraldehyde 3-phosphate; *1, 3-BPG*, 1, 3-bisphosphoglycerate; *G3PDH*, glyceraldehyde 3-phosphate dehydrogenase; *MG*, methylglyoxal; *Gly-I*, glyoxalase I; *TCA*, tricarboxylic acid

Cell culture

Human prostatic cancer PC-3 cells [7] (American Type Culture Collection, Rockville, Md., USA) were maintained in RPMI-1640 medium, supplemented with 10% fetal bovine serum, penicillin (100 U/ml) and streptomycin (100 µg/ml). For experiments, cells were seeded in 6-well plates or T-75 flasks at the initial cell density of 5×10^5 cells/ml and were cultured with specified concentrations of MG and other agents. Cell number and viability were determined using the trypan blue exclusion method and the AlamarBlue viability test (Biosource International, Camarillo, Calif., USA), respectively.

Preparation of cell extracts

All procedures were performed on ice or at 4°C. Approximately 50 mg of prostate tissue was removed from each specimen and homogenized using a tissue grinder, followed by three cycles of freeze-thaw in liquid nitrogen. Cell extracts (cytosolic proteins) were obtained by centrifugation and stored at –80°C, while the protein concentration was determined using the Pierce protein assay reagent (Pierce, Rockford, Ill., USA). For PC-3 cells, cell extracts were directly obtained from freeze-thaw (without homogenization), followed by centrifugation.

Glyoxalase I assay

The glyoxalase I (Gly-I) activity was measured by the spectrophotometric method described by Ranganathan and Tew [16]. Following preparation of the reaction mixture (200 mM imidazole HCl, pH 7.0, 16 mM $MgSO_4$, 7.9 mM MG, 1 mM GSH), the reaction was started by the addition of cell extracts (40 µg) from each specimen. The increase in absorbance at 240 nm, due to a production of S-D-lactoylglutathione ($E_{240}=3.37\ mM^{-1}cm^{-1}$), was measured with times on a spectrophotometer. The Gly-I activity was then expressed by units/mg protein where 1 unit is defined to catalyze the formation of 1 µmol of S-D-lactoylglutathione per min under assay conditions.

Glyceraldehyde 3-phosphate dehydrogenase assay

The method of Heinz and Freimuller [6] was used for glyceraldehyde 3-phosphate dehydrogenase (G3PDH) activity assay. The reaction mixture was prepared in a final volume of 3 ml containing 2 ml of 50 mM triethanolamine chloride buffer (pH 7.5), 50 µl of 14 mM NADH, 40 µl of 500 mM $MgSO_4$, 200 µl of 16.5 mM ATP, 100 µl of 93 mM glycerate 3-phosphate, 100 µl of 114 mM L-cysteine, and 5 µl of 3-phosphoglycerate kinase (5,000 units/ml). The reaction was started with the addition of cell extracts (20 µg), and the change in absorbance at 340 nm, due to the NADH-NAD^+ conversion, was recorded using a spectrophotometer. G3PDH activity was then expressed as units/mg protein using the absorbance coefficient of 6.22 at 340 nm (units = Δ absorbance/min/6.22).

Results

Gly-I activity in prostate tissue specimens

The Gly-I activity in 66 prostate tissues was quantitatively determined by the established method [16] and was plotted against CAP or NCP (normal and BPH) specimens (Fig. 2). Gly-I activities (0.6–2.9 units/mg protein;

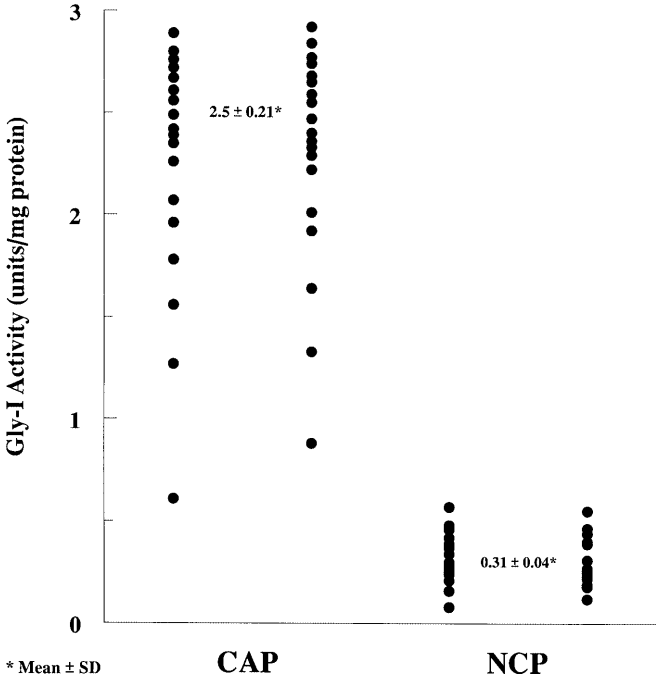

Fig. 2 Gly-I activity in prostate tissues. Cell extracts from 37 CAP and 29 NCP (normal and BPH) specimens were analyzed for Gly-I activity by the spectrophotometric method. Gly-I activity was then plotted against CAP and NCP specimens, respectively. The data are mean ± SD from three independent experiments

mean 2.5 ± 0.21SD) in all 37 CAP specimens were significantly (>eightfold) higher than those (0.08–0.57 units/mg protein; mean 0.31 ± 0.04SD) in NCP specimens ($n = 29$). Thus, these results confirm that Gly-I activity is consistently and distinctly higher in CAP specimens.

Involvement of Gly-I in MG-induced PC-3 cell death

To explore a significance of such a high Gly-I activity in CAP specimens, the effects of MG on cell viability and Gly-I were studied in PC-3 cells used as an in vitro experimental model. MG is a substrate for Gly-I [18] and exhibits cytotoxic effect on various cancer cells [4, 8, 17]. A dose-response study of MG (1–3 mM) showed that 1 or 2 mM MG caused a marginal (10–15%) reduction in the viable cell number (cell viability) at 24 h (Fig. 3a). In contrast, the cells treated with 3 mM MG showed some cell death after 6 h, which extended progressively and reached nearly 100% by 24 h (Fig. 3a). This rapid and potent cytotoxic effect of MG led us to assume that some key event must have taken place at the early phase. The cells exposed to MG (3 mM) for 1, 3 and 6 h were then assayed for Gly-I. The initial Gly-I activity (4.0 ± 0.46 units/mg protein) was profoundly (~77%) lost within 3 h and further lost to ~91% by 6 h (Fig. 3b). Therefore, MG-induced cell death was indeed accompanied by the inactivation of Gly-I at the early phase of death process.

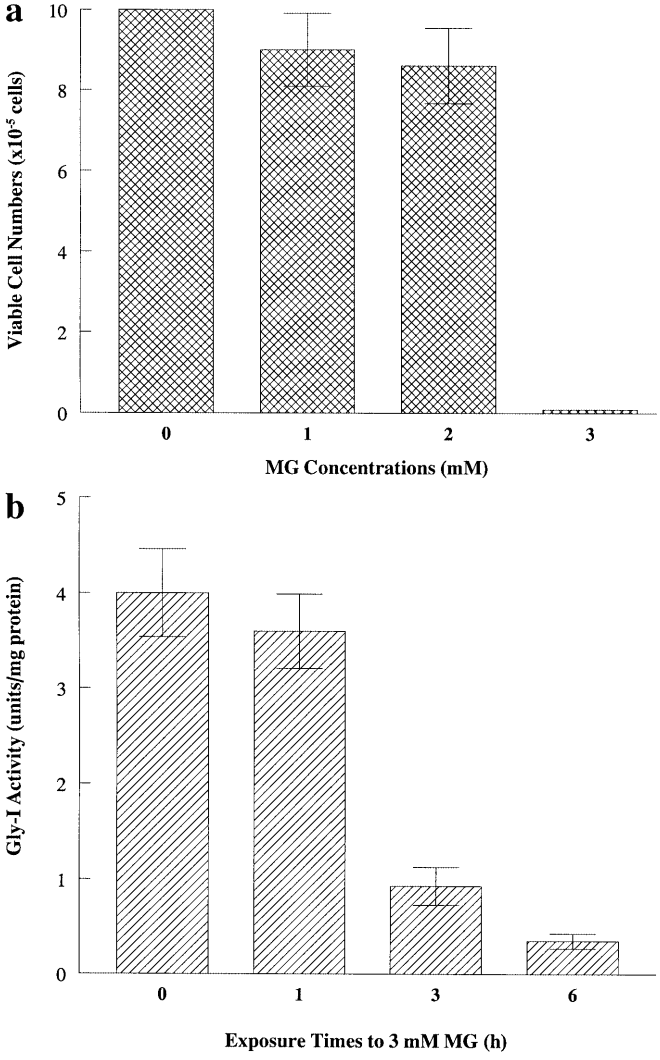

Fig. 3 **a** Effect of MG on PC-3 cell growth. PC-3 cells were cultured with 1, 2 and 3 mM MG and cell number was determined at 24 h. **b** Effect of MG on Gly-I activity. After cells were exposed to 3 mM MG for 1, 3 and 6 h, Gly-I activity (units/mg protein) was measured and plotted. The data are mean ± SD from three separate experiments

Detoxification of MG by Gly-I

We next examined whether exogenously supplemented N-acetylcysteine (NAC), a cell-permeable precursor of GSH (an essential cofactor for Gly-I) [18], might help maintain Gly-I activity to overcome MG cytotoxicity. PC-3 cells were cultured with MG (3 mM) in the presence of NAC (1–5 mM) for 24 h and cell viability was assessed. NAC (1 mM) was capable of preventing MG-induced cell death with ~57% cell viability, and NAC ≥3 mM has restored over 83% cell viability against MG (Fig. 4a). Gly-I activity was also maintained ~90% with NAC (3 mM) even after 6-h MG (3 mM) exposure, whereas its activity was abolished by over 90% without it (Fig. 4b). Thus, Gly-I appears to play a vital role in MG detoxification for cell viability, although its activity

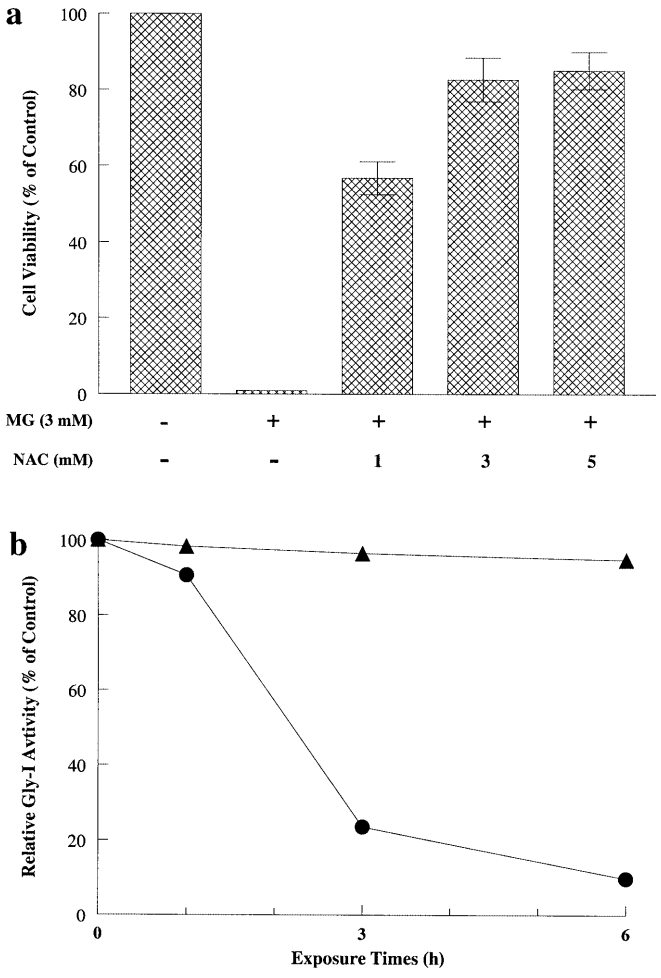

Fig. 4 **a** Cytoprotection against MG with NAC. Cells were cultured with 3 mM MG alone or supplemented with 1, 3, and 5 mM NAC and cell viability (% of control) was assessed at 24 h. **b** Restoration of Gly-I activity with NAC. Following exposure of cells to 3 mM MG (*filled circles*) or 3 mM MG/3 mM NAC combination (*filled squares*) for 1, 3 and 6 h, Gly-I activity (%) relative to control (100% = 4.0 ± 0.46 units/mg protein) was plotted

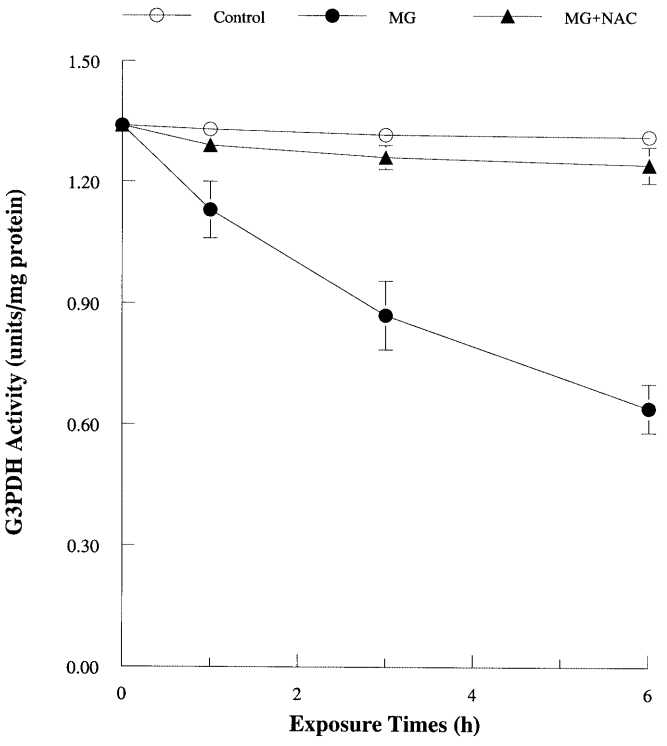

Fig. 5 Inhibition of glyceraldehyde 3-phosphate dehydrogenase (G3PDH) by MG. Cells were exposed to MG (3 mM) or MG (3 mM)/NAC (3 mM) combination for 1, 3, and 6 h and assayed for G3PDH activity. The data shown are mean ± SD from three separate experiments

relies primarily upon the availability of cellular GSH (converted from NAC).

Blocking of glycolysis by MG

Since MG is also a potent inhibitor of G3PDH in glycolysis (Fig. 1) [4], the glycolytic process could be interrupted if MG is not detoxified by Gly-I. It is thus important to assess the effect of MG on G3PDH. The cells exposed to MG (3 mM) for 1, 3 and 6 h were subjected to G3PDH assay. Compared to controls, G3PDH activity progressively declined with MG exposure times, resulting in an almost 50% reduction in its activity by 6 h (Fig. 5). In contrast, a similar study supplemented with NAC (3 mM) revealed that G3PDH activity was well preserved (>90%) with NAC at 6-h MG exposure, plausibly because of maintaining Gly-I activity (Fig. 5). These results indicate that without MG detoxification by Gly-I, MG could interfere with glycolysis through the inhibition of G3PDH. Thus, this further supports the notion that Gly-I may play a detoxifying role in glycolysis to sustain normal cellular activity and viability of prostate cancer.

Discussion

In the present study, we investigated whether a detoxifying enzyme, Gly-I, might play an important role in the growth and development of prostate cancer. Since Gly-I activity has been shown to be altered in several human cancer cells [2, 3, 16], we examined it in prostate cancer (CAP) and non-cancerous (NCP) prostate tissues. Gly-I activity was found to be markedly (>eightfold) higher in all CAP specimens than in NCP specimens. This augmented Gly-I activity appears to be a unique characteristic of CAP specimens, suggesting that change in such activity could be a useful parameter for assessing or differentiating CAP from NCP status.

To elicit the significance of high Gly-I activity in CAP specimens, we explored a potential cytotoxic effect of MG on prostatic cancer PC-3 cells. Cell viability was almost completely diminished after 24-h MG exposure, accompanied by a dramatic inactivation of Gly-I by 6 h, implying the involvement of Gly-I in a MG-mediated

death process. Since GSH is essentially required for Gly-I activity [18], such a Gly-I inactivation (Fig. 3b) might have resulted from a rapid depletion of cellular GSH used by Gly-I for excessive MG detoxification. This possibility was tested by exogenously supplementing NAC to the cell culture receiving MG, because NAC is cell-permeable and can be intracellularly converted to GSH. NAC was capable of restoring ~85% cell viability with ~90% Gly-I activity, confirming that cellular GSH is indeed essential to Gly-I, which may play a critical role in sustaining cell viability through a cellular detoxification process. It has also been reported that MG-induced apoptosis was partially prevented by NAC in human monocytic leukemia U937 cells [15], suggesting possible activation of some GSH-dependent enzymes including Gly-I. Therefore, cytoprotection against MG appears to be primarily provided by Gly-I in prostatic cancer cells, although it cannot rule out the possibility that other GSH-dependent enzymes could play a secondary role in protection against MG cytotoxicity. Additional studies are yet required for further clarification.

We then examined a consequence of Gly-I inactivation or which cellular event might be subsequently affected by non-detoxified MG, because such a study may provide a clue to a possible mechanism of MG-induced cell death. Activity of G3PDH, a crucial glycolytic enzyme, was found to be significantly (~50%) down-regulated by MG. This is indicative of a defective glycolysis likely leading to acute cellular energy depletion, which could have a significant causal effect on cell death. However, it is yet possible that a growth cessation prior to cell death could partially affect glycolysis. Nevertheless, these results are highly suggestive of a critical role of Gly-I in glycolysis to sustain cancer cell growth and viability; i.e. the detoxification of MG.

Taken together, in vitro studies using MG indicate that the inactivation of Gly-I by MG is likely to trigger the inhibition of G3PDH, blocking the glycolytic pathway, shutting down energy (ATP) production, and resulting in PC-3 cell death.

In addition, it is tempting to speculate that a higher Gly-I activity in CAP specimens could be linked to a *higher* cellular MG level compared to normal prostate levels, although the exact reason remains unclear. Due to the inadequate vascularization, cancer cells are often under a hypoxic state and are forced to undergo inefficient anaerobic glycolysis via a lactate route (Fig. 1) for energy production [21, 22]. To compensate such an energy deficiency, cancer cells including CAP have been shown to have an abnormally high glycolytic activity compared to normal cells [10, 12], resulting in a rapid accumulation of toxic MG in these cancer cells. Thus, accelerated glycolysis in CAP may at least account for a higher Gly-I activity required for detoxifying overproduced MG. In this study, a relatively high concentration (3 mM) of MG was required for demonstrating the cytotoxic activity in vitro, which might not be directly relevant to physiological conditions, but its effective dose and potential side effects in vivo need to be properly assessed using animals. Meanwhile, taking advantage of a potent MG cytotoxicity, several inhibitors of Gly-I [5, 11, 14] to "acutely" raise the cellular MG level have been developed and shown to have a better efficacy on some cancer cases [11, 19]. Further studies on such a treatment modality for prostate cancer are thus warranted.

In conclusion, Gly-I consistently exhibits its higher activity in CAP than in NCP specimens and may play a critical detoxifying role in glycolysis to maintain normal cellular activity and viability in prostate cancer.

References

1. Davidson SD, Cherry JP, Choudhury MS, Tazaki H, Mallouh C, Konno S (1999) Glyoxalase I activity in human prostate cancer: a potential marker and importance in chemotherapy. J Urol 161:690
2. Di Ilio C, Del Boccio G, Casaccia R, Aceto A, DiGiacomo F, Federici G (1987) Selenium and glutathione-dependent enzyme activities in normal and neoplastic human lung tissues. Carcinogenesis 8:281
3. Di Ilio C, Angelucci S, Pennelli A, Zezza A, Tenaglia R, Sacchetta P (1995) Glyoxalase activities in tumor and non-tumor human urogenital tissues. Cancer Lett 96:189
4. Halder J, Ray M, Ray S (1993) Inhibition of glycolysis and mitochondrial respiration of Ehrlich ascites carcinoma cells by methylglyoxal. Int J Cancer 54:443
5. Hamilton DS, Creighton DJ (1992) Inhibition of glyoxalase I by the enediol mimic S-(N-hydroxy-N-methylcarbamoyl) glutathione: the possible basis of a tumor-selective anticancer strategy. J Biol Chem 267:24933
6. Heinz F, Freimuller B (1982) Glyceraldehyde 3-phosphate dehydrogenase from human tissues. Methods Enzymol 89:301
7. Kaighn ME, Narayan KS, Ohnuki Y, Lechner JF, Jones LW (1979) Establishment and characterization of a human prostate carcinoma cell line (PC-3). Invest Urol 17:16
8. Kang Y, Edwards LG, Thornalley PJ (1996) Effect of methylglyoxal on human leukemia 60 cell growth: Modification of DNA, G_1 growth arrest and induction of apoptosis. Leuk Res 20:397
9. Larsen K, Aronsson AC, Marmstal E, Mannervik B (1985) Immunological comparison of glyoxalase I from yeast and mammals with quantitative determination of the enzyme in human tissues by radioimmunoassay. Comp Biochem Physiol 82B:625
10. Lazo PA (1981) Amino acids and glucose utilization by different metabolic pathways in ascites-tumor cells. Eur J Biochem 117:19
11. Lo TWC, Thornalley PJ (1992) Inhibition of proliferation of human leukaemic 60 cells by diethyl esters of glyoxalase inhibitors in vitro. Biochem Pharmacol 44:2357
12. Macbeth RAL, Bekesi JG (1962) Oxygen consumption and anaerobic glycolysis of human malignant and normal tissue. Cancer Res 22:244
13. McLellan AC, Thornalley PJ (1989) Glyoxalase activity in human red blood cells fractioned by age. Mech Ageing Dev 48:63
14. Murthy NSRK, Bakeris T, Kavarana MJ, Hamilton DS, Lan Y, Creighton DJ (1994) S-(N-aryl-N-hydroxycarbamoyl) glutathione derivatives are tight-binding inhibitors of glyoxalase I and slow substrates for glyoxalase II. J Med Chem 37:2161
15. Okado A, Kawasaki Y, Hasuike Y, Takahashi M, Teshima T, Fujii J, Taniguchi N (1996) Induction of apoptotic cell death by methylglyoxal and 3-deoxyglucosone in macrophage-derived cell lines. Biochem Biophys Res Commun 225:219

16. Ranganathan S, Tew KD (1993) Analysis of glyoxalase I from normal and tumor tissue from human colon. Biochim Biophys Acta 1182:311
17. Ray M, Halder J, Dutta SK, Ray S (1991) Inhibition of respiration of tumor cells by methylglyoxal and protection of inhibition by lactaldehyde. Int J Cancer 47:603
18. Thornalley PJ (1990) The glyoxalase system: new developments towards functional characterization of a metabolic pathway fundamental to biological life. Biochem J 269:1
19. Thornalley PJ (1995) Advances in glyoxalase research: glyoxalase expression in malignancy, antiproliferative effects of methylglyoxal, glyoxalase I inhibitor diesters and S-D-lactoylglutathione, and methylglyoxal-modified protein binding and endocytosis by the advanced glycation endoproduct receptor. Crit Rev Hematol Oncol 20:99
20. Thornalley PJ (1996) Pharmacology of methylglyoxal: formation, modification of proteins and nucleic acids, and enzymatic detoxification: a role in pathogenesis and antiproliferative chemotherapy. Gen Pharmacol 27:565
21. Vaupel P, Kallinowski F, Okunieff P (1989) Blood flow, oxygen and nutrient supply, and metabolic microenvironment of human tumors: a review. Cancer Res 49:6449
22. Wike-Hooley JL, Haveman J, Reinhold HS (1984) The relevance of tumor pH to the treatment of malignant disease. Radiother Oncol 2:343