ELSEVIER

GLYOXALASE I PHENOTYPE AS A POTENTIAL RISK FACTOR FOR PROSTATE CARCINOMA

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ABSTRACT

Objectives. To elicit a possible link between glyoxalase I (Gly-I), a detoxifying enzyme, and the incidence of prostate cancer (PCa), we investigated Gly-I phenotypic expression in the prostatic tissue and red blood cells (RBCs) from patients with PCa.

Methods. Eighty-seven clinical specimens, including 42 PCa tissue samples, 20 RBC samples, and 25 matched pair (prostate and RBC) samples from patients at prostatectomy were examined. The Gly-I phenotypes in these specimens were assessed by nondenaturing starch-polyacrylamide gel electrophoresis.

Results. Of the 87 patients, 63 (72.4%) were white, 15 (17.2%) were black, and 9 (10.4%) were another ethnicity (eg, Hispanic, Asian, Indian). Three Gly-I phenotypes were detected in these specimens as fast, intermediate, and slow-moving bands on the gel. The fast phenotype was the most common form found in the white (34 [54%] of 63) and black (8 [53.3%] of 15) patients, but the third ethnic group was too small for proper analysis. To validate this finding, the data from the white patients were compared with the Gly-I phenotypic frequencies in U.S. populations. The data analysis confirmed that a higher incidence (54%) of the fast type in our white patients was statistically significant ($P < 0.0001$) compared with its phenotypic frequency of 30.6% in the general U.S. white population.

Conclusions. The significantly high frequency ($P < 0.0001$) of the fast Gly-I phenotype was detected among patients with PCa, suggesting it is a potential risk factor for PCa. Whether its increased incidence in whites reflects the lack of sample numbers for other ethnic groups needs additional investigation. UROLOGY **57:** 183–187, 2001. © 2001, Elsevier Science Inc.

Although prostate cancer (PCa) is the second-leading cause of cancer death in elderly men in the United States,[1] the etiologies and risk factors of PCa are not fully understood. Extensive epidemiologic studies have shown that the incidence of PCa increases rapidly with age and multiple factors, including 5-alpha-dihydrotestosterone, genetic predisposition, race, diet, lifestyle, occupation, environment, and specific diseases, were implicated in the oncogenesis of PCa.[2–4] However, no single or specific genetic factor has yet served as a reliable diagnostic/prognostic marker for the development of PCa. Thus, we were interested in exploring a potential marker or factor associated with a genetic predisposition to PCa that could be useful in better understanding the disease prevalence.

Our main focus was on glyoxalase I (Gly-I), a vital detoxifying enzyme present in the cytosol of all human tissue.[5] Its major biologic function is to detoxify toxic electrophilic metabolites, such as methylglyoxal or other alpha-oxoaldehydes.[6] Gly-I is also known to exist in three phenotypes, GLO 1-1, GLO 1-2, and GLO 2-2, representing the homozygous and heterozygous expression of two alleles, GLO^1 and GLO^2, located on chromosome 6.[7] This distinct phenotypic expression was originally found in red blood cells (RBCs) and subsequently confirmed for a variety of human tissues.[6] The usefulness of such Gly-I expression in RBCs has led to numerous recent studies of Gly-I. Gly-I phenotyping has been used as a genetic marker for polymorphism in various ethnic populations,[8] in surveys of susceptibility to certain diseases/disorders (eg, diabetes mellitus and chronic alcoholism),[9,10] and in

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forensic science.[11] Moreover, the altered activity and expression of Gly-I has been documented in various human malignancies, including colon,[12] lung,[13] bladder, and renal[14] carcinoma. For instance, an increase in Gly-I activity and its expression (GLO 2-2) was observed in colon carcinoma compared with the corresponding normal tissue.[12] However, possible differences in Gly-I phenotypic expression have not yet been explored in various colon cancer specimens.

Recently, we reported that Gly-I activity was consistently and significantly higher in PCa tissue compared with normal or benign prostatic hyperplasia (BPH) tissue.[15] This finding suggests that an increase in Gly-I activity could be a useful parameter for differentiating PCa from a nonmalignant status. However, the significance of the different Gly-I phenotypes expressed in PCa specimens has not been explored or documented. It is conceivable that such differences in phenotypic expression may have clinical implications or may provide insight into the nature of PCa. To our knowledge, this is the first study investigating the Gly-I phenotypes in PCa tissue and RBCs from patients with PCa. Our data were also compared with the distributions of Gly-I phenotypes in general U.S. populations[16] for additional confirmation. Such studies suggest that Gly-I polymorphism could be considered a potential risk factor for prostate carcinogenesis, which may also provide a clue to the incidence of PCa.

MATERIAL AND METHODS

Specimen Collection

Of 87 patients (median age 67 ± 3.8 years), 63 (72.4%) were white, 15 (17.2%) were black, and 9 (10.4%) were another ethnicity (eg, Hispanic, Asian, Indian). The clinical specimens, including 42 PCa tissue samples, 20 blood samples, and 25 matched-pair (prostate and blood) samples, were freshly obtained from patients with PCa (who had not undergone any type of prior treatment) at prostatectomy. The most probable tissue (3 to 5 g) with PCa was removed from a whole prostate and snap-frozen in liquid nitrogen, and the adjacent portion of the specimen was sent to the pathology department for histologic examination. All 67 prostate specimens were subsequently confirmed to be cancerous by the pathology report and were used in this study. The freshly collected preoperative peripheral blood samples were centrifuged at 2000 rpm for 5 minutes. Isolated RBCs were mixed with a 4-volume of water and kept at 4°C for 20 minutes to complete hemolysis. Hemolysates were then stored in liquid nitrogen until use.

Cell Extract Preparation

Approximately 50 mg prostatic tissue was excised from each PCa specimen and homogenized using a tissue grinder in cell lysis buffer (10 mM HEPES-KOH, pH 7.4, 90 mM KCl, 1.5 mM $Mg(OAc)_2$, 5% glycerol, 0.5% NP-40, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride). The homogenate was further subjected to three freeze-thaw cycles in liquid nitrogen. Cell extracts (cytosolic proteins) were then obtained by centrifugation at 12,000 rpm for 15 minutes and stored at −80°C. Five-microliter aliquots of cell extracts were subjected to protein determination using the Pierce protein assay reagent (Pierce, Rockford, Ill).

Gly-I Phenotype Analysis

The phenotypic expression (qualitative assessment) of Gly-I was analyzed using nondenaturing starch-polyacrylamide gel electrophoresis (PAGE) with minor modifications, following the protocol described by McLellan and Thornalley.[17] In brief, 7 μg of cell extract or 5 μL of hemolysate (RBCs) from each specimen was subjected to PAGE at a constant 100 V until a dye front ran completely off the gel (~1.5 hours). The gel was then incubated with a staining solution (100 mM Na_2HPO_4, pH 6.6, 600 mM methylglyoxal, 20 mM reduced glutathione) at 37°C for 10 minutes in a humidified incubator. Three Gly-I phenotypes were detected as the appearance of dark blue bands at the distinct migratory positions on the gel, using a developing solution (94 mM KI, 2 mM I_2), and were photographed for documentation.

Statistical Analysis

The chi-square and Normal test statistics were performed to determine the statistical significance of the differences between the experimental and reference groups. Significance was defined as $P < 0.05$.

RESULTS

Phenotypic Expression of Gly-I in Clinical Specimens

Qualitative analysis of the Gly-I phenotypes by the PAGE method[17] relies on the formation of the starch-iodine inclusion complex, detected as dark blue stains on the gel. The analysis of 25 matched-pair (prostatic tissue and RBC) samples showed that the Gly-I phenotypes detected in all paired samples were comparable; several representatives are illustrated in Figure 1. For convenience, the three Gly-I phenotypes, generally named GLO 1-1, GLO 1-2, and GLO 2-2, were denoted by the slow, intermediate, and fast-migrating bands on the gel, respectively. Each phenotype with a different traveling rate can be readily detected by its distinct migratory pattern on the gel (Fig. 1). The compatibility found in the prostate and blood specimens also suggests the clinical utility of blood samples for such phenotypic analysis.

After PAGE analysis of all 87 specimens (including prostatic tissue and blood samples), the overall Gly-I phenotypic frequencies were as follows: fast, 47 (54.0%) of 87; intermediate, 28 (32.2%) of 87; and slow, 12 (13.8%) of 87 samples; thus, the fast type appeared to be the most common form in PCa (Table I). Because most (72% or 63 of 87) of the patients were white, when analysis is performed on samples from white patients only, the frequency (34 [54.0%] of 63 samples) of the fast type is consistent with the overall fast frequency (Table II). Despite the small number of blacks (17%) in the study, a similarly large percentage (53.3%) of the fast type was found (Table II). The third group with only 9 patients of other ethnic groups was too small for proper analysis. Thus, these results suggest that the fast phe-

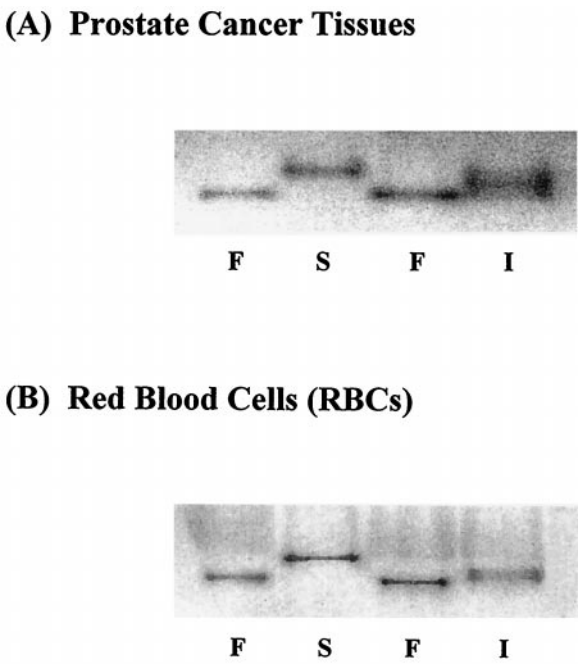

FIGURE 1. *Gly-I phenotypes in clinical specimens. Phenotypic expressions of Gly-I in (A) PCa tissue and (B) RBCs from the same patients with PCa were analyzed using PAGE. Three Gly-I phenotypes are denoted as F (fast), I (intermediate), and S (slow)-moving bands on the gel.*

TABLE I. *Overall Gly-I phenotypic frequencies in clinical specimens*

Gly-I Phenotype	No. of Specimens (n = 87)	Phenotype Percentage
Fast	47	54.0 (47/87)
Intermediate	28	32.2 (28/87)
Slow	12	13.8 (12/87)

KEY: *Gly-I = glyoxalase I.*

notype of Gly-I is more likely to be associated with the incidence or risk of PCa.

To validate this finding, the frequencies of Gly-I phenotypes in U.S. populations were examined, because the higher frequency of the fast phenotype in these patients might result simply from its greater distribution in the general population. We compared our data with previously published data, obtained from a completely ascertained, randomly selected, countrywide (more than 10 states) population of several ethnic groups.[16] The statistical analysis revealed that the frequency (54%) of the fast type in white patients with PCa was statistically different and greater ($P < 0.0001$) than that (30.6%) in the general U.S. white population (Table II). The sample size of black patients with PCa was too small for a similar analysis. Therefore, the high frequency (greater than 50%) of the fast type among white patients was substantial and unlikely to be a result of simple random probability.

ABO Blood Types Versus Gly-I Phenotypes

In conjunction with Gly-I, it was of interest to examine whether another RBC-related factor, the ABO blood type (antigen), might also have a link to PCa incidence or some clinical relevance to Gly-I phenotypic expression. Forty-five preoperative blood samples from patients with PCa were available for the ABO blood study. The patients were again mostly white (78%; n = 35), with fewer blacks (11%; n = 5) and other ethnicities (11%; n = 5). The blood types of the white patients were 42.9% type A, 40.0% type O, 14.3% type B, and 2.8% type AB. However, the values were not statistically different ($P > 0.435$) from those in the general U.S. white population (n = 140,669), with 41.4% type A, 44.5% type O, 10.3% type B, and 3.8% type AB.[18] These data suggest that blood types are unlikely to be related to the incidence of PCa, although additional studies with large sample numbers are needed for confirmation.

In addition, we examined a possible relation between blood type and Gly-I phenotype among white patients with PCa. Although the fast phenotype was found more often in patients with type A or O blood (~22% each) (data not shown), this may simply reflect the wide distribution of these blood types in our white patients (~83%) and in the U.S. white population (~86%).[18] Thus, no inherent relationship between blood type and Gly-I phenotype was demonstrated.

COMMENT

In the present study, we investigated a potential risk factor associated with a genetic predisposition to PCa to gain insight into the etiology of PCa. Gly-I has been extensively studied in ethnic polymorphism and in several human malignancies[8,12–14]; however, its significance in PCa has not been adequately addressed. We evaluated the phenotypic expression of Gly-I in clinical specimens from patients with PCa for a possible correlation with the incidence of PCa.

First, the applicability of the established analytical method of Gly-I investigation (PAGE)[17] was examined using prostatic tissues and blood (RBC) samples from patients with PCa. The analysis of 25 matched specimens revealed that three phenotypes (fast, intermediate, and slow) were readily distinguishable by their specific migratory patterns on the gel. All Gly-I phenotypes detected in both the prostatic tissue and RBC specimens were comparable, indicating that the same Gly-I phenotype is expressed in the prostate and blood (RBC). This is rather useful and practical because of the easy sam-

TABLE II. ***Comparison of Gly-I phenotypic frequencies between white and black patients and general U.S. populations***

Ethnic Group	Gly-I Phenotype Frequency		
	Fast	Intermediate	Slow
Whites with PCa (n = 63)*	54.0% (34/63)†	30.1% (19/63)	15.9% (10/63)
General U.S. white population (n = 1812)	30.6%†‡	50.4%	19.0%
Blacks with PCa (n = 15)*	53.3% (8/15)	33.3% (5/15)	13.4% (2/15)
General U.S. black population (n = 1825)	47.5%‡	41.3%	11.2%

KEY: Gly-I = glyoxalase I; PCa = prostate cancer.
** Both prostatic tissue and blood samples were analyzed for Gly-I phenotypes in patients with PCa.*
† P *<0.0001.*
‡ P *<0.00001; data from Gaensslen* et al.[16]

pling of the blood for such an analysis. However, these phenotypic expressions are almost undetectable in normal and BPH tissue samples and only become highly detectable in PCa specimens,[15] but they can be consistently detected in RBCs regardless of whether the subject has PCa. Therefore, Gly-I phenotyping using RBCs could be useful in screening for a risk of possible PCa development, but diagnostic assessment of PCa must rely on prostate biopsy (tissue samples).

PAGE analysis of all specimens revealed that the fast phenotype was the most common form (54%) expressed in the major ethnic group (72%) of white patients. It should be noted that subgroups of these white patients included a wide variety of backgrounds (eg, Italian, Irish, Jewish, Polish) without one primary ethnic group. A similar trend (fast type prevalence) was also found in the black patients and the third group; however, the sample numbers were too small to draw any affirmative conclusions. More specimens from these ethnic groups are required for proper assessment. A comparative statistical analysis was also performed to corroborate the significance of the fast type in white patients. A high frequency (54%) of the fast type in our white patients was indeed statistically greater ($P < 0.0001$) than that in the general U.S. white population, indicating a correlation between the fast phenotypic expression and the incidence of PCa. In addition, the same U.S. population study[16] revealed that the fast phenotype frequency (47.5%) in U.S. blacks was ~17% higher and statistically greater ($P < 0.00001$) than that (30.6%) in U.S. whites (Table II). This difference may become more evident when an annual PCa incidence per 100,000 men (in a standard survey) is considered. Thus, an ~50% higher PCa incidence in blacks (compared with whites)[19] could in part be linked to their greater frequencies of fast phenotypic expression. Taken together, the fast phenotype of Gly-I may be a useful indicator for the risk of PCa development.

Specific blood types have been shown to be linked to the incidences of several human malignancies; for example, type A blood was more frequently found in the patients with endometrial/ovarian,[20] upper urogenital,[21] pancreatic,[22] and gastric[23] cancer. However, to our knowledge, little information is available for such a link between blood type and PCa incidence. We found that no specific blood type was associated with patients with PCa or specific Gly-I phenotypes. These findings suggest that the ABO blood types and Gly-I phenotypes are independent factors, although both are expressed in RBCs.

In conclusion, the fast phenotype of Gly-I was more frequently (greater than 50%) expressed in patients with PCa and could be useful in PCa screening. However, because of the unequal ethnic distributions of patients in our study, whether such an increased incidence ($P < 0.0001$) in white men with PCa compared with their counterparts in the general U.S. population is also reflected in other ethnic groups requires further investigations. Nevertheless, this Gly-I phenotypic analysis may provide valid information for those expressing the fast type and encourage them to take early prophylactic measures such as proper diet, lifestyle, and routine examinations.

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