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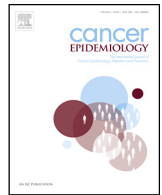

Short communication

Glutathione S-transferase P1 mRNA expression in plasma cell disorders and its correlation with polymorphic variants and clinical outcome

Flavia Stella [a,1,*], Natalia Weich [b,1], Julieta Panero [a], Dorotea Beatriz Fantl [c], Natalia Schutz [c], Ariela F. Fundia [b,2], Irma Slavutsky [a,2]

[a] *Laboratorio de Genética de Neoplasias Linfoides, Instituto de Medicina Experimental, CONICET-Academia Nacional de Medicina, Buenos Aires, Argentina*
[b] *Laboratorio de Genética Hematológica, Instituto de Medicina Experimental, CONICET-Academia Nacional de Medicina, Buenos Aires, Argentina*
[c] *Departamento de Clínica Médica, Sección Hematología, Hospital Italiano de Buenos Aires, Argentina*

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ABSTRACT

Background: Glutathione S-transferase P1 (*GSTP1*) is an important phase II enzyme involved in detoxification of carcinogens. *GSTP1* gene overexpression has been observed in a variety of human cancers but there are no studies in plasma cell disorders. The aim of this study was to examine *GSTP1* mRNA expression level in multiple myeloma (MM) and monoclonal gammopathy of undetermined significance (MGUS). In addition, we have determined *GSTP1* polymorphic variants in order to estimate MM risk and their relationship with the expression level. Results were also correlated with laboratory parameters and clinical outcome.
Methods: Bone marrow mononuclear cells from 125 patients with plasma cell disorders were studied. Peripheral blood samples of 110 age and sex matched healthy controls were also evaluated. Real-Time Quantitative RT-PCR and PCR-RFLP assays were used.
Results: Upregulation of *GSTP1* was observed in 37.7% MM and in 22.6% MGUS patients. A significant increase of *GSTP1* expression in MM with respect to MGUS was detected ($p = 0.0427$). Most MM patients that achieved complete remission had low transcription levels (77.8%) compared to those who did not reach this condition (44.4%) ($p = 0.0347$). *GSTP1* heterozygous carriers showed reduced expression compared to those with homozygous wild type genotype ($p = 0.0135$).
Conclusion: Our findings suggest, for the first time, a role for *GSTP1* expression in development and/or progression of plasma cell disorders, and a probable influence of functional capacity of the enzyme on clinical outcome. These results and those of the literature support *GSTP1* as an interesting tumor marker and a potential therapeutic target.

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1. Introduction

Multiple myeloma (MM) is a malignant disorder characterized by the accumulation of clonal plasma cells in the bone marrow (BM). Almost all patients evolve from a previous premalignant condition, named monoclonal gammopathy of undetermined significance (MGUS). The disease shows significant heterogeneity with regards to clinical presentation, biologic characteristics, response to treatment, and outcome [1].

Glutathione S-transferase P1 (GSTP1) is an important phase II enzyme involved in detoxification of carcinogens [2]. GSTP1 protein overexpression has been reported in a variety of human cancers, including plasma cells from MM and MGUS patients [3]. Differences in the expression and activity of GSTP1 have been related to genetic polymorphisms including *GSTP1* rs1695 (c.313 A > G, p.105 Ile > Val), that results in reduced catalytic activity and detoxification capacity of the enzyme [2]. Different studies have evaluated the role of this polymorphism on MM risk and evolution showing discordant results [4]. To our knowledge, there is no information about *GSTP1* gene expression in plasma cell disorders. Therefore, the aim of this study was to examine *GSTP1* mRNA expression level in MM and MGUS patients. In addition, we have determined *GSTP1* polymorphic variants in order to estimate MM risk and genotype influence on the transcriptional level. Results were also correlated with laboratory parameters and clinical evolution.

* Corresponding author at: Laboratorio de Genética de Neoplasias Linfoides, Instituto de Medicina Experimental, CONICET-Academia Nacional de Medicina, J.A. Pacheco de Melo 3081, C1425AUM – Buenos Aires, Argentina.
Tel.: +54 11 4805 8803x241; fax: +54 11 4803 9475.
E-mail addresses: fla_stella@yahoo.com.ar, fla_stella@hotmail.com (F. Stella).
[1] These authors contributed equally to this study.
[2] These authors contributed equally as last authors to this work.

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2. Materials and methods

2.1. Population studied

A total of 125 consecutive patients with plasma cell disorders: 94 with MM and 31 with MGUS, were prospectively studied. All patients were evaluated at diagnosis before any intervention. The diagnosis was based on the International Myeloma Working Group Criteria [1]. Age and sex distribution and clinico-pathological characteristics of all patients are summarized in Table 1. Patients under the age of 65 years and fit were treated with an induction therapy with thalidomide or bortezomib plus hematopoietic stem-cell transplantation. Conventional therapy combined with thalidomide or bortezomib were administered in patients older than 65 years or unfit for high dose treatment. A small number of cases received VAD chemotherapy alone. The median of follow up was 27.6 month (range 1–67 months). Eight patients were evaluated both at diagnosis and during complete remission (CR). For comparative analysis of polymorphic variants, a total of 110 unrelated healthy blood donors with a comparable age range (24–85 years), gender distribution (59 males) and ethnicity as patients, were also studied. In a number of patients the sample was not enough to obtain high quality DNA and RNA leading to differences in the number of cases studied for genotyping (73 MM and 19 MGUS) and those evaluated for mRNA expression (69 MM and 31 MGUS). Forty-nine MM and 17 MGUS patients had both studies. All individuals provided their informed consent according to institutional guidelines. The study was approved by the Ethics Committee of our Institution.

Table 1
Clinical characteristics of patients with plasma cell disorders.

Clinical characteristics	MM	MGUS
N° of cases	94	31
Sex (F/M)	55/39	22/9
Mean age (range), years	66.2 (24–86)	70.3 (41–84)
Paraprotein isotype (%)		
IgG	61	72
IgA	20	7
IgM	4	21
Others	15	0
Type of light chain (%)		
Kappa	58.4	63
Lambda	41.6	37
DS Stage (%)		
I	22	–
II	9	–
III	69	–
ISS (%)		
I	45	–
II	32	–
III	23	–
Bone marrow infiltration (%)		
<30	52.1	100
30–60	22.5	–
>60	25.4	–
Lytic bone lesions (%)	51	–
	Mean (range)	Mean (range)
β_2 microglobulin (μg/ml)	0.36 (0.12–5.03)	0.30 (0.11–0.77)
Lactate dehydrogenase (U/l)	184.1 (82–1265)	148.7 (94–231)
Hemoglobin (g/dl)	11.09 (6.9–15.30)	12.42 (9–15.8)
Serum albumin (g/dl)	3.35 (1.8–4.6)	3.74 (3–4.4)
Creatinine (g/dl)	1.99 (0.57–11.8)	0.91 (0.46–1.82)
Serum calcium (mg/dl)	9.26 (6.8–13.8)	9.17 (7.8–10.3)
Paraprotein M (g)	2.81 (0.9–9.4)	0.62 (0.18–1.73)

F, female; M, male; DS, Durie and Salmon [Durie BG, Salmon E. *Cancer* 1975; 36(3): 842–854]; ISS, International Staging System [Greipp PR, et al. *J Clin Oncol* 2005; 23(15):3412–3420].

2.2. GSTP1 genotyping

Genomic DNA was obtained from BM mononuclear cells of patients and peripheral blood of normal individuals. *GSTP1* rs1695 was analyzed by PCR-RFLP with *Alw261* restriction enzyme. PCR was done in a final volume of 25 μl containing 0.4 μM of previously published primers [2] (Supplementary Table A) and 100 ng of genomic DNA. The cycling conditions were: 94 °C for 5 min, 30 cycles of 94 °C, 55 °C, 72 °C for 30 s each, and 5 min at 72 °C. PCR products (10 μL) were digested overnight with 10 units of *Alw261* restriction enzyme at 37 °C. Electrophoresis was performed on 4% 3:1 NuSieve/agarose gels (Supplementary Figure A1). Cases and controls were genotyped blinded. All genotypes were independently scored by two reviewers, and 10% of the samples were randomly reanalyzed, yielding identical results. Negative controls were included in each assay.

2.3. GSTP1 expression

Total RNA were obtained from BM mononuclear cells of patients and peripheral mononuclear cells of healthy individuals. RT-PCR was carried out using 1X RT Buffer, 200 U/μL of Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT), 250 ng/μL random primer, 10 mM each dNTP and 1.5 μg/μL of the total RNA. The PCR conditions were: 10 min at 95 °C, 60 min at 37 °C and 10 min at 95 °C. Expression analysis was performed by real-time quantitative PCR (qRT-PCR) in a Rotor-Gene Q (Qiagen) equipment, based on EVAGreen methodology. The PCR reaction was done using 2 μL of cDNA, 10 μL of *Mezcla Real*™ 2× (Biodynamics, Buenos Aires, Argentina) and 10 pmol/μL (0.8 μL) of each primer [5,6] in a 20 μL final volume (Supplementary Table A). The PCR conditions were 5 minutes at 95 °C, followed by 45 cycles at 95 °C for 20 s, 60 °C for 20 s and 72 °C for 45 s, and a holding at 50 °C for 30 s. Each cDNA sample was analyzed in duplicate in parallel using *beta-actin* gene (*ACTB*) as control. The cycle threshold (Ct) values of target and control genes were computed. Relative gene expression were presented as $2^{(-\Delta\Delta Ct)}$, where $\Delta\Delta Ct = \Delta Ct$ *GSTP1* $- \Delta Ct$ *ACTB*. The specificity of the PCR products were monitored by dissociation curves with single peak of each amplicon, and also confirmed by electrophoresis on 2% agarose gel (Supplementary Figure A2). Standard curves were measured by fivefold duplicated serial dilutions of RT templates from K562 cell line.

2.4. Statistical analysis

For statistical analysis, Mann–Whitney test, Student t test, χ^2 or Fisher's exact tests and Kendall's coefficient were performed using GraphPad Prism Version 5.0 (2008). The 2 × 2 contingency Fisher's exact test was used for estimating odds ratios and 95% confidence intervals. The cut-off point for mRNA expression was determined by receiver operating characteristic (ROC) analysis. Hardy–Weinberg equilibrium was tested by the χ^2 test. For all tests, $p < 0.05$ was regarded as statistically significant.

3. Results and discussion

Genotyping analysis revealed that allelic frequencies among controls were in Hardy–Weinberg equilibrium ($p = 0.43$). In MM, no statistical differences were found in the genotype distribution between patients and controls (Table 2) and no correlation with laboratory parameters or patient outcome were observed. In addition, an exploratory study of 19 patients with MGUS, whose DNAs were available, was also performed. Among them, 13/19 (68.4%) patients with homozygous wild type (*GSTP1*-AA) genotype and 6/19 (31.6%) heterozygous (*GSTP1*-AG) carriers

Table 2
Genotyping *GSTP1* gene in MM patients and controls.

GSTP1		Controls $N = 110$ (%)		Patients $N = 73$ (%)		OR (95% CI)	*P*
Genotypes	AA	52	(47.3)	31	(42.5)	Reference	
	AG	48	(43.6)	40	(54.8)	1.398 (0.758–2.57)	0.352
	GG	10	(9.1)	2	(2.7)	0.335 (0.069–1.633)	0.206
	AG + GG	58	(52.7)	42	(57.5)	1.215 (0.669–2.205)	0.547
Alelles	A	152	(69.4)	102	(69.9)	Reference	
	G	72	(30.6)	44	(30.1)	0.910 (0.579–1.430)	0.731

OR, odds ratios; CI, confidence intervals; AA, homozygous wild-type genotypes; AG, heterozygous genotypes; GG, homozygous variant genotype.

were identified. No MGUS cases with homozygous variant (*GSTP1*-GG) genotype were detected. Carriers of *GSTP1*-AA genotype were numerically higher than controls and MM, but no significant differences were found ($p > 0.07$) probably due to the scarce number of patients evaluated. Our findings suggest that *GSTP1* rs1695 polymorphism is not associated with MM risk or evolution. The lack of association between *GSTP1* variants and MM susceptibility was also reported by Maggini et al. [7]. However, discordant results were found by other authors regarding treatment response and outcome [4]. To our knowledge, the role of *GSTP1* variants in etiology and outcome of MGUS was not previously examined. Further investigations are required in view of the scarce and conflicting results achieved to date in plasma cell disorders.

GSTP1 mRNA expression was significantly increased in MM compared to MGUS cases ($p = 0.0427$) (Table 3). Eleven healthy controls were also evaluated to obtain a baseline value of *GSTP1* expression with our approach (0.02 ± 0.002). In both pathologies, expression analysis showed a high inter-individual heterogeneity. Thus, for a better analysis, patients were divided into two expression groups: high and low expression, according to the cut-off value obtained using ROC curves analysis (0.03). Upregulation of *GSTP1* was observed in 37.7% of MM and in 22.6% of MGUS cases. Our results suggest a role of this gene in the multiple step process of progression from MGUS to MM. The only report about GSTP1 protein expression in MM and MGUS did not find differences in the intensity of positivity between plasma cells of both entities [3]. In line with our findings, increased *GSTP1* expression in relation to stages was also reported in other tumors [8,9], supporting *GSTP1* upregulation as a risk factor for cancer progression. *GSTP1* overexpression has been correlated with the development of the multidrug-resistant phenotype, not only by its detoxification capacity but also due to the influence on signaling pathways that control cell proliferation and survival [10]. Particularly, *GSTP1* inhibits JNK (c-jun N-terminal kinase) and other protein kinases involved in stress response, cell proliferation and apoptosis, suggesting a possible role of this isoenzyme in resistance to apoptosis during anticancer therapy [11].

In our series, *GSTP1* mRNA expression did not correlate with laboratory parameters of the disease. Interestingly, no association between gene expression and the percentage of BM plasma cell infiltration was observed, indicating that *GSTP1* transcription level would not be influenced by the tumor burden.

Although a small number of cases could be evaluated both at diagnosis and during CR (8 cases), lower *GSTP1* mRNA levels were found at CR (0.029 ± 0.006) compared to the moment of diagnosis (0.087 ± 0.032). The evaluation of clinical evolution showed that CR was achieved in 52% of patients with low *GSTP1* expression compared to 22% of those with high mRNA levels ($p = 0.06$). In concordance, when only patients with CR were analyzed, 77.8% of cases had reduced *GSTP1* expression compared to 44.4% of those who did not reach this condition ($p = 0.0347$) (Fig. 1a), independently of the treatment used, supporting this gene as a possible prognostic marker in MM patients. In agreement with our results, *GSTP1* overexpression was associated to poor treatment response and outcome in different hematological neoplasias like acute non-lymphoblastic leukemia [12], chronic lymphocytic leukemia [13], non-Hodgkin lymphomas [14,15], and also solid tumors [16]. Moreover, differences among B-cell lymphomas have also been observed, with high *GSTP1* expression in mantle cell lymphoma compared to marginal zone lymphoma, follicular lymphoma and diffuse large B cell lymphoma [15,17],

Table 3
GSTP1 mRNA expression in MM and MGUS patients.

Group	No. of cases (%)	Gene expression (mean ± ES)
MM	69	0.087 ± 0.032
MM-HE	26 (37.7%)	0.22 ± 0.08
MM-LE	43 (62.3%)	0.011 ± 0.001
MGUS	31	0.031 ± 0.014 [a]
MGUS-HE	7 (22.6%)	0.10 ± 0.04
MGUS-LE	24 (77.4%)	0.007 ± 0.001

HE, high expression; LE, low expression.
[a] Significant differences, with respect to MM: $p = 0.0427$ (Mann–Whitney test).

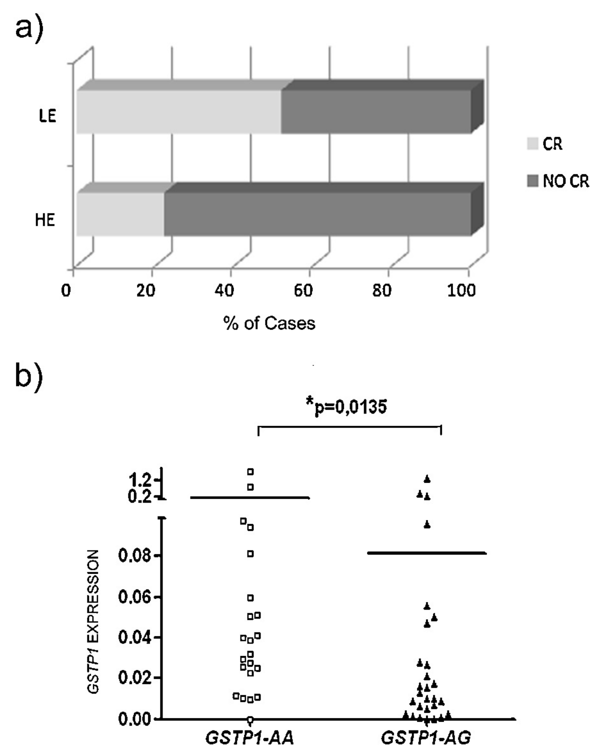

Fig. 1. Analysis of *GSTP1* mRNA expression. (a) Percentage of cases that achieve complete remission (CR) in MM expression groups. HE: high expression; LE: low expression; (b) Distribution of MM genotypes according to the expression level showing that patients with *GSTP1*-AA wild type genotype had higher mean expression than *GSTP1*-AG heterozygous carriers ($p = 0.0135$).

probably reflecting different transcriptional or posttranscriptional mechanisms.

Finally, the comparison between *GSTP1* mRNA expression and genotype distribution was evaluated in 49 cases with MM, and in 17 patients with MGUS. *GSTP1* overexpression was significantly more frequent in MM cases with *GSTP1*-AA genotype (54.5%; 12/22) while low expression was mainly observed in *GSTP1*-AG genotype (77.8%; 21/27) ($p = 0.036$). In addition, the mean mRNA level of *GSTP1*-AA wild type patients was significantly higher (0.15 ± 0.08) compared to *GSTP1*-AG heterozygous carriers (0.08 ± 0.05) ($p = 0.0135$) (Fig. 1b). No data of mRNA transcripts from the two MM patients with *GSTP1*-GG genotype were obtained. In MGUS patients, a similar association between genotype and mRNA expression was found, but no significant differences were observed. It is of interest to note that all MM patients with *GSTP1*-AG genotype achieving CR showed low transcriptional levels at diagnosis. Few studies had correlated transcription levels with *GSTP1* genotype but the results are discordant. While different authors found reduced mRNA expression in carriers of the variant allele [18,19] others observed that it was associated to overexpression [20]. These contradictory findings could be explained by the existence of polymorphisms at the *GSTP1* promoter region that may modulate the expression levels of this gene and confer drug resistance [21,22]. Interestingly, in our series most MM patients with CR had low transcription levels and all cases with heterozygous genotypes also have reduced expression, suggesting the importance of *GSTP1* activity in clinical evolution. This low activity resulting from a variant genotype and to a lesser transcription level may increase the effectiveness of chemotherapy due to lower drug conjugation and inactivation.

In conclusion, our results suggest, for the first time, a role for *GSTP1* expression in development and/or progression of plasma cell disorders, and a probable influence of functional capacity of the enzyme on clinical outcome. Our findings and those of the literature support *GSTP1* as an interesting tumor marker and a potential therapeutic target. Nevertheless, further studies are needed to clarify the effect of *GSTP1* expression in MM patients.

Conflict of interest statement

The authors have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.canep.2013.07.004.

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