Polymorphisms of p53, GSTM1 and GSTT1, and HPV in uterine cervix adenocarcinoma

C.R. Nogueira de Carvalho[1], I.D.C. Guerreiro da Silva[2], J.S. Pereira[2], N.C. Nogueira de Souza[2], G. Rubino de Azevedo Focchi[3], J.C.L. Ribalta[1]

[1]Department of Gynecology, Universidade Federal de São Paulo/Escola Paulista de Medicina
[2]Laboratory of Molecular Gynecology Universidade Federal de São Paulo/Escola Paulista de Medicina
[3]Department of Pathology, Universidade Federal de São Paulo/Escola Paulista de Medicina (Brazil)

Summary

Objective: To analyze the participation of glutathione-S-transferase (GST) M1 and T1 polymorphisms associated or not with protein p53 polymorphism at codon 72 and in the presence of HPV in the carcinogenesis of uterine cervix adenocarcinoma. *Methods:* Forty-three samples of uterine cervix adenocarcinoma were studied and 86 samples of endocervical cells of women without tumors formed the control group. The presence of HPV was determined in order to genotype the isoforms of p53 at codon 72, GSTM1, GSTM1*0, GSTT1 and GSTT1*0 which were evaluated by the PCR method. *Results:* HPV was present in 97.67% of the adenocarcinoma cases and in 31.40% of the control group. Statistical analysis showed differences ($p = 0.001$) and an OR of 113.3 (CI 95%: 13.67-947.14). GSTT1 and GSTT1*0 analysis showed a significant difference between the groups ($p = 0.001$) with an OR of 4.58 (CI 95%: 2.041-10.28) ($p < 0.001$) for the presence of GSTT1*0. When it was associated with HPV OR was 6.6 (CI 95%: 0.04-0.50). Analyses of p53 and GSTM1 and GSTM1*0 either alone or associated with HPV were not significant. *Conclusion:* The presence of GSTT1*0 increased the risk for uterine cervix adenocarcinoma development while the allele GSTT1 had a protective action. The other isoforms did not appear to participate in the carcinogenesis of uterine cervix adenocarcinoma.

Key words: p53; GSTM1; GSTT1; HPV; Adenocarcinoma.

Introduction

Polymorphisms are structural DNA modifications of a certain gene, inherited and transmitted to a part of the population. Repetitions of microsatellites, insertion, inversion and deletion of small segments may occur. The simple exchange of a nucleotide for another, called single nucleotide polymorphism (SNP) is the most frequent occurrence [1]. Variations of p53 and of the metabolizing glutathione-S-transferase M1 and T1 (GSTM1 and GSTT1) are examples of polymorphisms [2, 3]. Protein p53 is a phosphoprotein whose function is related to blockade of cell cycle progression and to the start of the apoptosis chain. In humans polymorphism at codon 72 occurs with substitution of the amino acid proline (PRO) for arginine (ARG). Both alleles may be functionally homozygous or heterozygous [4, 5]. Women with ARG homozygosis when infected by human papillomavirus of high oncogenic risk have a seven-fold higher risk of developing squamous cell carcinoma of the uterine cervix than the others [2].

Glutathione-S-transferases (GST) are part of a phase II superfamily of metabolizing enzymes [6]. Their fundamental role is due to the detoxification of endogenous and exogenous compounds, forming non-toxic and more soluble derivatives ready to be excreted or transported and stocked by phase III metabolism transporters. In humans there are seven cytosol GST superfamilies. Among them the GSTM and GSTT superfamilies should be pointed out [7, 8].

Revised manuscript accepted for publication March 12, 2008

The GST-M family has five isoforms or subfamilies GSTM1 to GSTM5. The GSTM1 gene, localized in 1p13.3, has three alleles, GSTM1*A, GSTM1*B and GSTM1*0. The first two differ regarding substitution of the guanine for cytosine in nucleotide 534 of exon 7, so that, in position 172 of the enzyme there is substitution of one lysine (GSTM1*A) for asparagine (GSTM1*B). The third allele (GSTM1*0) is the result of an unequal exchange between GSTM1 and GSTM2 loci which are physically near and share 99% of the nucleotide sequence, with the occurrence of a 15 kb deletion which contains the whole GSTM1 gene [9].

The GST-T family, instead, has two isoforms or subfamilies, GSTT`and SGTT2, separated by 50 kb. The GST1 gene is localized in 22q11-2, and presents three alleles, GSTT1*A, GSTT1*B and GSTT1*0 or null allele. The two first result from an exchange of the arginine for cytosine in nucleotide 310 of exon 3, which exchanges the threonine (GSTT1*A) of residue 104 for proline (GSTT1*B). GSTT1 is located in a region of extensive homology and is flanked by two 18 kb regions called HA3 and HA5 which each have in their central region 403 bp with 100% identity. Recombination of 403 bp to the right with those to the left results in a 54 kb deletion containing the whole GSTT1 gene; as a result allele GSTT1*0 or null allele emerges [9].

Several authors demonstrate or refute the association of the null alleles with higher susceptibility to develop intraepithelial neoplasias and squamous cell carcinoma of the uterine cervix associated or not with p53 polymorphisms [10-15].

Epidemiologic studies confirmed that HPV is the causal factor of uterine cervix cancer associated with several cofactors, being found in 95% of adenocarcinomas of the uterine cervix.

Uterine cervix adenocarcinomas are little known tumors when compared to the squamous cell variety. They have a worse prognosis and are more resistant to the immunologic reaction of the host body when compared to the cell variety [20-23].

The present aim was to analyze an eventual association of polymorphisms present in the p53 gene, as well as the metabolizing enzymes GST1/GSTM1*0 and GSTT1/GSTT1*0 with the emergence of cervical adenocarcinoma.

Materials and Methods

The study group consisted of tumor fragments obtained from 44 women with uterine cervix adenocarcinoma at any clinical stage, proven by histopathologic examination and still without treatment. The fragments were processed histologically with a final diagnosis of adenocarcinoma.

The control group consisted of 100 samples of endocervical scrapings obtained with a brush. Smears were made on a slide for the cytopathologic examination and the remainder dispersed in a cytosolic Tris EDTA solution. Absence of any intraepithelial and inferior genital tract invading neoplasia was proven by cytologic and colposcopic examination.

Biomolecular analyses were performed in the Laboratory of Molecular Gynecology of the Department of Gynecology, UNIFESP-EPM. After paraffin removal from the histopathologic sections of both the study and control groups, DNA was extracted [25], presence of HPV was searched [26], polymorphism at codon 72 of the TP53 gene was analyzed [27] and alleles of GSTM1 and GSTT1 families was studied [28] using polymerase chain reaction (PCR).

Statistical analysis was performed using the following tests: Student's *t*-test to compare age between groups; chi-square test to compare presence of HPV in the study and control groups to verify the participation of the polymorphic varieties of GSTM1 and GST1 families in the two groups; Fisher's exact test for the analysis of p53 isoforms in the study and control groups; analysis of the association of HPV with p53 protein isoforms and with GMST1 and GSTT1 enzyme alleles in the genesis of uterine cervix adenocarcinoma, as well as analysis of the association of p53 isoforms with alleles of the GSTM1 and GSTT1 families; analysis of maximum likelihood estimation to evaluate the association of GSTM1 with the T1 enzyme family in the carcinogenesis of uterine cervix. In all performed and applicable tests rejection of the null hypothesis was equal to or less than 5% (0.05).

Results

Of the 44 selected specimens for the study group one case was excluded because of non-amplification of DNA, so that 43 samples remained. In the control group 14 cases were excluded for the same reason so that this group consisted of 86 DNA samples of endocervical cells. The age range of the study group varied from 23 to 86 years, with a mean of 52.48 years and median of 45 years. The analysis of the groups using the Student's *t*-test showed that they were homogeneous (p = 0.1402) (CI 95%).

Table 1. — *Distribution of 129 women with and without uterine cervix adenocarcinoma according to groups, presence of HPV and statistical evaluation.*

	Study		Control		Total	
	n	%	n	%	n	%
HPV+	42	97.67	27	31.40	69	100.0
HPV-	1	2.33	59	68.60	60	100.0
Total	43	100.0	86	100.0	129	100.0

Chi-square test p = 0.01(CI 95%); n = number of cases; HPV+ = presence of human papillomavirus; HPV- = absence of human papillomavirus.

Table 2. — *Distribution of 129 women with and without uterine cervix adenocarcinoma according to groups, isoforms of GSTT1 and statistical analysis.*

	Study		Control		Total	
	n	%	n	%	n	%
GSTT1	21	48.84	70	81.40	91	100.0
GSTT1*0	22	51.16	16	18.60	38	100.0
Total	43	100.0	86	100.0	129	100.0

Chi-square test p = 0.01 (CI 95%); n = number of cases; GSTT1 = glutathione-S-transferase isoform T1; GSTT1*0 = glutathione-S-transferase isoform null T1.

Table 3. — *Distribution of the association of presence of human papillomavirus with GSTT1 and GSTT1*0 with or without adenocarcinoma according to groups and statistical evaluation.*

	Study				Control				Total	
	GSTT1		GSTT1*0		GSTT1		GSTT1*0			
	n	%	n	%	n	%	n	%	n	%
HPV+	20	46.51	22	51.16	24	27.91	3	3.49	69	100
HPV-	1	2.33	0	0.00	46	53.48	13	15.11	60	100
Total	21	48.84	22	51.16	70	81.40	16	18.60	129	100

Fisher test p < 0.001 (CI de 95%); n = number of cases; HPV+ = presence of human papillomavirus; HPV- = absence of human papillomavirus; GSTT1 = allele T1 of glutathione-S-transferase; GSTT1*0 = null allele of glutathione-S-transferase.

Presence of HPV in the study and control groups is shown in Table 1. The statistical analysis by the chi-square test showed a significant difference between the two groups (p = 0.001). Analysis of maximum likelihood estimation, odds ratio (OR) with 95% confidence interval (CI) showed a 113.79-fold risk (CI 95%: 13.67 - 947.14) for adenocarcinoma development in the presence of HPV.

The p53 polymorphism at codon 72 in the two groups was evaluated by Fisher's exact test and there was no statistical difference (p = 0.397) (CI 95%). Association between the presence of virus and the different p53 isoforms also did not show any statistical difference (p = 653; CI 95%).

Similarly the distribution of GSTM1 and GSMT1*0 alleles did not show a statistically significant difference by the chi-square test (p = 0.374; CI 95%). The presence of HPV associated with the GSTM1 family, verified by Fisher's exact test, did not show any significant difference (p = 0.256; CI 95%). Association of the p53 isoforms with the family of the GSTM1 enzymes, analyzed by Fisher's exact test also did not show significant differences (p = 1.000; CI 95%).

Evaluation of enzyme GSTT1 with alleles GSTT1 and GSTT1*0 (Table 2) evidenced a difference between the study and control groups by the chi-square test (p = 0.001; CI 95%). Analysis of maximum likelihood estima-

tion, OR with 95% CI showed a 4.58-fold risk (CI 95%: 2.041-10.28) (p = 0.001) for acquiring uterine cervix adenocarcinoma with GSTT1*0. Analysis of HPV-associated with enzymes GSTT1 and GSTT1*0 (Table 3) revealed a significant difference between the two groups (p = 0.001; CI 95%) using Fisher's exact test. Analysis of maximum likelihood estimation, OR with CI 95% showed a 6.6-fold risk (CI 95%: 1.99-22.17; p < 0.001) of the association HPV with GSTT1*0 and 0.15 times (CI 95%: 0.04-0.50; p = 0.0021) for the association HPV with GSTT1.
The association of the protein p53 isoforms with the GSTT1 enzyme family did not show significance by Fisher's exact test (p = 0.56; CI 95%).

Evaluation of the association between GSTM1 and GSTT1 families was performed using a logistic regression model. This fact allowed us to conclude that the GSTM family is not a prognostic factor for uterine cervix cancer either alone or associated with the GSTT1 family.

Discussion

In this study, the mean age of patients with adenocarcinoma was 52.48 years, superior to the reports by Pirog *et al.* [17] and Bulk *et al.* [29], but was similar to those by Lea *et al.* [30] and Baalbergen *et al.* [31].

The significant difference of HPV presence (p = 0.001) in the study and control groups and the 113.8-fold risk (p < 0.001) of developing adenocarcinoma agree with Pirog *et al.* [17] and Castellsagué *et al.* [19].

The lack of statistical significance between the study and control groups suggests the lack of participation of p53 polymorphism at codon 72 in the generation of uterine cervix adenocarcinoma in the presence of HPV in the studied population. These findings agree with the results obtained by Hildesheim *et al.* [32] and Gustafsson *et al.* [33] and are at variance with those obtained by other authors [34, 35].

In the studied population, phase II metabolizing enzymes GSTM1 and their null allele GSTM1*0 did not participate in adenocarcinoma genesis. Analyses of the association of these enzymes with HPV and with p53 isoforms were also not significant.

In contrast the risk of a woman with the GSTT1*0 genotype of developing uterine cervix adenocarcinoma was 4.6 times higher compared to those with GSTT1. The risk of women with HPV infection associated with genotype GSTT1*0 of developing adenocarcinoma was 6.6 higher than for women with the GSTT1 isoform. In the face of a HPV infection the presence of the metabolizing enzyme GSTT1 would exert a protecting function according to what the applied statistical tests revealed.

The trend toward participation of the p53 Arg/Arg isoform associated with the alleles GSTT1 and GSTT1*0 in the genesis of adenocarcinomas was noted in this study, however confirming trials are needed. It seems that only the GSTT1 family would have a prognostic value for adenocarcinomas.

Our results are similar to those described by Ueda *et al.* [27] although the histological type analyzed by those authors was squamous type.

Association studies between complex genic traits and common diseases, although popular, are marked by lack of reproducibility. The possible causes would include a false-positive association which corrected, would not be replicated; true association which would fail on replication in a low statistical power study, constituting a false-negative study; true association in a certain population would not occur in another due to genetic heterogeneity or environmental variables [36].

In our research it was not possible to establish real comparisons regarding reproducibility. Although focusing on uterine cervix carcinoma, the comparisons of the associations were performed with the squamous variety in spite of the fact that up to now the correct identification of the differences or similarities between both varieties of the cervical neoplasia is lacking.

This study is the first to analyze the association between HPV and the isoforms of the metabolizing enzymes GSTM1 and GSTT1 in uterine cervix adenocarcinoma. Other studies should be performed to confirm or refute the data reported in this research.

Acknowledgements

To Dr. Marilena Melo for referral of the control group cases; to Prof. Helena Miller, Prof. João Norberto Stavale, Prof. José Donato de Próspero, Prof. João Carlos Sampaio Góes and Dr. Sergio Ricardo Rocha de Araujo for referral of the study group.

References

[1] Romero R., Kuivaniemi H., Tromp G., Olson J.N.: "The design, execution, and interpretation of genetic association studies to decipher complex diseases". *Am. J. Obstet. Gynecol.,* 2002, *187,* 1299.
[2] Storey A., Thomas M., Kalita A. *et al.*: "Role of a p53 polymorphism in the development of human papillomavirus-associated cancer". *Nature,* 1998, *393,* 229.
[3] Ueda M., Terai Y., Kanda K. *et al.*: "Germline plymorphism of p53 codon 72 in gynecological cancer". *Gynecol. Oncol*., 2006, *100*, 173.
[4] Haupt S., Berge M., Goldberg Z., Haupt Y.: "Apoptosis – the p53 network". *J. Cell Sci.*, 2003, *116*, 4077.
[5] Pietsch E.C., Humbey O., Murphy M.E.: "Polymorphisms in the p53 pathway". *Oncogene,* 2006, *25,* 1602.
[6] Xu C., Li C.Y.-T., Kong A.-N.T.: "Induction of phase I, II and III drug metabolism/transport by xenobiotics". *Arch. Pharm. Res.,* 2005, *28,* 249.
[7] Hayes J.D., Flanagan J.U., Jowsey I.R.: "Glutathione Transferase". *Annu. Rev. Pharmacol. Toxicol.,* 2005, *45,* 51.
[8] Frova C.: "Glutathione transferase in genomics era: news insights and perspectives". *Biomol. Eng.,* 2006, *23,* 149.
[9] Parl F.F.: "Glutathione S-transferase genotype and cancer risk". *Cancer Lett.*, 2005, *221*, 123.
[10] Rebbeck T.R.: "Molecular epidemiology of the human glutathione-S-transferase genotypes GSTM1 and GSTT1 in cancer susceptibility". *Cancer Epidemiol. Biomarker. Prev.,* 1997, *6,* 733.
[11] Habdous M., Siest G., Herbeth B., Vincent-Viry M., Visvikis S.: "Polymorphismes des glutathion S-transferases et pathologies humaines: bilan des études épidémiologiques". *Ann. Biol. Clin.,* 2004, *62*, 15.
[12] Goodman M.T., McDuffie K., Hernandez B., Bertram C.C., Wilkens L.R., Guo C. *et al.*: "*CYP1A1, GSTM1,* and *GSTT1*, polymorphisms and risk of cervical squamous intraepithelial lesions in a multiethnic population". *Gynecol. Oncol.,* 2001, *81,* 263.
[13] Au W.W., Sierra-Torres C.H., Tyring S.K.: "Acquired and genetic susceptibility to cervical cancer". *Mutat. Res.,* 2003, *544,* 361.

[14] Lee S.A., Kim J.W., Roh J.W., Choi J.Y., Lee K.-M., Yoo K.Y. *et al.*: "Genetic polymorphisms of *GSTM1*, p21, p53 and HPV infection with cervical cancer in Korean women". *Gynecol. Oncol.,* 2004, *93,* 14.
[15] Sharma A., Sharma J.K., Murthy N.S., Mitra A.B.: "Polymorphisms at GSTM1 and GSTT1 gene loci and susceptibility to cervical cancer in Indian population". *Neoplasma,* 2004, *51,* 12.
[16] Haverkos H., Rohrer M., Pickworth W.: "The cause of invasive cervical cancer could de multifactorial". *Biomed. Pharmacoter.*, 2000, *54,* 54.
[17] Pirog E.C., Kleter B., Olgac S., Bobkiewicz P., Lindeman J., Quint W.G. *et al.*: "Prevalence of human papillomavirus DNA in difference histological subtype of cervical adenocarcinoma". *Am. J. Pathol.,* 2000, *157,* 1055.
[18] Danaei G., Hoorn S.V., lopez A.D., Murray C.J., Ezzati M.: "Comparative Risk Assessment Collaborating Group. Causes of cancer in the world: comparative risk assessment of nine behavioural and environmental risk factors". *Lancet,* 2005, *366,* 1784.
[19] Castellsagué X., Díaz M., Sanjosé S., Muñoz N., Herrero R., Franceschi S. *et al.*: "Worldwide human papillomavirus etiology of cervical adenocarcinoma and its cofactors: Implications for screening and prevention". *J. Natl. Cancer Inst.*, 2006, *98*, 303.
[20] Altekruse S.F., Lacey J.V. Jr., Brinton L.A., Gravitt P.E., Silverberg S.G., Barners W.A. Jr.: "Comparison of human papillomavirus genotypes, sexual, and reproductive risk factors of cervical adenocarcinomas and squamous cell carcinoma: Northeastern United States". *Am. J. Obstet. Gynecol*., 2003, *188,* 657.
[21] Ayhan A., Al R.A., Baykal C., Demirtas E., Yüce K., Ayhan A.: "A comparison of prognoses of FIGO stage IB adenocarcinoma and squamous cell carcinoma". *Int. J. Gynecol. Cancer,* 2004, *14*, 279.
[22] Recoules-Arche A., Rouzier R., Rey A., Villefranque V., Haie-Meder C., Pautier P. *et al.*: "Les adenocarcinomas du col utérin ont-ils plus mauvais prognostic que les carcinomas épidermoïdes?". *Gynecol. Obstet. Fertil.,* 2004, *32*, 116.
[23] Chao A., Wang T.H., Lee Y.S., Hsueh S., Chao A.S., Chang T.C. *et al.*: "Molecular characterization of adenocarcinoma and squamous carcinoma of the uterine cervix using microarray analysis of gene expression". *Int. J. Cancer,* 2006, *119,* 91.
[24] Cawkwell L., Quirke P.: "Direct multiplex amplification of DNA from a formalin fixed, paraffin wax embedded tissue". *Mod. Pathol.*, 2000, *53,* 51.
[25] Morgan K., Lam L., Kalsheker N.: "A rapid and efficient method for DNA extraction from paraffin wax embedded tissue for PCR amplification". *J. Clin. Pathol. Mol. Pathol.,* 1996, *49,* M179.
[26] Manos M.M., Ting Y., Wright D.K., Lewis A.J., Broke T.R., Wolinsky S.M.: "Use of polymerase amplification for the detection of genital human papillomaviruses". *Cancer Cells,* 1989, *7,* 209.
[27] Soulitzis N., Sourvinos G., Dokiniakis D.N., Spandidos D.A.: "p53 codon 72 polymorphism and its association with bladder cancer". *Cancer Lett.*, 2002, *179,* 175.
[28] Ueda M., Hung Y.C., Terai Y., Saito J., Nunobiki O., Noda S. *et al.*: "Glutathione-S-transferase and p53 polymorphisms in cervical carcinogenesis". *Gynecol. Oncol.*, 2005, *96,* 736.
[29] Bulk S., Visser O., Rozendaal L., Verheijen R.H., Meijer C.J.: "Incidence and survival rate of women with cervical cancer in the Greater Amsterdam area". *Br. J. Cancer,* 2003, *89*, 834.
[30] Lea J.S., Sheets E.E., Wenham R.M., Duska L.R., Coleman R.L., Miller D.S. *et al.*: "Stage IIB-IVB cervical adenocarcinoma: prognostic factors and survival". *Gynecol. Oncol.*, 2002, *84,* 115.
[31] Baalbergen A., Ewing-Graham P.C., Hop W.C., Struijk P., Helmerhorst T.J.: "Prognostic factors in adenocarcinomas of uterine cervix". *Gynecol. Oncol.*, 2004, *92,* 262.
[32] Hildesheim A., Schiffman M., Brinton L., Fraumeni J.F. Jr., Herrero R., Bratti M.C.: "p53 polymorphism and risk of cervical cancer". *Nature,* 1998, *396*, 531.
[33] Gustafsson A.C., Guo Z., Hu X., Ahmadian A., Brodin B., Nilsson A. *et al.*: "HPV-related cancer susceptibility and p53 codon 72 plymorphism". *Acta Derm. Venerol.,* 2001, *81,* 125.
[34] Andersson S., Rylander E., Strand A., Sällström J., Wilander E.: "The significance of p53 codon 72 polymorphism for the development of cervical adenocarcinomas". *Br. J. Cancer,* 2001, *85,* 1153.
[35] Yang Y.C., Chang C.L., Chen M.L.: "Effect of p53 polymorphism on the susceptibility of cervical cancer". *Gynecol. Obstet. Invest.,* 2001, *51*, 197.
[36] Newton-Cheh C., Hirchhorn J.N.: "Genetic association studies of complex traits design and analysis issues". *Mutat. Res.,* 2005, *573,* 54.

Address reprint requests to:
C.R. NOGUEIRA DE CARVALHO, M.D.
Department of Gynecology
Univ. Fed. de São Paulo
Rua Tutoia 349/102
São Paulo (SP) 04007-001 (Brazil)
e-mail: carmenrcarvalho@uol.com.br