Medical Microbiology and Immunology (2019) 208:49–58
https://doi.org/10.1007/s00430-018-0557-y

ORIGINAL INVESTIGATION

Polymorphisms in the *TGFB1* signal peptide influence human papillomavirus infection and development of cervical lesions

Kleber Paiva Trugilo[1] · Guilherme Cesar Martelossi Cebinelli[1] · Fernanda Costa Brandão Berti[1] · Nádia Calvo Martins Okuyama[1] · Fernando Cezar-dos-Santos[1] · Michelle Mota Sena[1] · Luis Fernando Lásaro Mangieri[2] · Maria Angelica Ehara Watanabe[1] · Karen Brajão de Oliveira[1]

Received: 25 April 2018 / Accepted: 23 August 2018 / Published online: 30 August 2018

Abstract

The main purpose was to assess the effect of c.29C>T and c.74G>C polymorphisms in the *TGFB1* signal peptide on HPV infection and development of cervical lesions. Cervical swabs and blood samples were obtained from 349 outpatient women, along with socio-demographic and sexual behavioral data. The study population was stratified by absence or presence of HPV DNA, as tested by PCR, as well as by lesion grade. *TGFB1* signal peptide polymorphisms were genotyped using PCR-restriction fragment length polymorphism. HPV DNA was detected in 172 (49.3%) patients. c.74GC and the combined c.29CC+CT/c.74GC genotype were more frequent in infected patients (35.1 and 15.7%) than in uninfected women (6.2 and 14.7%). Accordingly, these genotypes were associated with a higher risk of HPV infection, with odds ratio and 95% confidence interval of 2.81 and 1.35–5.86 ($P=0.004$) for c.74GC and 3.14 and 1.42–6.94 ($P=0.004$) for the combined genotype, respectively. High-grade lesions were also 2.48 times more likely to occur in c.29CC patients than in c.29TT patients, with a 95% confidence interval of 1.01–6.08 ($P=0.047$). The data demonstrate that c.74G>C and c.29C>T polymorphisms are significantly associated with risk of HPV infection and high-grade squamous intraepithelial lesions, respectively. Thus, *TGFB1* signal peptide polymorphisms are potential susceptibility markers.

Keywords TGFB1 combined genotype · Susceptibility markers · rs1800470 · rs1800471

Introduction

Human papillomavirus (HPV), specifically high-risk HPV, is strongly associated with cervical intraepithelial lesions and cancer [1, 2]. HPV is mainly transmitted by skin or mucosa contact, which enables virions to infect basal layer cells in the epithelium via micro-wounds. In the cervix, most HPV infections are cleared or suppressed by cell-mediated immunity within 1–2 years. However, persistent infections are strongly linked to the development of precancerous squamous intraepithelial lesions, which are typically scored as low-grade or high-grade according to the Bethesda System (2001) [3, 4].

Infection with high-risk HPV is necessary, but not sufficient for cell immortalization and subsequent malignancy. Genetic modifications in the host caused by viral DNA integration, chemical, and physical mutagens may also contribute to these processes [5]. In addition, exogenous and endogenous factors such as tobacco use, parity, oral contraceptive use [6], immune system impairment, and immunological interactions at the site of infection [7] may all influence progression from HPV infection to high-grade cervical lesions.

Transforming growth factor-β (TGFB), one of several cytokines that regulate cell growth, maturation, and differentiation, is also implicated in cervical cancer, suggesting an association with HPV infection severity, malignancy, or both [8, 9]. We note, however, that TGFB may either suppress tumor formation or promote metastasis depending on tumor stage [10, 11]. Furthermore, sequence variations in *TGFB* and its receptors may alter expression and activity [12–16]. For instance, several single nucleotide polymorphisms

✉ Karen Brajão de Oliveira
karen.brajao@gmail.com

[1] Department of Pathological Science, Biological Science Center, State University of Londrina, Londrina, Paraná, Brazil

[2] Department of Gynecology and Obstetrics, Health Science Center, State University of Londrina, Londrina, Paraná, Brazil

(SNPs) have been described in *TGFB1*, including c.29C>T (rs1800470) and c.74G>C (rs1800471) in the signal peptide, a coding region. These SNPs cause amino acid substitutions, where we observe the proline to leucine (c.29C>T) and arginine to proline (c.74G>C) exchanges at positions 10 and 25, respectively [17]. Modifications in amino acid composition of the signal peptide could affect its polarity and hence different rates of protein export [16]. Both SNPs have been associated with non-HPV cancer, as breast [12, 18–20], oral [21], and lung cancers [22], as well as HPV-positive oropharyngeal cancer [9], in which combined variant genotypes were more frequent among HPV16-positive patients than negative ones. However, the c.29C>T and c.74G>C polymorphisms influence on HPV infection and cervical carcinogenesis is not totally clear. The few studies that evaluated the relation between *TGFB1* signal peptide SNPs and HPV infection [23–25] or cervical cancer [26, 27] in different ethnicities did not find any significant association. Here, we assess for the first time the significance of c.29C>T and c.74G>C polymorphisms in HPV infection and development of cervical lesions.

Materials and methods

Ethical approval

This study was approved by the Institutional Ethics Committee Involving Humans of the State University of Londrina, Londrina, PR, Brazil (CEP/UEL 133/2012; CAAE 05505912.0.0000.5231). The purpose of the study and the procedures involved were explained to all participants, and written informed consent was obtained prior to sample collection and interview.

Patients and samples

In this case–control study, we analyzed 349 women who underwent outpatient cytology testing between 2013 and 2015 at an ambulatory colposcopy facility of the Intermunicipal Consortium of Health of the Middle Paranapanema, at the University Hospital and Clinic Center of the State University of Londrina, and at two Basic Healthcare Units in Londrina—PR, Brazil. After sample collection, cytobrushes were stored in 2 mL of TE buffer (10 mM Tris–HCl, 1 mM EDTA pH 8.0) at 4 °C until analysis. Peripheral blood was drawn into sterile syringes containing EDTA as anticoagulant, and stored at 4 °C until analysis. Patients were interviewed using a structured questionnaire to collect sociodemographic and sexual behavioral data. Participants were stratified based on presence or absence of HPV DNA, as tested by PCR, and based on lesion grade, as determined by cervical cytology.

Cervical cytology

Cytology samples were graded according to the Bethesda System (2001) at the Public Health System Laboratory. Patients were deemed to have low-grade squamous intraepithelial lesions, high-grade squamous intraepithelial lesions or no lesions if cytology samples were normal, i.e., were not indicated as having low- or high-grade squamous intraepithelial lesions, cervical carcinomas, atypical squamous cells of undetermined significance, or other atypical squamous cells that cannot be excluded as high-grade squamous intraepithelial lesions [28].

DNA extraction

Genomic DNA was obtained from cervical cytobrushes using DNAzol (Invitrogen Inc., Carlsbad, CA, USA) according to the manufacturer's instructions, and stored at − 20 °C until use. Genomic DNA was also extracted from peripheral blood using a Biopur Mini Spin Plus Kit (Biometrix, Curitiba, Paraná, Brazil). DNA concentration was measured at 260 nm on a NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA), and purity was assessed by the A260/A280 ratio.

HPV detection by PCR

HPV was detected by PCR using the primers MY09 (5′-CGTCCMAARGGAWACTGATC-3′) and MY11 (5′-GCMCAGGGWCATAAYAATGG-3′), which are designed to amplify a conserved region of approximately 450 bp in the HPV L1 gene (GenBank Accession number: AJ236888) [29]. This method was selected because it targets very small fragments, and consequently is more sensitive than several other molecular techniques [28]. A fragment of human b-globin with a length of 268 bp was co-amplified as an internal control using primers GH20 (5′-GAAGAGCCA AGGACAGGTAC-3′) and PC04 (5′-CAACTTCATCCA CGTTCACC-3′) [28]. Reactions without template DNA were used as a negative control to test for contamination, and DNA from HeLa cells, which are stably integrated with HPV18, was used as positive control. PCR products were electrophoresed on 10% polyacrylamide and stained with silver nitrate.

Genotyping of *TGFB1* signal peptide polymorphisms

TGFB1 signal peptide polymorphisms were genotyped by PCR-restriction fragment length polymorphism according to Wood et al. (2000) [16]. In this method, a single amplicon is digested with *MspA1-I* and *Bgl-I* to detect c.29C>T

and c.74G>C polymorphisms, respectively. Digestion with *MspA1-I* produces fragments of 149, 67, 40, 26, and 12 bp for the C allele at the c.29C>T site, and 161, 67, 40 and 26 bp for allele T. In contrast, digestion with *Bgl-I* generates fragments of 131, 103, and 60 bp for the G allele at the c.74G>C site, and 163 and 131 bp for allele C.

Statistical analysis

Continuous data were tested for normality by Kolmogorov–Smirnov test and non-normal data were analyzed by Mann–Whitney test to compare groups. Data were expressed by median and interquartile range (IQR). Differences in socio-demographic and sexual behavioral data between infected and uninfected women were examined using contingency tables and Pearson's χ^2 test. Hardy–Weinberg equilibrium in infected and uninfected women was tested using the χ^2 test. Differences in the distribution of genotypes were assessed by the χ^2 test between infected and uninfected women, and among women with or without low- and high-grade squamous intraepithelial lesions. The crude and adjusted odds ratio with 95% confidence interval were calculated to estimate the association between polymorphisms and HPV infection or lesion grade based on a binary or multinomial logistic regression model, respectively, without or with adjustment for confounding factors (knowledge of HPV, age, marital status, number of full-term pregnancies, and parturition for HPV infection analysis and HPV status, age, and tobacco use for lesion grade analysis). Data were analyzed in SPSS Statistics 22.0 (SPSS Inc., Chicago, Illinois, USA). $P < 0.05$ was considered statistically significant.

Results

The study population consisted of 349 women, of whom 172 (49.3%) tested positive for HPV DNA, and 177 (50.7%) did not. The median age of all women was 38 years, with interquartile range of 19 years. Infected women were significantly younger ($P < 0.001$) at 35 (20) years than uninfected women, who were age 41 (18) years.

Socio-demographic and sexual behavioral data

Socio-demographic and sexual behavioral data are listed in Tables 1 and 2, respectively. Compared with the uninfected group, HPV infection was frequently observed in women who were ≤ 24 years old ($P = 0.006$), single ($P = 0.014$), and had not been pregnant ($P = 0.008$) or given birth ($P = 0.008$). In addition, HPV infection was more frequent among patients who did not had knowledge about the virus and its transmission ($P = 0.018$). Notably, using the group with no cervical lesions as a reference, low-grade squamous intraepithelial lesions were more frequent in women aged 24 years or less ($P = 0.039$), whereas high-grade intraepithelial lesions were more common among women who had at least four sexual partners in their lifetime ($P < 0.001$). Low- and high-grade lesions were also more frequent among women who smoked ($P = 0.002$).

TGFB1 signal peptide polymorphisms and HPV infection

We found that *TGFB1* signal peptide polymorphisms were in Hardy–Weinberg equilibrium ($P > 0.05$) in infected and uninfected women. Among uninfected women, 64 (36.2%) had genotype TT at the c.29C>T site, whereas 83 (46.9%) and 30 (16.9%) had genotype CT and CC, respectively. Among infected women, 50 (29.1%) had genotype TT, 81 (47.1%) had genotype CT, and 41 (23.8%) had genotype CC. The distribution of c.29C>T genotypes was comparable between these groups ($P = 0.185$).

However, the distribution of c.74G>C polymorphism was significantly different between infected and uninfected women ($P = 0.004$). In particular, 166 (93.8%) uninfected and 145 (84.3%) infected women had genotype GG, whereas 11 (6.2%) uninfected and 27 (15.7%) infected patients had genotype GC. The CC genotype was not observed (Table 3). Accordingly, based on binary logistic regression models adjusted for knowledge of HPV, age, marital status, number of full-term pregnancies, and parturition, these women were at increased risk of infection, with an adjusted odds ratio of 2.42 and 95% confidence interval of 1.12–5.39, with b 0.90, χ^2_{Wald} ($df = 1$) 4.99, and $P = 0.026$. Higher susceptibility was also observed with the combined genotypes c.29CC+CT/c.74GC and c.29TT/c.74GG. For instance, infected patients were more likely to have genotype c.29CC+CT/c.74GC than uninfected women, with b 1.03, χ^2_{Wald} ($df = 1$) 5.18, and $P = 0.023$, and were 2.80 times more likely to be infected than those with the c.29TT/c.74GG genotypes, with a 95% confidence interval of 1.15–6.82 (Table 4).

TGFB1 signal peptide polymorphisms and cervical lesions

Based on cervical cytology, 343 women were included in the further analysis, of whom 259 did not have cervical lesions, whereas 22 and 62 had low- and high-grade squamous intraepithelial lesions, respectively (Table 5). Four of the excluded patients were diagnosed with cervical cancer, and another two were excluded because of missing cervical cytology results. Significant differences in the distribution of c.29C>T polymorphism were noted between women without lesions and those with high-grade squamous intraepithelial lesions ($P = 0.042$). Based on a multinomial logistic regression model adjusted for HPV

Table 1 Socio-demographic data of HPV-uninfected and HPV-infected women

Variable	HPV-uninfected		HPV-infected		*P* value*
	n = 177**	%	*n* = 172**	%	
Knowledge of HPV					0.018
No	32	18.1	46	26.7	
Have ever heard	96	54.2	73	42.4	
Yes	44	24.9	26	15.1	
Missing data	5	2.8	27	15.8	
Age range (years)					0.006
≤ 24	13	7.3	31	18.0	
25–34	46	26.0	53	30.8	
35–44	45	25.4	40	23.3	
45–54	50	28.3	28	16.3	
≥ 55	23	13.0	20	11.6	
Missing data	0	0.0	0	0.0	
Ethnicity					0.157
Caucasian	91	51.4	67	39.2	
Brown	58	32.8	63	36.8	
Black	22	12.4	13	7.6	
Missing data	6	3.4	29	16.4	
Monthly income[a]					0.143
< 1 minimum wage	42	23.7	43	25.0	
1–< 3 minimum wages	106	59.9	77	44.8	
3–< 5 minimum wages	10	5.6	11	6.4	
≥ 5 minimum wages	4	2.3	0	0.0	
Missing data	15	8.5	41	23.8	
Smoking status					0.177
No	143	80.8	118	68.6	
Yes	32	18.1	38	22.1	
Missing data	2	1.1	16	9.3	
Education[b]					0.236
Incomplete elementary	57	32.2	43	25.0	
Complete elementary	15	8.5	21	12.2	
Incomplete secondary	21	11.9	24	14.0	
Complete secondary	58	32.7	45	26.2	
Incomplete higher education	6	3.4	6	3.5	
Complete higher education	14	7.9	5	2.9	
Missing data	6	3.4	28	16.2	
Marital status					0.014
Single	18	10.2	39	22.7	
Married/civil partner	127	71.7	103	59.9	
Divorced	23	13.0	18	10.5	
Widowed	9	5.1	8	4.6	
Missing data	0	0.0	4	2.3	

[a]Based on Brazilian minimum wage, approximately US$ 220.00

[b]Based on Brazilian educational system

*By two-sided χ^2 test, with $P < 0.05$ considered significant

**For socio-demographic characteristics analysis between HPV-uninfected and HPV-infected women not all 349 women were included, with variations depending on the characteristic analyzed

Table 2 Sexual behavioral characteristics of HPV-uninfected and HPV-infected women

Variable	HPV-uninfected		HPV-infected		*P* value*
	n = 177**	%	*n* = 172**	%	
Contraception					0.137
No	102	57.6	91	52.9	
Yes, hormonal	60	33.9	54	31.4	
Yes, condom	13	7.3	13	7.6	
Yes, both	0	0.0	5	2.9	
Missing data	2	1.2	9	5.2	
Number of full-term pregnancies					0.008
0	13	7.4	30	17.4	
1	29	16.4	43	25.0	
2	56	31.6	38	22.1	
3	45	25.4	36	20.9	
4	19	10.7	12	7.0	
≥ 5	15	8.5	12	7.0	
Missing data	0	0.0	1	0.6	
Parturition					0.008
No	15	8.5	35	20.3	
Natural birth	69	39.0	68	39.6	
Cesarean birth	59	33.3	43	25.0	
Both	34	19.2	25	14.5	
Missing data	0	0.0	1	0.6	
Abortion					0.654
No	129	72.9	114	66.3	
Yes	35	19.8	27	15.7	
Not applicable[a]	13	7.3	30	17.4	
Missing data	0	0.0	1	0.6	
Age at first sexual intercourse (years)					0.107
≤ 17	93	52.5	99	57.6	
≥ 18	82	46.3	61	35.5	
Missing data	2	1.2	12	6.9	
Age at menarche (years)					0.153
≤ 11	37	20.9	42	24.4	
12	43	24.3	47	27.3	
13	48	27.1	28	16.3	
≥ 14	48	27.1	44	25.6	
Missing data	1	0.6	11	6.4	
Sexual partners during lifetime					0.072
1	67	37.9	39	22.7	
2–3	48	27.1	51	29.7	
≥ 4	57	32.2	55	32.0	
Missing data	5	2.8	27	15.6	
Sexual partners in past 6 months					0.454
0	22	12.4	23	13.4	
1	147	83.1	118	68.6	
≥ 2	3	1.7	5	2.9	
Missing data	5	2.8	26	15.1	

*By two-sided χ^2 test, with $P < 0.05$ considered significant

**For sexual behavioral characteristics analysis between HPV-uninfected and HPV-infected women not all 349 women were included, with variations depending on the characteristic analyzed. [a]Women who had not been pregnant

Table 3 Distribution of *TGFB1* signal peptide polymorphisms genotypes by HPV infection

TGFB1 genotype	HPV-uninfected		HPV-infected		*P* value*
	n = 177 [a]	%	*n* = 172 [b]	%	
c.29C>T					0.185
TT	64	36.2	50	29.1	
CT	83	46.9	81	47.1	
CC	30	16.9	41	23.8	
c.74G>C					0.004
GG	166	93.8	145	84.3	
GC	11	6.2	27	15.7	
CC	0	0.0	0	0.0	
c.29C>T/c.74G>C[1]					0.004
TT/GG	64	85.3	50	64.9	
CC + CT/GC	11	14.7	27	35.1	

*By two-sided χ^2 test, with $P < 0.05$ considered significant

[1]Combined *TGFB1* signal peptide polymorphisms genotypes

[a]Hardy–Weinberg equilibrium χ^2: c.29C>T = 0.122, $P > 0.05$; c.74G>C = 0.182, $P > 0.05$

[b]Hardy–Weinberg equilibrium χ^2: c.29C>T = 0.531, $P > 0.05$; c.74G>C = 1.248, $P > 0.05$

status, age, and tobacco use, the CC genotype was found to be associated with high-grade lesions, with b 0.91, χ^2_{Wald} ($df = 1$) 3.96, and $P = 0.047$. Indeed, the CC genotype increased the probability of developing high-grade lesions by 2.48-fold compared to the TT genotype, with a 95% confidence interval of 1.01–6.08. In contrast, c.74G>C polymorphism was not significantly associated with lesion grade ($P > 0.05$, Table 6).

Discussion

HPV infection and progression to cervical lesions involve multiple factors, both intrinsic and extrinsic, which are determinant for the good or bad outcome of the disease. Environmental factors as well as the participation of immunoregulatory mechanisms are important in this context. Therefore, in this study, we identified the main socio-demographic and sexual behavior characteristics of our study population and verified whether *TGFB1* polymorphisms c.29C>T and c.74G>C are associated with HPV infection and development of cervical lesions in women from Paraná, Brazil.

Socio-demographic and sexual behavioral profiles demonstrated that HPV was associated with women who were not aware of the virus ($P = 0.018$), suggesting that lack of information about HPV and how to avoid exposure probably predisposes women to infection. HPV was also associated with younger women ≤ 24 years of age ($P = 0.006$), who, in this study, were also typically single ($P = 0.014$) and had never been pregnant ($P = 0.008$) or had given birth ($P = 0.008$). Indeed, young age is a well-known independent factor associated with HPV infection [30], perhaps because of increased sexual activity at this age and cervical ectopy, an anatomical feature in young women that exposes the columnar epithelium in the ectocervix and renders the tissue more vulnerable to infection [30, 31].

Although high-grade squamous intraepithelial lesions were expected to be more frequent in the same younger age group at higher risk of HPV, low-grade lesions were actually found to be more frequent ($P = 0.039$). Similarly, Nunes et al. (2014) [32] reported that high-grade lesions were more prevalent in older women than in younger women,

Table 4 *TGFB1* signal peptide polymorphisms genotypes and susceptibility to HPV infection

TGFB1 genotype	χ^2_{Wald} ($df = 1$)		*P* value		Odds ratio and $CI_{95\%}$			
	Crude*	Adjusted**	Crude*	Adjusted**	Crude*		Adjusted**	
c.29C>T								
TT	–	–	–	–	1.00	Reference	1.00	Reference
CT	0.83	0.12	0.364	0.735	1.25	0.77–2.10	1.10	0.65–1.86
CC	3.35	1.71	0.067	0.191	1.75	0.96–3.18	1.54	0.81–2.96
c.74G>C								
GG	–	–	–	–	1.00	Reference	1.00	Reference
GC	7.58	4.99	0.006	0.026	2.81	1.35–5.86	2.42	1.12–5.39
c.29C>T/c.74G>C[a]								
TT/GG	–	–	–	–	1.00	Reference	1.00	Reference
CC+CT/GC	8.01	5.18	0.005	0.023	3.14	1.42–6.94	2.80	1.15–6.82

*Binary logistic regression, with "uninfected group" as reference and $P < 0.05$ considered significant

**Binary logistic regression adjusted for knowledge of HPV, age, marital status, number of full-term pregnancies, and parturition, with "uninfected group" as reference and $P < 0.05$ considered significant

[a]Combined *TGFB1* signal peptide polymorphisms genotypes

Table 5 Distribution of *TGFB1* signal peptide polymorphisms genotypes by lesion grade

TGFB1 genotype	NL[a]		LSIL[b]		HSIL[c]		*P* value*
	n = 259	%	*n* = 22	%	*n* = 62	%	
c.29C>T							0.042
TT	95	36.7	6	27.3	13	21.0	
CT	120	46.3	11	50.0	29	46.8	
CC	44	17.0	5	22.7	20	32.3	
c.74G>C							0.149
GG	235	90.7	19	86.4	51	82.3	
GC	24	9.3	3	13.6	11	17.7	
CC	0	0.0	0	0.0	0	0.0	

*By two-sided χ^2 test, with $P < 0.05$ considered significant

[a]No lesion

[b]Low-grade squamous intraepithelial lesion

[c]High-grade squamous intraepithelial lesion

Table 6 *TGFB1* signal peptide polymorphisms genotypes and susceptibility to low- and high-grade squamous intraepithelial lesions

Lesion	*TGFB1* genotype	χ^2_{Wald} (*df* = 1)		*P* value		Odds ratio and $CI_{95\%}$			
		Crude*	Adjusted**	Crude*	Adjusted**	Crude*		Adjusted**	
LSIL[a]	c.29C>T								
	TT	–	–	–	–	1.00	Reference	1.00	Reference
	CT	0.50	1.40	0.479	0.237	1.45	0.52–4.07	2.08	0.62–7.00
	CC	0.86	1.04	0.353	0.307	1.80	0.52–6.21	2.06	0.51–8.30
	c.74G>C								
	GG	–	–	–	–	1.00	Reference	1.00	Reference
	GC	0.44	0.56	0.507	0.452	1.55	0.43–5.60	1.69	0.43–6.67
HSIL[b]	c.29C>T								
	TT	–	–	–	–	1.00	Reference	1.00	Reference
	CT	2.48	2.13	0.115	0.144	1.77	0.87–3.58	1.80	0.82–3.97
	CC	9.00	3.96	0.003	0.047	3.32	1.51–7.28	2.48	1.01–6.08
	c.74G>C								
	GG	–	–	–	–	1.00	Reference	1.00	Reference
	GC	3.57	0.85	0.059	0.357	2.11	0.97–4.58	1.52	0.62–3.72

*Multinomial logistic regression, with "no lesion" as reference and $P < 0.05$ considered significant

**Multinomial logistic regression adjusted for HPV status, age, and tobacco use, with "no lesion" as reference and $P < 0.05$ considered significant

[a]Low-grade squamous intraepithelial lesion

[b]High-grade squamous intraepithelial lesion

suggesting either that HPV infections are spontaneously cleared more often in women under 30 years of age and do not progress to high-grade lesions, or that high-grade lesions regress in younger women. Interestingly, a high number of sexual partners over the lifetime was not associated with HPV infection in our cohort, in contrast to previous studies. Nevertheless, a high number of sexual partners can reasonably be expected to increase the risk of infection, and hence to increase the risk of high-grade lesions.

Notably, low- and high-grade lesions were more frequent in women who self-reported smoking than in non-smokers ($P = 0.002$), in agreement with previous reports indicating that cigarette use synergizes with HPV infection to promote malignant progression of cervical lesions [33, 34]. In particular, tobacco carcinogens such as benzo[*a*]pyrene have been detected in the cervical mucus of women who smoke and who develop cervical dysplasias [35]. Indeed, benzo[*a*]pyrene has been shown to enhance virus replication and persistence in a dose-dependent manner, or otherwise to correlate with HPV genome amplification, potentially resulting in increased expression of the E6 and E7 oncoproteins [33].

Nevertheless, only a subset of individuals exposed to HPV develops persistent infection that may progress to high-grade lesions and cervical cancer, implying that other

genetic factors may contribute to the process. Such genetic factors may include sequence variations in *TGFB1*, as TGFB is a potent modulator of cell growth, maturation, and differentiation, and an important cytokine in the infection microenvironment [11, 36]. Thus, using molecular techniques, we assessed whether the *TGFB1* signal peptide polymorphisms c.29C>T and c.74G>C are associated with HPV infection and development of cervical lesions.

To eliminate confounding effects in the susceptibility analysis, logistic regression was used to adjust for confounding factors. According to the adjusted results, the c.74GC genotype and the combined c.29CC+CT/c.74GC genotype were associated with infected women. Our results are in agreement with Guan and colleagues (2010) [9], who reported that patients (both gender) with HPV16-positive squamous cell carcinoma of the oropharynx were more likely to have c.29CC+CT genotype and c.29CC+CT/c.74GC+CC combined genotypes than HPV-negative patients. Furthermore, even statistically non-significant, they observed that c.74GC+CC genotype distribution was slightly more frequent in HPV16-positive tumor patients than HPV-negative ones. In our case, besides c.74GC genotype, c.29CC was also more frequent in HPV-infected women than in uninfected, however, this difference did not reach statistical significance. Therefore, we note that the c.29C>T and c.74G>C genotypes by themselves were similarly distributed in infected and uninfected women in our cohort, and thus, analysis of combined polymorphisms was more informative, as has been previously noted [37]. Hence, a panel of polymorphisms may be more reliable as a marker of susceptibility to HPV infection and other diseases.

Remarkably, only c.29C>T polymorphism was associated with lesion grade. Adjusted results confirmed that women with the c.29CC genotype were 2.5 times more likely to develop high-grade squamous intraepithelial lesions compared to women with the c.29TT genotype ($P = 0.047$).

One overarching possibility is that c.29C>T and c.74G>C polymorphisms profoundly impact TGFB1 production, which was found in several studies to gradually increase during progression from normal tissue to cervical cancer [38–40]. For instance, the c.74G and c.29C alleles upregulate TGFB1 in vitro [12, 41, 42], and c.29C is associated with high serum levels of TGFB1 [43–45]. Because TGFB1 may autoregulate HPV gene expression in infected genital epithelial cells by suppressing LCR-driven transcription activity and transcription of early genes [46], we believe that women who produce TGFB1 more abundantly as a result of their c.74GG genotype may have some protection against HPV infection. Conversely, women with the c.74GC genotype are at an elevated risk of HPV infection, as we observed in our cohort, presumably because this genotype suppresses TGFB production [41]. We note that TGFB may additionally control HPV replication early in infection by cell cycle arrest in the G1 phase, a process that depends on inducing the small cyclin-dependent kinase inhibitors p15, p21, and p27 and downregulating the oncogene c-myc through Smads and E2F4/5 [10].

However, in the event that HPV infection persists and lesions develop, increased TGFB production resulting from the c.29CC genotype may then worsen prognosis, as TGFB is also the most powerful immunosuppressor in mammals [47]. In particular, TGFB suppresses the differentiation of T cells [48] and induces naive T cells to express the transcription factor forkhead box P3 (Foxp3), which drives conversion to regulatory T cells [49, 50]. Notably, Foxp3 expression shows an increasing tendency with the progression of cervical lesions and is higher in metastatic lymph nodes than in normal ones [51]. Therefore, the *TGFB1* c.29CC genotype may drive HPV-caused lesions progression as a result of increased immunosuppression.

Although the small number of patients with low-grade squamous intraepithelial lesions and the exclusion of patients with cervical cancer may have limited the sensitivity of the present study, the strengths of our analyses lie in the analysis of combined polymorphisms and in the adjustment for potential confounding factors including HPV, age, and smoking.

To our knowledge, we demonstrated for the first time that the c.74G>C and c.29C>T polymorphisms are significantly associated with susceptibility to HPV infection and high-grade squamous intraepithelial lesions, respectively. Although further studies with a larger number of patients are warranted to confirm the results, our study suggests c.74G>C and c.29C>T polymorphisms in the *TGFB1* signal peptide as potential susceptibility markers.

Acknowledgements The authors thank the volunteers who participated in the study, the Intermunicipal Consortium of Health of the Middle Paranapanema, the University Hospital and Clinic Center of State University of Londrina, the Municipal Health Department of Londrina, Londrina—PR, Brazil, and Londrina State University Coordination for Postgraduation.

Funding This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (470137/2013-4), Fundação Araucária—Programa Pesquisa para o SUS (34935.406.36850.19112012). This work received fellowship support from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, and financial support for language editing from FAEPE/UEL-PUBLIC 2016.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ, Muñoz N (1999) Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J Pathol 189:12–19
2. Smith JS, Lindsay L, Hoots B, Keys J, Franceschi S, Winer R, Clifford GM (2007) Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions: a meta-analysis update. Int J Cancer 121:621–632
3. Schiffman M, Castle PE, Jeronimo J, Rodriguez AC, Wacholder S (2007) Human papillomavirus and cervical cancer. Lancet 370:890–907
4. Roberts JN, Buck CB, Thompson CD, Kines R, Bernardo M, Choyke PL, Lowy DR, Schiller JT (2007) Genital transmission of HPV in a mouse model is potentiated by nonoxynol-9 and inhibited by carrageenan. Nat Med 13:857–861
5. zur Hausen H (2000) Papillomaviruses causing cancer: evasion from host-cell control in early events in carcinogenesis. J Natl Cancer Inst 92:690–698
6. Castellsagué X, Muñoz N (2003) Chap. 3: Cofactors in human papillomavirus carcinogenesis—role of parity, oral contraceptives, and tobacco smoking. J Natl Cancer Inst Monogr 20–28
7. Patel S, Chiplunkar S (2009) Host immune responses to cervical cancer. Curr Opin Obstet Gynecol 21:54–59
8. Alcocer-González JM, Berumen J, Taméz-Guerra R, Bermúdez-Morales V, Peralta-Zaragoza O, Hernández-Pando R, Moreno J, Gariglio P, Madrid-Marina V (2006) In vivo expression of immunosuppressive cytokines in human papillomavirus-transformed cervical cancer cells. Viral Immunol 19:481–491
9. Guan X, Sturgis EM, Lei D, Liu Z, Dahlstrom KR, Wei Q, Li G (2010) Association of TGF-beta1 genetic variants with HPV16-positive oropharyngeal cancer. Clin Cancer Res 16:1416–1422
10. Lebrun J-J (2012) The dual role of TGFβ in human cancer: from tumor suppression to cancer metastasis. ISRN Mol Biol 381428
11. Zarzynska JM (2014) Two faces of TGF-beta1 in breast cancer. Mediators Inflamm 141747
12. Dunning AM, Ellis PD, McBride S, Kirschenlohr HL, Healey CS, Kemp PR, Luben RN, Chang-Claude J, Mannermaa A, Kataja V, Pharoah PD, Easton DF, Ponder BA, Metcalfe JC (2003) A transforming growth factorbeta1 signal peptide variant increases secretion in vitro and is associated with increased incidence of invasive breast cancer. Cancer Res 63:2610–2615
13. Grainger DJ, Heathcote K, Chiano M, Snieder H, Kemp PR, Metcalfe JC, Carter ND, Specter TD (1999) Genetic control of the circulating concentration of transforming growth factor type beta1. Hum Mol Genet 8:93–97
14. Healy J, Dionne J, Bélanger H, Lariviere M, Beaulieu P, Labuda D, Sinnett D (2009) Functional impact of sequence variation in the promoter region of TGFB1. Int J Cancer 125:1483–1489
15. Shah R, Rahaman B, Hurley CK, Posch PE (2006) Allelic diversity in the TGFB1 regulatory region: characterization of novel functional single nucleotide polymorphisms. Hum Genet 119:61–74
16. Wood NA, Thomson SC, Smith RM, Bidwell JL (2000) Identification of human TGF-beta1 signal (leader) sequence polymorphisms by PCR-RFLP. J Immunol Methods 234:117–122
17. Martelossi Cebinelli GC, Paiva Trugilo K, Badaró Garcia S, Brajão de Oliveira K (2016) TGF-1 functional polymorphisms: a review. Eur Cytokine Netw 27:81–89
18. Pooja S, Francis A, Rajender S, Tamang R, Rajkumar R, Saini KS, Megu K, Goel MM, Surekha D, Rao DR, Rao L, Ramachandra L, Kumar S, Kumar S, Vishnupriya S, Satyamoorthy K, Negi MP, Thangaraj K, Konwar R (2013) Strong impact of TGF-β1 gene polymorphisms on breast cancer risk in Indian women: a case-control and population-based study. PLoS One 8:e75979
19. Shin A, Shu XO, Cai Q, Gao YT, Zheng W (2005) Genetic polymorphisms of the transforming growth factor-beta1 gene and breast cancer risk: a possible dual role at different cancer stages. Cancer Epidemiol Biomarkers Prev 14:1567–1570
20. Ziv E, Cauley J, Morin PA, Saiz R, Browner WS (2001) Association between the T29> C polymorphism in the transforming growth factor beta1 gene and breast cancer among elderly white women: the study of osteoporotic fractures. JAMA 285:2859–2863
21. Carneiro NK, Oda JMM, Losi-Guembarovski R, Ramos G, Oliveira BV, Cavalli IJ, de SF Ribeiro, Gonçalves EM, Watanabe MS MAE (2013) Possible association between TGF-β1 polymorphism and oral cancer. Int J Immunogenet 40:292–298
22. Fan H, Yu H, Deng H, Chen X (2014) Transforming growth factor-β1 rs1800470 polymorphism is associated with lung cancer risk: a meta-analysis. Med Sci Monit 20:2358–2362
23. Lima SF, Tavares MMF, Macedo JL, Oliveira RS, Heráclio SA, Maia MMD, Souza PRE, Moura R, Crovella S (2016) Influence of IL-6, IL-8, and TGF-β1 gene polymorphisms on the risk of human papillomavirus-infection in women from Pernambuco, Brazil. Mem Inst Oswaldo Cruz 111:663–669
24. Marangon AV, Guelsin GAS, Visentainer JEL, Borelli SD, Watanabe MAE, Consolaro MEL, Caleffi-Ferracioli KR, Rudnick CCC, Sell AM (2013) The association of the immune response genes to human papillomavirus-related cervical disease in a Brazilian population. Biomed Res Int 146079
25. Fernandes APM, Gonçalves MAG, Simões RT, Mendes-Junior CT, Duarte G, Donadi EA (2008) A pilot case–control association study of cytokine polymorphisms in Brazilian women presenting with HPV-related cervical lesions. Eur J Obstet Gynecol Reprod Biol 140:241–244
26. Wang Q, Zhang C, Walayat S, Chen HW, Wang Y (2011) Association between cytokine gene polymorphisms and cervical cancer in a Chinese population. Eur J Obstet Gynecol Reprod Biol 158:330–333
27. Stanczuk GA, Tswana SA, Bergstrom S, Sibanda EM (2002) Polymorphism in codons 10 and 25 of the transforming growth factor-beta 1 (TGF-β1) gene in patients with invasive squamous cell carcinoma of the uterine cervix. Eur J Immunogenet 29:417–421
28. da Silva MC, Martins HP, de Souza JL, Tognim MCB, Svidzinski TIE, Teixeira JJV, Consolaro MEL (2012) Prevalence of HPV infection and genotypes in women with normal cervical cytology in the state of Paraná, Brazil. Arch Gynecol Obstet 286:1015–1022
29. Bauer HM, Ting Y, Greer CE, Chambers JC, Tashiro CJ, Chimera J, Reingold A, Manos MM (1991) Genital human papillomavirus infection in female university students as determined by a PCR-based method. JAMA 265:472–477
30. Coser J, da Rocha Boeira T, Wolf JM, Cerbaro K, Simon D, Lunge VR (2016) Cervical human papillomavirus infection and persistence: a clinic-based study in the countryside from South Brazil. Braz J Infect Dis 20:61–68
31. Hwang LY, Lieberman JA, Ma Y, Farhat S, Moscicki AB (2012) Cervical ectopy and the acquisition of human papillomavirus in adolescents and young women. Obstet Gynecol 119:1164–1170
32. Nunes JD, Vidal FC, Ferraro CT, Chein MBC, Brito LMO, Monteiro SCM (2014) Molecular detection of human papillomavirus in Brazilian women with cervical intraepithelial neoplasia in a northeast Brazilian city. Genet Mol Res 13:9077–9085
33. Alam S, Conway MJ, Chen HS, Meyers C (2007) The cigarette smoke carcinogen benzo[a]pyrene enhances human papillomavirus synthesis. J Virol 82:1053–1058
34. Castellsagué X, Bosch FX, Muñoz N (2002) Environmental cofactors in HPV carcinogenesis. Virus Res 89:191–199

35. Melikian AA, Sun P, Prokopczyk B, El-Bayoumy K, Hoffmann D, Wang X, Waggoner S (1999) Identification of benzo[a]pyrene metabolites in cervical mucus and DNA adducts in cervical tissues in humans by gas chromatography-mass spectrometry. Cancer Lett 146:127–134
36. Torres-Poveda K, Bahena-Román M, Madrid-González C, Burguete-García AI, Bermúdez-Morales VH, Peralta-Zaragoza O, Madrid-Marina V (2014) Role of IL-10 and TGF-β1 in local immunosuppression in HPV-associated cervical neoplasia. World J Clin Oncol 5:753–763
37. Crawford DC, Nickerson DA (2005) Definition and clinical importance of haplotypes. Annu Rev Med 56:303–320
38. Ki K-D, Tong S-Y, Huh C-Y, Lee JM, Lee SK, Chi SG (2009) Expression and mutational analysis of TGF-beta/Smads signaling in human cervical cancers. J Gynecol Oncol 20:117–121
39. Peghini BC, Abdalla DR, Barcelos ACM, Teodoro LDGVL, Murta EFC, Michelin MA (2012) Local cytokine profiles of patients with cervical intraepithelial and invasive neoplasia. Hum Immunol 73:920–926
40. Xu Q, Wang S, Xi L, Wu S, Chen G, Zhao Y, Wu Y, Ma D (2006) Effects of human papillomavirus type 16 E7 protein on the growth of cervical carcinoma cells and immuno-escape through the TGF-beta1 signaling pathway. Gynecol Oncol 101:132–139
41. Awad MR, El-Gamel A, Hasleton P, Turner DM, Sinnott PJ, Hutchinson IV (1998) Genotypic variation in the transforming growth factor-beta1 gene: association with transforming growth factor-beta1 production, fibrotic lung disease, and graft fibrosis after lung transplantation. Transplantation 66:1014–1020
42. Gu X, Ji X, Shi L-H, Yi CH, Zhao YP, Wang AH, Lu LG, Yu WB, Gao CF (2012) Transforming growth factor beta1 gene variation Leu10Pro affects secretion and function in hepatic cells. Dig Dis Sci 57:2901–2909
43. Guo W, Dong Z, Guo Y, Chen Z, Yang Z, Kuang G, Shan B (2011) Polymorphisms of transforming growth factor-β1 associated with increased risk of gastric cardia adenocarcinoma in north China. Int J Immunogenet 38:215–224
44. Taubenschuß E, Marton E, Mogg M, Frech B, Ehart L, Muin D, Schreiber M (2013) The L10P polymorphism and serum levels of transforming growth factor β1 in human breast cancer. Int J Mol Sci 14:15376–15385
45. Yokota M, Ichihara S, Lin TL, Nakashima N, Yamada Y (2000) Association of a T29> C polymorphism of the transforming growth factor-beta1 gene with genetic susceptibility to myocardial infarction in Japanese. Circulation 101:2783–2787
46. Woodworth CD, Notario V, DiPaolo JA (1990) Transforming growth factors beta 1 and 2 transcriptionally regulate human papillomavirus (HPV) type 16 early gene expression in HPV-immortalized human genital epithelial cells. J Virol 64:4767–4775
47. Gorelik L, Flavell RA (2002) Transforming growth factor-beta in T-cell biology. Nat Rev Immunol 2:46–53
48. Flavell RA, Sanjabi S, Wrzesinski SH, Licona-Limón P (2010) The polarization of immune cells in the tumour environment by TGF beta. Nat Rev Immunol 10:554–567
49. Chen W, Jin W, Hardegen N, Lei KJ, Li L, Marinos N, McGrady G, Wahl SM (2003) Conversion of peripheral CD4 + CD25- naive T cells to CD4 + CD25 + regulatory T cells by TGF-beta induction of transcription factor Foxp3. J Exp Med 198:1875–1886
50. Tone Y, Furuuchi K, Kojima Y, Tykocinski ML, Greene MI, Tone M (2008) Smad3 and NFAT cooperate to induce Foxp3 expression through its enhancer. Nat Immunol 9:194–202
51. Luo Q, Zhang S, Wei H, Pang X, Zhang H (2015) Roles of Foxp3 in the occurrence and development of cervical cancer. Int J Clin Exp Pathol 8:8717–8730