Presence of Human Papilloma Virus in a Series of Breast Carcinoma from Argentina

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Abstract

Background: The etiology and the molecular mechanisms related to breast carcinogenesis remain poorly understood. Some recent reports have examined the role of Human Papillomavirus (HPV) in this disease. The purpose of this study was to determine the prevalence of HPV in breast cancer.

Methods: Sixty one fresh frozen breast cancers samples were analyzed. Samples were tested for HPV by PCR, and products were automatically sequenced. Findings were correlated with clinical and pathological characteristics.

Results: The HPV DNA prevalence in the breast cancer samples was 26% (16/61). Clinical parameters were not statistically associated with HPV presence (p ≥ 0.05 χ² test). Sequence analysis in a subgroup of cases indicates the prevalence of low risk HPV11, followed by high risk HPV16. We found no HPV transcriptional activity.

Conclusion: The present study demonstrated for the first time in Argentina the presence of HPV in a proportion of the malignant breast tissues. This finding suggests that HPV may have a biological significance in breast carcinogenesis.

Introduction

Breast cancer is the most frequently diagnosed malignancy in women in many populations [1], and the incidence of breast carcinoma has increased by 2.5% over the last 50 years [2]. Many risk factors have been associated with the pathogenesis of this disease; including family history, hormones, cigarette smoking and alcohol consumption [3], but the molecular mechanisms related to the breast carcinogenesis remain poorly understood. In Argentina, deaths caused by breast carcinoma raise up to 21.8 per every 100,000 women, the second place in Latin America, and represent 5,400 deaths per year [4].

Currently a large number of infectious agents have been described to either cause or contribute to specific human cancers [5]. Indeed, 10–15% of the cancers worldwide can be linked to viral infections [6]. Many studies have recently suggested that certain viruses might be involved in the pathogenesis of breast cancer, such as specific types of Human papillomavirus (HPV), Epstein Barr virus (EBV) and Mouse mammary tumor virus (MMTV), but viral involvement in breast carcinogenesis still remains controversial. While some groups have suggested that high risk HPV could be implicated in breast tumor development [3,7–22], other groups detracted this proposal [23–26]. However, HPV immortalization of normal breast epithelium has been demonstrated in vitro [27], raising the possibility that HPV might be etiologically related to a proportion of breast cancers.

Human papillomaviruses (HPVs) species, family Papillomaviridae, genus Alphapapillomavirus, are small DNA viruses that infect epithelial cells of the skin and mucosa. HPV is the causal agent of cervix-uterine cancer and anogenital malignancies. However, HPV has been detected in extragenital tumors such as oral, esophageal, tonsillar, laryngea and lungs [1]. The HPV types that infect the genital mucosa can be divided into two groups. The high-risk types, including HPV16, 18, 31 and others, are frequently found in cervical cancers. By contrast, the low-risk types, including HPV6 and 11, also infect the genital epithelia, but are rarely detected in malignancies [28].

Considering the controversial reports on the association of HPV in breast carcinomas, the aim of this study was to explore HPV presence in this neoplasia among a female Argentinean population. Also to examine HPV genotype, particularly those of high risk and correlate their presence with clinical characteristics. This study demonstrated for the first time in Argentina the presence of HPV in a proportion of the malignant breast tissues, with a prevalence of low risk HPV11, followed by high risk HPV16.
Materials and Methods

Patients

The study was conducted on 61 fresh tissue biopsies of breast carcinoma, collected without any preselection criteria from the pathological archives of the Pathology Service of M. Villegas de Martínez Hospital. Tumors, which were typed according to the American Joint Committee on Cancer, included: 43 invasive ductal carcinomas, 10 invasive lobular, 2 papillary, 2 tubular, 1 adenoid cystic, 1 mucinous, 1 apocrine and 1 comedocarcinoma. Patients’ age ranged from 35 to 92 years (median age 57 years).

This study has the approval of the Institutional Review Board and the Ethics Board of both M. Villegas de Martínez Hospital and Ricardo Gutierrez Children Hospital and is also in accordance with the Helsinki Declaration of 1975. A written informed consent was obtained from every patient.

Polymerase Chain Reaction (PCR) for HPV Detection and Sequencing

DNA was extracted from tumor fresh biopsies using QIAamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany) following manufacturer’s instructions.

The presence of HPV DNA sequences was verified by amplification with two sets of primers. A first round with degenerated primers MY09, 5'-CGTCCMARRGGAWACT-GATC-3' and MY11, 5'-GCMCAAGGWCATAAAATGG -3', amplifying 450 bp long fragment in highly conserved region in L1 gene; and a second round with consensus primers GP5+, 5'-TTTGTACTGTGATAGATAC-3' and GP6+, 5'-CTTATCTAAATGTCGAAATAAAA-3' generate 140 to 150 bp fragment of the L1 region of the virus [29]. Both systems of primers detect a broad spectrum of oncogenic and non-oncogenic HPV types. HPV positive HeLa cell line (ATCC® Number: CCL-2) was used as positive control in all PCR reactions.

In a separate reaction tube, a set of primers for the beta-globin gene, 5'-GAGAGGCGAAG GACAGGTAC-3' and 5'-CAACTTTCATCCACGTTCACCC-3', were incubated with the template DNA. The presence of a 260- base pair (bp) amplification product served as a control to monitor the amplification ability of a single copy gene.

HPV PCR products were separated by electrophoresis in 2% agarose gel stained with ethidium bromide and purified with QIAEXII gel extraction kit (Qiagen GmbH) according to manufacturer instructions. These purified PCR products were directly sequenced using Big Dye Terminator v3.1 kit (Applied Biosystems, Foster City, CA) in an automated Genetic Analyzer 3130×1 (Applied Biosystems). HPV sequences were compared with published ones of known HPV types. At least two independent sequencing reactions were performed with the inner primers to confirm each sequence.

E6 and E7 Analysis by RT-PCR

Total RNA in fresh biopsies was extracted using Trizol according to manufacturer instructions. In order to confirm efficient extraction, the quality of RNA from each sample was assessed by RT-PCR amplification of the ubiquitously expressed phosphoglycerate kinase gene (PGK), which acted as a control to ensure that only RNA was amplified (247 pb fragment), along with the absence of contaminating DNA (additional 600 bp fragment). Good quality RNA samples were chosen and 2 ug were used for cDNA synthesis using Superscript II RT kit (Invitrogen Inc., California, USA) according to the manufacturer’s instructions. Amplification was performed with specific primers (kindly given by Dr Luis Jave Suárez) for E6 HPV16 fwd 5' CAGAGCTTGCAA-

CACTTAC3', rev 5' AGTGGC TTGGACAGTTATA C 3', E7 HPV16 fwd 5' GACAGGAGACGACGGAG 3', rev5' ATTCCTAGTGTGCCCCATTAACA 3', E6 HPV18 fwd 5' GCCACGCTACAG CTAACGCTGA T 3', rev 5' GCAGCG-CAGGCACCTTATA 3', E7 HPV18 fwd 5' TGTCAAGGAG-CATTAAAGGACT 3', rev 5' CACACAAAGGAG-CAGGATTGTTCA 3'.

Results and Discussion

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 4 software (GraphPad Software, Inc., San Diego California USA). Fisher’s exact test or Chi square tests (2) were used for statistical analysis when appropriate.

Table 1. HPV status according to clinical parameters in breast carcinoma cases.

| Breast carcinoma characteristics | HPV status | N | pos | % | p |
|---------------------------------|------------|---|-----|---|---|
| Tumor histology                 |            |   |     |   |   |
| Invasive ductal                  |            | 43 | 12  | 28 | 0.2487 |
| Invasive lobular                 |            | 10 | 1   | 11 |   |
| Papillary                       |            | 2  | 2   | 100|   |
| Tubular                         |            | 2  | 1   | 50 |   |
| Adenocystic                     |            | 1  | 0   | 0  |   |
| Mucinous                        |            | 1  | 0   | 0  |   |
| Apocrine                        |            | 1  | 0   | 0  |   |
| Comedocarcinoma                 |            | 1  | 0   | 0  |   |
| Total                           |            | 61 | 16  | 26 |   |
| Steroid hormone receptors*      |            |   |     |   |   |
| Positive                        |            | 32 | 10  | 31 | 0.6956 |
| Negative                        |            | 10 | 2   | 20 |   |
| Axillary lymph node status†      |            |   |     |   |   |
| Positive                        |            | 14 | 4   | 29 | 1.0000 |
| Negative                        |            | 22 | 6   | 27 |   |

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Mexico [26], described no evidence of HPV association with breast carcinoma. The findings reported by our study also support the notion for a viral association with breast carcinoma. In fact, in Argentina our group has previously also described EBV presence in 31% breast carcinoma patients, with a particular latency profile, not already observed in EBV-associated tumors [30]. Interestingly, 1/61 patients of this analyzed series harboured both HPV and EBV, pointing out that both viruses might contribute to the neoplastic process.

In this report, clinical parameters such as tumor histology, axillary lymph node status or estrogen and progesterone receptors were not statistically associated with HPV presence (p>0.05 χ² test), in accordance with previous reports [7,11,14,15]. However, Kroupis et al described the only breast cancer series harbouring high-risk HPV DNA sequences related to clinical parameters; in which those HPV+ cases displayed less estrogen-receptor and were proliferative [31]. Even though in the present study HPV presence was not statistically significant among histological tumor types, it is noteworthy that both papillary breast carcinoma cases, a rare histologic type, were HPV positive.

At present, about 130 HPV types were identified by their sequence of the gene encoding the major capsid protein L1 isolated from HPV associated diseases. Moreover, they can be also classified into high- and low-risk types depending upon their oncogenic potential. This is shown most clearly in the genital tract, in which there is regular or sporadic infection with about 30–40 types. These can be divided into those predominately associated with benign anogenital warts or condylomata, low-risk HPV types 6, 11 and their relatives, and those associated with anogenital cancers (intraepithelial neoplasia), particularly of the cervix, HPV 16, 18, 31, 33, 35, 45, and minor types. The most important players of cervical cancers are HPV 16, found in 50–70% of cases, and HPV 18, found in 7–20% of cases [32]. When HPV typing was performed by sequence analysis in a subgroup of 9 cases of our series, 4 cases were characterized as HPV 11, 3 as HPV 16 and 1 as HPV13. Notably, 1 case showed a co-infection with HPV 11 together with HPV 87. The presence of HPV11, a low risk type, was very remarkable since mostly high risk HPV 16 and 18 types were reported in breast carcinoma [17,21,22]. In Iran, high-risk HPV genotypes in breast cancer patients were the predominant types, but other genotypes (HPV-6, HPV-11, HPV-15, HPV-23, and HPV-124) were also detected [16]. Only de Villiers et al, in a German series, demonstrated that the most prevalent type in both breast carcinomas and nipples from the same patient was HPV 11, followed by HPV 6 [3]. In Argentina, the prevalence of HPV16 in high grade intraepithelial cervical lesions samples is 48.5% and 16.9% for HPV18; instead, invasive cervical cancer shows a prevalence of 59.5% for HPV16 and 17.6% for HPV18 [33].

HPV types in Latin American breast carcinoma patients, displayed prevalence for high risk HPV. In fact, in Chile only the HPV-16 genotype was present in positive breast carcinomas without co-infections with other HPV genotypes [14], while in Mexico, 66.6% were positive for HPV 16, 20% for HPV 18, and 13.4% were positive for both. High risk HPV types also prevail in breast carcinoma from other population. Damin et al [15] reported 56% HPV 16 and 40% HPV 18. In line with this, Li et al [12], in a meta-analysis, found that the four most commonly identified HPV types, in order of decreased prevalence, were HPV33, 18, 16, and 53. In Japan, the most frequently detected HPV genotype was HPV 16 (92%), followed by HPV 6 (46%), HPV 18 (12%), and HPV 33 (4%) [7]. Kroupis et al [31] identified a total of 21 high-risk viruses: 67% HPV 16, 14% HPV 39, 10% HPV 50, 5% HPV 73 and 5% HPV 82.

HPV analysis performed so far in this report enables exclusively evaluation of the presence of viral DNA, but do not inform on the possible viral activity or infection productivity. Therefore, evaluation of E6 and E7 mRNA in high risk genotypes may represent a good marker for HPV involvement in malignant transformation. In fact, a strong association between high risk mRNA-HPV presence and risk of neoplastic progression in cervical lesions has been described [34,35]. In consequence, E6 and E7 mRNA-HPV presence in high risk phenotypes was suggested as a tool for early identification of cervical lesions that would evolve toward neoplastic transformation. Furthermore, HPV transcriptional activity was also found in most oropharyngeal squamous cell carcinoma [36]. E6 and E7 mRNA from high risk HPV16 and HPV18 evaluated in 9 cases with quality RNA from our series by RT-PCR were negative, demonstrating no transcriptional activity of high risk HPV types, as previously observed in Italy [37].

Although the route of transmission for the virus has not been determined, women positive for both breast and cervical cancers were found to be infected with the same HPV type in both tumors [37]. Furthermore, women with squamous precancer and women with glandular precancer in the cervix had a significantly higher risk of malignant breast tumors than the general female population from Norway [18]. Unfortunately, in our series HPV data in cervix tissue were not available to give rise to this assumption.

Conclusions

The present study demonstrated for the first time in Argentina the presence of HPV in a significant proportion of the malignant breast tissues analyzed. Presence of low risk types, together with the absence of transcriptional activity for high risk types suggest that HPV presence may have a biological significance in breast carcinogenesis as a contributing factor to tumor development, but not as the tumor driven force. It should be pointed out that these observations need additional studies to be substantiated, especially by monitoring future breast cancer incidence amongst women vaccinated against high risk HPV type. Meanwhile, the present results may represent an important issue in virus-associated cancer, which represent potential tools for the development of specific therapies leading for patients with breast cancer.

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Author Contributions

Conceived and designed the experiments: ALPS MVP PC. Performed the experiments: MAL RGL MC. Analyzed the data: HG PMV PG. Contributed reagents/materials/analysis tools: HG. Wrote the paper: ALPS PC.

Secretaria de Salud. Available: http://www.dgepi.salud.gob.mx/boletin/2008/sem40/pdf/edit4008.pdf. Accessed 2013 March 26.

References

1. Amarante MK, Watanabe MA (2009) The possible involvement of virus in breast cancer. J Cancer Res Clin Oncol 135: 329–337.
2. Sistema Único de Información para la Vigilancia Epidemiológica de la Dirección General de Epidemiología, Anuarios de Morbilidad 2000–2006.
3. de Villers EM, Sandstrom RE, zur Hausen H, Bux CE (2005) Presence of papillomavirus sequences in condylomatosus lesions of the mammary and in invasive carcinoma of the breast. Cancer Res 7: 1–11.

4. Viniegra Marta (2010) Cáncer de mama en Argentina: organización, cobertura y cálculo de las acciones de prevención y control. Informe final julio 2010: diagnóstico de situación del Programa Nacional y Programas Provinciales. Viniegra M; Paulino M; Arrossi S-1a ed. Buenos Aires: Organización Panamericana de la Salud – OPS, p 141.

5. Zúñi Zur H (2009) The search for infectious causes of human cancers: where and why. Virology 392: 1–10.

6. Parkin DM (2006) The global health burden of infection-associated cancers in the year 2002. Int J Cancer 118: 3030–3041.

7. Khan NA, Castillo A, Koriyama C, Kijima Y, Umekita Y, et al (2008) Human papillomavirus DNA in breast cancer tissues in female breast carcinomas in Japan. Br J Cancer 99: 408–414.

8. Kan CY, Iacopetta BJ, Lawson JS, Whitaker NJ (2005) Identification of human papillomavirus DNA gene sequences in human breast cancer. Br J Cancer 93: 946–948.

9. Heng B, Glenn WK, Ye Y, Tran B, Delprado W, et al (2009) Human papilloma virus is associated with breast cancer. Br J Cancer 101: 1345–1350.

10. Mendizábal Ruiz AF, Morales JA, Ramírez-Jimeno LJ, Pardilla-Rosales M, Morón-Muñoz MC, et al (2009) Low frequency of human papillomavirus DNA in breast cancer tissue. Breast Cancer Res Treat 114: 189–194.

11. de León DC, Monteil DP, Nencova J, Mykytova I, Turcios E, et al (2009) Human papillomavirus (HPV) in breast tumors: prevalence in a group of Mexican patients. BMC Cancer 9: 26.

12. Li N, Bi X, Zhang Y, Zhao P, Zheng T, et al (2011) Human papillomavirus DNA gene sequences in human breast cancer. Br J Cancer 93: 1541–1546.

13. Abe J, Kan Y, Hasegawa Y, Kato S, Kato Y, et al (2009) Kshv genes indicate a role for human papilloma virus in breast cancer. Br J Cancer 101: 1351–1356.

14. Aguayo F, Kuhn N, Koriyama C, Gonzalez C, Ampuero S, et al (2011) Human papillomavirus and Epstein Barr virus infections in breast cancer from Chile. Breast Cancer Res Treat 126: 515–520.

15. Damin AP, Karaman R, Zeitzler CG, Galleli M, Alexandre CO (2004) Evidence for an association of human papillomavirus and breast carcinomas. Breast Cancer Res Treat 84: 151–157.

16. Sugarová A, Nadílka NA, Naghdvár F, Nategh R, Emami H, et al (2012) Human papillomavirus is associated with breast cancer in the north part of Iran. ScientificWorldJournal 2012: 837191.

17. Glenn WK, Heng B, Delprado W, Iacopetta B, Whitaker NJ, et al (2012) Epstein-Barr virus, human papillomavirus and mouse mammary tumour virus as multiple viruses in breast cancer. PLoS One 7: e48788.

18. Hansen BT, Nygård M, Falk RS, Hønvind S (2012) Breast cancer and ducal carcinoma in situ among women with prior squamous or glandular precancer in the cervix: a register-based study. Br J Cancer 107: 1451–3.

19. Freiga A, Lorenzoni L, Bononi M, De Cesare A, Ciardi A, et al (2012) Evaluation of E6 and E7 mRNA expression in HPV DNA positive breast cancer. Eur J Gynaecol Oncol 33: 164–7.

20. Smořa PW, Medeiros LR, Smořa Pires PD, Edelweiss MI, Rosa DD, et al (2012) Prevalence of human papillomavirus in breast cancer: a systematic review. Int J Gynecol Cancer 22: 343–7.

21. Mou X, Chen L, Liu F, Shen Y, Wang H, et al (2011) Low prevalence of human papillomavirus (HPV) in Chinese patients with breast cancer. J Int Med Res 39: 1636–44.

22. Antonsson A, Spurr TP, Chen AC, Francis GD, McMullan NA, et al (2011) High prevalence of human papillomavirus in fresh frozen breast cancer samples. J Med Virol 83: 2157–63.

23. Hachana M, Ziadl S, Amara K, Touni I, Korbi S, et al (2010) No evidence of human papillomavirus DNA in breast carcinoma in Tunisian patients. Breast 19: 541–544.

24. Hedlau S, Kumar U, Hussain S, Shukla S, Pande S, et al (2011) Breast cancer and human papillomavirus infection: no evidence of HPV etiology of breast cancer in Indian women. BMC Cancer 11: 27.

25. Silva RG Jr, da Silva BB (2011) No evidence for an association of human papillomavirus and breast carcinoma. Breast Cancer Res Treat 125: 261–264.

26. Herrera-Gerpert R, Khan NA, Koriyama C, Aikča S, Pérez-Sánchez VM (2011) High-risk human papillomavirus in mammary gland carcinomas and non-neoplastic tissues of Mexican women: no evidence supporting a cause and effect relationship. Breast 20: 104–109.

27. Wazer DE, Liu XL, Chu Q, Gao Q, Band V (1995) Immortalization of distinct human mammary epithelial cell types by human papilloma virus 16 E6 or E7. Proc Natl Acad Sci U S A 92: 3687–3691.

28. Bondy J, Laimins LA (2011) Persistence of human papillomavirus infection: keys to malignant progression. Trends Microbiol 19: 33–39.

29. Sozial K, Demer D, Detlefsen A, Hak Y, Stahmer A, et al (2004) Detection and typing of human papillomavirus by E6 nested multiplex PCR. J Clin Microbiol 42: 3176–3184.

30. Lorenzetti MA, De Matteo E, Gasićć H, Martínez-Vázquez P, Lara J, et al (2010) Characterization of Epstein-Barr virus latency pattern in Argentine breast carcinoma. PLoS One 5: e13603.

31. Kroupa C, Markou A, Pourlinś N, Dionysiss-Astieriu A, Lainidou ES (2006) Presence of high-risk human papillomavirus sequences in breast cancer tissues and association with histopathological characteristics. Clin Biochem 39: 727–731.

32. Stanley M (2010) Pathology and epidemiology of HPV infection in females. Gynecol Oncol (Suppl 2): 5–10.

33. Giapponi A, Bardach A, Ghigojly D, Gibbons L, Picconi MA (2011) Type-Specific HPV Prevalence in Cervical Cancer and High-Grade Lesions in Latin America and the Caribbean: Systematic Review and Meta-Analysis. PLoS One 6: e25493.

34. Tinelli A, Leo G, Pisanò M, Storelli F, Leo S, et al (2009) HPV viral activity by mRNA-HPV molecular analysis to screen the transforming infections in precancer cervical lesions. Curr Pharm Biotechnol 10: 767–771.

35. Broccolo F, Fusetti L, Rosiini S, Caraceni D, Zappacosta R, et al (2013) Comparison of oncogenic HPV type-specific viral DNA load and E6/E7 mRNA detection in cervical samples: Results from a multicenter study. J Med Virol 85: 472–482.

36. Gao G, Chenneck RD, Gay HA, Torstad WL, Zhang TR, et al (2013) A novel RT-PCR method for quantification of human papillomavirus transcripts in archived tissues and its application in oropharyngeal cancer prognosis. Int J Cancer 132: 802–809.

37. Freiga A, Lorenzoni L, Bononi M, De Cesare A, Ciardi A, et al (2012) Evaluation of E6 and E7 mRNA expression in HPV DNA positive breast cancer. Eur J Gynaecol Oncol 33: 164–167.