



Short communication

Detection of Merkel cell polyomavirus and human papillomavirus DNA in porocarcinoma



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ABSTRACT

Background: Increasing evidences support the role of Merkel cell polyomavirus (MCPyV) and human papillomavirus (HPV) in non-cutaneous and cutaneous tumours. Porocarcinoma is a rare malignant neoplasm that arises from the intraepidermal ductal portion of the eccrine sweat glands. The aetiology of porocarcinoma is largely unknown and no systematic studies have been done to investigate the implication of infectious agents in the pathogenesis of this tumour.

Objectives: To investigate the possible association between MCPyV and/or HPV infection and porocarcinoma.

Study design: Forty-four formalin-fixed paraffin-embedded (FFPE) porocarcinomas (40 primary and 4 metastatic) and 10 healthy skin specimens (controls), were analysed for the presence of MCPyV and HPV DNA using molecular detection methods.

Results: MCPyV DNA was found in 27/40 (68%) primary porocarcinomas and in 3/10 (30%) controls (Fisher exact test: $p < 0.04$). No significant difference in viral load was observed between tumours and healthy skin. Moreover, 2/40 primary porocarcinomas tested positive for high-risk HPV16. Cutaneous beta-HPV infection was detected in 16/40 (40%) porocarcinomas and in 6/10 (60%) controls. No particular beta-HPV types were significantly associated with tumour or with healthy skin. Two out of 4 metastatic biopsies were MCPyV DNA positive. All metastatic samples had mixed infections with cutaneous HPV types.

Conclusions: This study demonstrated a significantly high prevalence of MCPyV and the presence of a broad spectrum of HPV types in porocarcinoma and provided the first available data about viral infections in this tumour. To understand the role, if any, of viral infections in the pathogenesis of porocarcinoma further studies are needed.

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1. Background

Porocarcinoma is a malignant epithelial tumour originating from or differentiating toward the intraepidermal eccrine duct (i.e. acrosyringium). Although considered the most frequent sweat gland carcinoma, the tumour is relatively rare, representing 0.005–0.01% of epithelial cutaneous neoplasms [1]. However, it is possible that the real incidence is underestimated, because of the difficult clinicopathologic distinction from the more acquainted squamous cell carcinoma [2,3]. About 20% of porocarcinomas can recur and metastasize to regional lymph nodes and rarely to dis-

tant organs; mortality rate has been estimated as high as 67% of patients with nodal metastases [4]. Although several cases of porocarcinoma have been associated with radiotherapy, the aetiology of this tumour is largely unknown.

Several studies have demonstrated that some small non-enveloped DNA viruses, such as MCPyV and HPV, have a role in the development of non-cutaneous and cutaneous tumours [5–11]. MCPyV was identified in 2008 as a new human polyomavirus associated with Merkel cell carcinoma (MCC) [5] and represents the likely causative agent of MCC [5,6]. Some alpha-HPV types, namely high-risk HPV (HR-HPV), are associated with cervical cancer, other anogenital carcinomas and with a subset of head and neck tumours [7]. Persistent infections of the skin with HR-HPV types may also represent a risk factor for non-melanoma skin cancers (NMSCs) [8].

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Epidemiological studies have shown the association of cutaneous beta-HPV infections and NMSCs in subjects with *epidermodysplasia verruciformis* [9] and in organ transplant recipients [10]. Moreover, there is accumulating evidence that beta-HPV may play a role in the pathogenesis of cutaneous cancer, in the general, immunocompetent population [11]. Finally, the presence of HPV20, 21 and 23 have been described in multiple eccrine poromas [12].

2. Objectives

To investigate the possible association between MCPyV and/or HPV infections and porocarcinoma we analysed the prevalence of MCPyV and HPV DNA in 44 porocarcinomas (40 primary and 4 metastatic) and 10 healthy skin (controls), using molecular detection methods.

3. Study design

Cases were selected using the following criteria [1,3]: 1) solid-cystic architecture; 2) invasive pattern and significant cytologic pleomorphism; 3) polyhedral cells with eosinophilic and/or clear cytoplasm; 4) duct formation and/or intracytoplasmic lumina; 5) necrosis and/or comedonecrosis.

In this study, 40 patients with primary porocarcinoma (27 males, 13 females; median age: 84 years; range 53–103 years) were included. The tumours were located in the head-neck region (28 cases), on the trunk (5 cases), on the lower extremities (3 cases) and on the hand (4 cases). The patients with metastatic porocarcinoma were 2 males and 2 females (median age: 80 years; range: 70–86 years); 2 patients had lymph node and 2 intraparotid metastases.

Ten healthy skin specimens were taken from 10 subjects (5 males, 5 females; median age: 79.3 years; range: 71–86 years). Seven of control samples were taken from the lower limbs and 3 from the upper limbs.

DNA was purified using QIAamp DNA FFPE Tissue kit (Qiagen). MCPyV detection and quantification were done by Droplet Digital PCR (ddPCR), Bio-Rad's QX200 ddPCR System, using TaqMan probe labelled with FAM. To normalize target amount, ddPCR assays for two housekeeping genes (AP3B1 and RPP30), using HEX labelled probes, were performed. The analytical sensitivity of the reaction, determined on 10-fold dilutions of cloned target, was equal to 1 copy per assay.

HPV detection and genotyping were done using line probe assays, based on the PCR amplification and reverse hybridization principle: (1) the RHA Kit Skin (beta) HPV (Diassay, Rijswijk, The Netherlands), which is able to identify 25 most common beta-HPV types, and (2) Ampliquality HPV-type Express v3.0 kit (AB Analytica, Italy) which is able to detect 40 mucosal alpha-HPVs. Fisher's exact test was used to compare the prevalence in sample subsets. A $p < 0.05$ was considered to be statistically significant.

4. Results

MCPyV DNA was detected in 27/40 (68%) primary porocarcinomas compared with 3/10 (30%) controls. This difference was statistically significant ($p < 0.04$). The average viral load was 2000 copies/ 10^5 cells (range: 30–21,200) in tumours and 700 copies/ 10^5 cells (range: 30–1,400) in controls.

The beta-HPV infection was detected in 16/40 (40%) primary porocarcinomas and in 6/10 (60%) controls (Table 1).

Two out of 40 primary porocarcinomas tested positive for alpha-HR-HPV16, and 1 harboured LR-HPV40. Two out of 10 healthy skin samples were infected with LR-HPV61.

There was no significant correlation between MCPyV or HPV positivity and sex, age, and anatomic localization of tumour.

Table 1
MCPyV and HPV DNA detection in primary porocarcinoma and control specimens.

| DNA detection | Porocarcinomas n = 40 (%) | Controls n = 10 (%) |
|-------------------------------|---------------------------|---------------------|
| MCPyV | 27 (68) | 3 (30) |
| All alpha-HPV | 3 (8) | 2 (20) |
| HPV16 | 2 | 0 |
| HPV40 | 1 | 0 |
| HPV61 | 0 | 2 |
| All beta-HPV | 16 (40) | 6 (60) |
| All beta-1HPV | 6 (15) | 2 (20) |
| HPV5 | 0 | 1 |
| HPV8 | 1 | 0 |
| HPV14 | 2 | 0 |
| HPV21 | 1 | 0 |
| HPV24 | 4 | 1 |
| HPV36 | 3 | 0 |
| All beta-2HPV | 7 (18) | 2 (20) |
| HPV9 | 3 | 2 |
| HPV15 | 1 | 0 |
| HPV17 | 1 | 0 |
| HPV23 | 1 | 0 |
| HPV37 | 1 | 0 |
| HPV38 | 1 | 0 |
| HPV80 | 1 | 0 |
| All beta-3HPV | 1 (3) | 0 |
| HPV75 | 1 | 0 |
| All beta-4HPV | 2 (5) | 0 |
| HPV92 | 2 | 0 |
| Undetermined HPV ^a | 6 | 3 |

^a Specimens which hybridize only with universal probe in RHA beta-HPV assay.

Two out of 4 metastatic samples (one from inguinal node and one from parotid) were MCPyV DNA positive. All metastatic samples had mixed infections with cutaneous HPV types and all were negative for mucosal HPV DNA. The beta-HPV types detected were HPV8, 12, 20, 24 and 93 (species 1) and HPV9, 23 and 37 (species 2). The viral load did not differ from that of primary tumours.

5. Discussion

The data published thus far indicate that MCPyV plays a role in MCC but not in other NMSCs, such as basal cell carcinoma (BCC) or squamous cell carcinoma (SCC). In fact, the prevalence of infection in MCC is very high (~80%) [13] and the viral load frequently reaches 1 viral copy per tumour cell [14]. On the other hand, both the prevalence of infection and the viral load found in other types of NMSCs are similar to those detected in healthy skin. In fact, the frequency of MCPyV DNA detection, reported by different authors, ranges from 0 to 38% [15–17] and the viral DNA copy number is lower than 1 per cell [18].

In the present study, the prevalence of MCPyV DNA in porocarcinomas, was significantly higher than that in healthy skin but the viral load was low and no significant difference was observed in MCPyV DNA levels between tumours and controls.

The prevalence of mucosal HPV DNA was very low. Interestingly, two porocarcinomas harboured HR-HPV16, which is a type most frequently found in HPV associated mucosal tumours [7] and has been also detected in cutaneous SCC [19].

The prevalence of beta-HPV was equally high in both kinds of samples and no particular beta-HPV types were significantly associated with tumours or with healthy skin. There was no significant difference in the frequency of mixed infections between tumours and controls. These results are in line with some previous studies, which frequently detected beta-HPV both in NMSCs and in healthy skin [20].

Both MCPyV and beta-HPV were frequently found in metastatic porocarcinomas. However, since it was not possible to analyse the primary tumours obtained from the same patients we could not verify the hypothesis of involvement, if any, of viral infections in the neoplastic progression [21].

To our knowledge, this is the first investigation aiming to determine the prevalence of viral infections in porocarcinoma. Our findings demonstrate a significantly high prevalence of MCPyV and the presence of a broad spectrum of HPV types in this tumour. However, the high prevalence of viral DNA does not definitively establish a direct causal role.

Several viral markers leading to MCPyV-associated MCC have been proposed: clonal integration of viral DNA into the cellular genome [5], LT-antigen expression in tumours, and the presence of tumour-associated mutations in the gene coding for LT-antigen [6].

In the HR-HPV associated tumours, the integration of viral DNA into the cell genome, expression of oncoproteins, and inactivation of tumour suppressor proteins has been demonstrated as the molecular mechanism underlying oncogenesis of these tumours. The mechanisms of beta-HPV oncogenesis have yet to be established. One possible hypothesis is that beta-HPV may impair host cell defences against excessive sun light exposure, thus interfering with DNA repair and apoptosis [22,23].

To understand the role, if any, of viral infection in porocarcinoma, further studies need to be performed to determine biological characteristics and activity of the virus present in tumour samples.

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

Ethical approval for the study was obtained from the Ethic Committee of Area Vasta Centro (Azienda Ospedaliero Universitaria Careggi, Florence, Italy), nr. 2015/00037100.

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References

- [1] A. Robson, J. Greene, N. Ansari, B. Kim, P.T. Seed, P.H. McKee, et al., Eccrine porocarcinoma (malignant eccrine poroma): a clinicopathologic study of 69 cases, *Am. J. Surg. Pathol.* 25 (2001) 710–720.
- [2] M. Mahalingam, J.E. Richards, M.-A. Selim, A. Muzikansky, M.P. Hoang, An immunohistochemical comparison of cytokeratin 7 cytokeratin 15, cytokeratin 19, CAM 5.2, carcinoembryonic antigen, and nestin in differentiating porocarcinoma from squamous cell carcinoma, *Hum. Pathol.* 43 (2012) 1265–1272.
- [3] C. Urso, Porocarcinoma: an exceedingly rare tumor or a tumor eclipse phenomenon? *Hum. Pathol.* 44 (2013) 448–449.
- [4] U. Marone, C. Caracò, A.M. Anniciello, G. Di Monta, M.G. Chiofalo, M.L. Di Cecilia, et al., Metastatic eccrine porocarcinoma: report of a case and review of the literature, *World J. Surg. Oncol.* 9 (2011) 32.
- [5] H. Feng, M. Shuda, Y. Chang, P.S. Moore, Clonal integration of a polyomavirus in human Merkel cell carcinoma, *Science* 319 (2008) 1096–1100.
- [6] M. Shuda, H. Feng, H.J. Kwun, S.T. Rosen, O. Gjoerup, P.S. Moore, T antigen mutations are a human tumor-specific signature for Merkel cell polyomavirus, *PNAS* 105 (2008) 16272–16277.
- [7] H. ZurHausen, Papillomaviruses in the causation of human cancers—a brief historical account, *Virology* 384 (2009) 260–265.
- [8] A. Iftner, S.J. Klug, C. Garbe, A. Blum, A. Stancu, S.P. Wilczynski, et al., The Prevalence of human papillomavirus genotypes in non-melanoma skin cancers of non-immunosuppressed individuals identifies High-Risk genital types as possible risk factors, *Cancer Res.* 63 (2003) 7515–7519.
- [9] S. Majewski, S. Jablonska, Do epidermodysplasia verruciformis human papillomaviruses contribute to malignant and benign epidermal proliferations? *Arch. Dermatol.* 138 (2002) 649–654.
- [10] E.M. de Villiers, D. Laverne, K. McLaren, E.C. Benton, Prevailing papillomavirus types in non-melanoma carcinomas of the skin in renal allograft recipients, *Int. J. Cancer* 73 (1997) 356–361.
- [11] A.S. Patel, M.R. Karagas, A.E. Perry, H.H. Nelson, Exposure profiles and human papillomavirus infection in skin cancer: an analysis of 25 genus β -types in a population-based study, *J. Invest. Dermatol.* 128 (2008) 2888–2893.
- [12] M.L. Diamantis, H.M. Richmond, P.L. Rady, S.K. Tying, J.E. Cutlan, C. Torres-Cabala, et al., Detection of human papillomavirus in multiple eccrine poromas in a patient with chronic graft-vs-host disease and immunosuppression, *Arch. Dermatol.* 147 (2011) 120–122.
- [13] V. Foulongene, O. Dereure, N. Kluger, J.P. Moles, B. Guillot, M. Segondy, Merkel cell polyomavirus DNA detection in lesional and non lesional skin from patients with Merkel cell carcinoma or other skin diseases, *Br. J. Dermatol.* 162 (2010) 59–63.
- [14] H.C. Laude, B. Jonchere, E. Maubec, A. Carlotti, E. Marinho, B. Couturaud, et al., Distinct Merkel cell polyomavirus molecular features in tumour and non tumour specimens from patients with Merkel cell carcinoma, *PLoS Pathog.* 6 (8) (2010) e1001076.
- [15] X. Sastre-Garau, M. Peter, M.F. Avril, H. Laude, J. Couturier, F. Rozenberg, et al., Merkel cell carcinoma of the skin: pathological and molecular evidence for a causative role of MCV in oncogenesis, *J. Pathol.* 218 (2009) 48–56.
- [16] A. Kassem, K. Technau, A.K. Kurz, D. Pantulu, M. Loning, G. Kayser, et al., Merkel cell polyomavirus sequences are frequently detected in non-melanoma skin cancer of immunosuppressed patients, *Int. J. Cancer* 125 (2009) 356–361.
- [17] D.E. Rollison, A.R. Giuliano, J.L. Messina, N.A. Fenske, B.S. Cherpelis, V.K. Sondak, Case-control study of merkel cell polyomavirus infection and cutaneous squamous cell carcinoma, *Cancer Epidemiol. Biomark. Prev.* 21 (1) (2012) 74–81.
- [18] M. Imajoh, Y. Hashida, H. Nakajima, S. Sano, M. Daibata, Prevalence and viral DNA loads of three novel human polyomaviruses in skin cancers from Japanese patients, *J. Dermatol.* 40 (8) (2013) 657–660.
- [19] M. Reuschenbach, T. Tran, F. Faulstich, W. Hartschuh, S. Vinokurova, M. Kloor, et al., High-risk human papillomavirus in non-melanoma skin lesions from renal allograft recipients and immunocompetent patients, *Br. J. Cancer* 104 (2011) 1334–1341.
- [20] M.M. Asgari, N.B. Kiviat, C.W. Critchlow, J.E. Stern, Z.B. Argenyi, G.J. Raugi, et al., Detection of human papillomavirus DNA in cutaneous squamous cell carcinoma among immunocompetent individuals, *J. Invest. Dermatol.* 128 (2008) 1409–1417.
- [21] A. Toll, B. Lloveras, E. Masferrer, C. Ferrandiz-Pulido, V. Garcia-Patos, T. Gheit, et al., Human beta papillomavirus DNA study in primary cutaneous squamous cell carcinomas and their corresponding metastases, *Arch. Dermatol. Res.* 306 (2014) 93–95.
- [22] S. Jackson, L. Ghali, C. Harwood, A. Storey, Reduced apoptotic levels in squamous but not basal cell carcinomas correlates with detection of cutaneous human papillomavirus, *Br. J. Cancer* 87 (2002) 319–323.
- [23] L. Struijk, E. van der Meijden, S. Kazem, J. terSchegget, F.R. de Gruij, D.M. Steenbergen, M.C.W. Feltkamp, Specific beta papillomaviruses associated with squamous cell carcinoma of the skin inhibit UVB-induced apoptosis of primary human keratinocytes, *J. Gen. Virol.* 89 (2008) 2303–2314.