ISSN 0025-7680

BRIEF COMMUNICATION MEDICINA (Buenos Aires) 2010; 70: 159-162

DETECTION OF POLYOMAVIRUS MAJOR CAPSID ANTIGEN (VP-1) IN HUMAN PILOMATRICOMAS

NORBERTO A. SANJUAN[1], SILVINA SIMULA[1], JOSE CASAS[2], ALBERTO WOSCOFF[3]

[1]*Laboratorio de Patología Experimental, Departamento de Microbiología y* [2]*Departamento de Patología, Facultad de Medicina, Universidad de Buenos Aires;* [3]*Departamento de Dermatología, Hospital Naval, Buenos Aires*

Abstract The family *Polyomaviridae* is composed of small, non-enveloped, double-stranded DNA viruses widely used to study cell transformation *in vitro* and tumor induction *in vivo*. The development of pilomatricomas in mice experimentally infected with polyomavirus led us to detect the viral major capsid protein VP-1 in human pilomatricomas. This tumor, even uncommon, is one of the most frequent benign hair follicle tumors in humans and is composed of proliferating matrix cells that undergo keratinization, and form cystic neoplasms. The detection of VP-1 was performed using the peroxidase-antiperoxidase technique in paraffin-embedded slides with a specific primary serum. Adjacent slides treated with normal rabbit serum as a primary were employed as internal control. Positive and negative controls were also employed as well as slides of lesions caused by human papillomavirus to rule out any unspecific cross-reactivity. In 4 out of 10 cases polyomavirus VP-1 was clearly detected in nuclei of human pilomatricomas proliferating cells, in a patchy pattern of distribution. The controls confirmed the specificity of the immunocytochemical procedure. These results could indicate either an eventual infection of the virus in already developed tumors or alternatively, a direct involvement of polyomavirus in the pathogenesis of some pilomatricomas. The recent discovery of a new human polyomavirus associated with Merkel cell carcinomas has been a strong contribution to better understand the pathogenesis of some human uncommon skin cancers. Hopefully the results reported in this work will encourage further research on the role of polyomavirus in other human skin neoplasms.

Key words: polyoma, pilomatricoma, hair-follicle tumor, human

Resumen ***Detección del antígeno mayor de la cápside de poliomavirus (VP-1) en pilomatricomas humanos***. La familia *Poliomaviridae* está compuesta por virus oncogénicos pequeños, no envueltos, con ADN de doble cadena. En un modelo experimental murino pudimos desarrollar pilomatricomas inducidos por la inoculación de virus polioma. Eso nos llevó a estudiar la posibilidad de que otro virus polioma estuviera involucrado en la génesis de pilomatricomas humanos. Los pilomatricomas son neoplasias benignas originadas en las células de la matriz del folículo piloso, que van madurando hacia la queratinización y terminan formando tumores quísticos. Se empleó la técnica de peroxidasa-antiperoxidasa para detectar VP-1 de polioma en cortes parafinados, usando un suero primario específico contra esa proteína. Como controles internos se trataron cortes adyacentes con suero normal de conejo como primario. Se emplearon, además, controles positivos y negativos y cortes de lesiones producidas por el virus papiloma humano. En 4 de los 10 casos estudiados se observó la presencia de VP-1 en los núcleos de células tumorales distribuidas en focos dispersos. Los controles empleados permitieron descartar cualquier inespecificidad de la reacción inmunocitoquímica. Estos resultados podrían indicar una eventual replicación del virus en tumores en desarrollo o, alternativamente, podrían significar un compromiso directo de poliomavirus en la etiopatogenia de algunos pilomatricomas. Recientemente se ha descubierto un nuevo virus polioma humano asociado al carcinoma de células de Merkel. Quizás los resultados descriptos en este trabajo permitan profundizar en el estudio de la asociación entre poliomavirus y neoplasias cutáneas humanas.

Palabras clave: polioma, pilomatricoma, folículo piloso, humano

The family *Polyomaviridae* is composed of small, non-enveloped, double-stranded DNA viruses widely used to study cell transformation *in vitro* and tumor induction *in vivo*[1]. Different polyomaviruses have been isolated from humans, and mice, hamsters and other animals[2]. Their ability to produce lytic infection is highly restricted to cells of their natural hosts, without replicating in cells of other animals. However, they can induce *in vitro* transformation in semi-permissive cells belonging to close-related species[3]. These experimental models have been useful to better understand several pathways involved in the interaction of viral antigens with key cellular regulatory factors such as telomerase[4], Src[5], Shc[6], PIP-3-kinase[7],

Received: 11-XI-2009 Accepted: 17-I-2010

Postal address: Dr. Norberto Sanjuán, Departamento de Microbiología, Facultad de Medicina, Universidad de Buenos Aires, Paraguay 2155, 1121 Buenos Aires, Argentina.

Fax: (54-11) 4508-3705 e-mail: patoexpe@fmed.uba.ar

14-3-3 proteins[8] and others, which finally lead to cell transformation. The murine polyomavirus genome encodes 3 "early proteins" (LT, mT and sT) and 3 "late" structural proteins: VP-1, VP-2 and VP-3. VP-1 is the most abundant viral capsid protein. It is accepted that mT and LT are the viral oncogenes responsible for cell transformation *in vitro* and tumor induction in mice. Polyomavirus isolates from several natural sources show a similar genome organization and VP-1 shares extensive amino-acid sequences among polyomaviruses found in different animal species, including humans[3]. Five polyomaviruses have been described in humans: BK, JC, KI, WU and MC. It is accepted that BK and JC polyomaviruses are ubiquitous, produce a life-time persistent infection and only reactivate after immunosupression. Under this condition BK has been associated to haemorrhagic cystitis and nephropathy while JC could be isolated from patients with progressive multifocal leukencephalopathy[2]. Polyomavirus KI and WU could be found in respiratory secretions and MC has been recently associated with Merkel tumors of the skin[2].

After inoculation into newborn C3H BiDa mice, the murine polyomavirus induces neoplasms arising from as many as 14 different cell types after a few months post-infection (pi). Mammary gland adenocarcinomas, thymomas, kidney sarcomas, parotid tumors and hair follicle tumors are developed as a consequence of polyomavirus infection in this mouse strain[9]. In our laboratory, the development of these polyomavirus-induced tumors has been studied in mice using the experimental model described by Benjamin and Dawe[9]. One of them was usually described as a "hair follicle tumor" and more recently, it has been referred to as "cystic trichoepithelioma"[10]. After a careful histological and histochemical study, we concluded that hair follicle tumors are, in fact, pilomatricomas (also called "pilomatrixomas"). Pilomatricomas are benign neoplasms originated in the matrix cells of the hair follicle. As these neoplasms grow up, they evolve to keratinized cells in a maturation pattern that ends in the presence of cells without a detectable nucleus ("ghost cells") and, furthermore, in a final transformation into amorphous keratin[11].

Considering that pilomatricomas are one of the most common benign skin tumors arising from hair follicles in humans (though they are not very frequent), we decided to explore if in these human tumors polyomavirus VP-1 could be detected by immunocytochemical methods. Ten paraffin-embedded blocks of human pilomatricomas obtained from archives were used, and several 5µm-thick serial slides from each paraffin-embedded tumor were prepared. In order to detect polyomavirus VP-1 in those samples, the classical peroxidase-antiperoxidase immunolabeling method (PAP) was performed using polyclonal rabbit anti-murine polyomavirus VP-1 as a primary serum, considering that VP-1 is the major and most abundant structural capsid protein in polyomavirus particles. After treating the slides with xylene, they were hydrated using decreased concentrations of ethanol and then soaked in 0.05 M HCl-Tris, pH 7.6. Endogenous peroxydase was blocked with 4% H_2O_2 diluted in methanol for 20 minutes. The primary serum was incubated overnight at 4 °C using a 1:1000 dilution in 0.05 M HCl-Tris buffer. The second and third sera (Dako®) were goat-anti-rabbit immunoglobulins diluted 1:50 and rabbit anti-peroxidase serum plus peroxidase, diluted 1:250, respectively. Extensive washings with 0.05 M HCl-Tris were performed among the applications of each serum. The presence of VP-1 was routinely detected by standard reactions containing 0.03% 3-3' diaminobenzidine and 2% H_2O_2 in a 0.05 M HCl-Tris buffer solution under microscopy control as described elsewhere. The primary serum was kindly provided by Thomas Benjamin (Harvard Medical School, Department of Pathology; Boston, MA, U.S.A.)[12]. This anti-VP-1 polyclonal serum has been widely used in several studies and has also demonstrated to be specific for polyoma VP-1 antigen[13, 14]. In every single experiment, normal rabbit serum was applied as primary serum in adjacent slides and processed with the PAP method as an internal control. Polyoma-induced pilomatricomas in mice were used as positive controls, while normal skin from mouse was employed as a negative control. Given the fact that some human papillomavirus (HPV) types can induce the development of benign skin tumors, we decided to check if the polyomavirus anti-VP-1 serum could eventually cross-react with HPV antigens. To achieve this, several slides obtained from one case of verruca vulgaris and another of condyloma acuminatum were treated. Those slides had been previously confirmed to express HPV structural antigens by the same PAP method employed in this study.

VP-1 positive labeling was detected in 4 out of 10 human pilomatricomas. Labeling was exclusively intranuclear with a patchy pattern of distribution in the neoplasms (Fig. 1). The adjacent slides, treated with normal rabbit serum as a primary serum, did not show any immunolabeling (Fig. 2). Positive and negative controls worked as expected and the slides that contained HPV were not labeled with the anti-VP-1 serum (not shown).

Our results demonstrated the presence of the major polyomavirus capsid protein in human pilomatricomas, with the same characteristics observed in the same type of tumors experimentally developed in mice after polyomavirus inoculation. The specificity of the reaction was confirmed since the adjacent slides treated with normal rabbit serum as a primary serum showed no labeling at all. The possibility of cross-reactivity with HPV was also ruled out since the anti-VP-1 serum did not label any of the representative tissues known to be infected with HPV. Furthermore, normal skin adjacent to the tumors showed no VP-1 labeling either. Thus, it should be assumed that

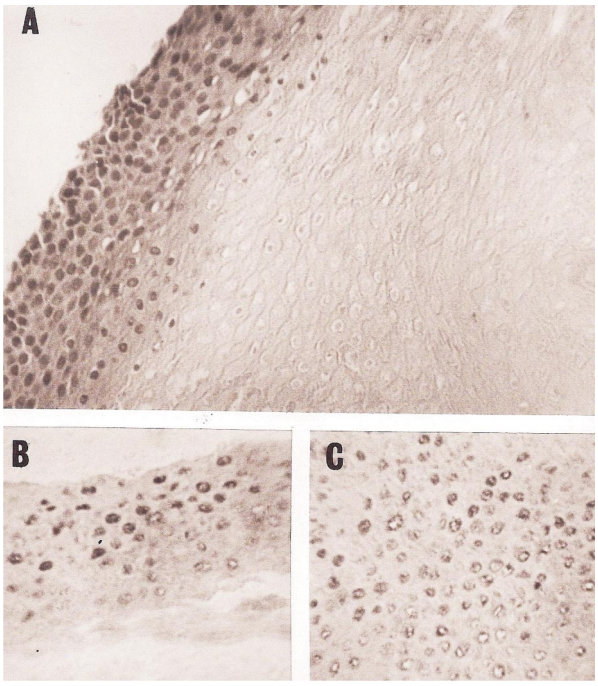

Fig. 1.– Histology and VP-1 immunolabeling of pilomatricomas. A: The proliferation of hair-follicle matrix cells is observed as well as their differentiation into keratinized "ghost" cells and, finally, their transformation in amorphous keratin (Hematoxilin-Eosin staining). B and C: 2 different human pilomatricomas showing polyomavirus VP-1 immunolabeling. The viral antigen is located exclusively in the nuclei. X 512.

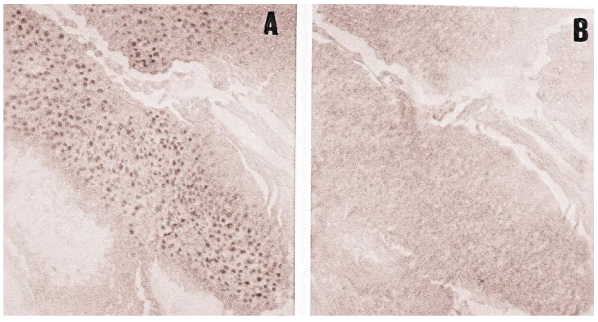

Fig. 2.– Polyomavirus VP-1 detection in a human pilomatricoma usig the PAP method. A: the tumor was treated with an anti-VP-1 rabbit polyclonal serum as a primary. B: the adjacent slide was treated with normal rabbit serum as an internal control. VP-1 is clearly observed in the nuclei of cells in the slide treated with the specific serum (A) but not in the adjacent, mock-treated slide (B). X 70.

the presence of Polyomavirus VP-1 in some human pilomatricomas is a real fact.

The simplest explanation of this phenomenon is to consider that human polyomaviruses (JC, BK, KI, WU and MC) are ubiquitous and could eventually replicate in actively proliferating tissues. Another possibility is that some of these viruses play a direct role in the development of human pilomatricomas and a third possibility is that a still unknown polyomavirus is associated with the development of human pilomatricomas. The lack of VP-1 detection in all the tumor cells but only in sheets of adjacent cells in the 4 positive cases suggests a clonal expansion of a tumor cell sub-population rather than at random infection by an eventual ubiquitous virus. The absence of VP-1 detection in the other 6 samples does not deny any of the possibilities described above. In this regard, it is widely accepted that the viruses known to be associated with human neoplasms are co-factors in their development; this means that their presence is necessary but not sufficient for the induction of tumor, being immunological, genetic and environmental factors also involved in the etiopathogenesis of these diseases. To note, this study describes the detection of VP-1 synthesis in human pilomatricomas, not the confirmation of polyomavirus replication in these tumors. This could be in agreement with a previous report, where we described the full expression of polyomavirus genome in experimental thymomas (including VP-1) with a simultaneous lack of viral encapsidation probably due to VP-1 underphosphorylation[13]. The same phenomenon could eventually explain the presence of VP-1 in pilomatricomas described here: the virus could be associated with the pathogenesis of the neoplasms without producing infectious viral particles and also, the lack of VP-1 expression in all the tumor cells in a single tumor or in all of the surgically-removed human pilomatricomas does not necessarily mean the absence of viral genome in them.

The only way to definitely establish the status of polyomavirus in pilomatricomas would be to isolate infectious polyomavirus or the different virus antigens and genomes from fresh pilomatricomas, before the fixative has been added to the surgical specimens. Even though this possibility has been considered to conduct this study, the low incidence of this kind of tumors and their clinical resemblance to other cystic skin diseases make this procedure hard to be done. However, polymerase-chain-reactions using specific primers to amplify DNA recovered from these paraffin-embedded samples are currently being performed to further characterize the virus. Anyhow, the detection of polyomavirus VP-1 in human pilomatricomas by a highly precise immunocytochemical method strongly suggests that, somehow, a Polyomavirus is associated with human pilomatricomas and that further studies leading to confirm this association have to be done.

Recently, a new human polyomavirus has been related with the etiology of Merkel cell carcinoma of the skin[15]. Hopefully, the preliminary report described here will encourage further research and characterization of human polyomavirus associated with the origin of human skin neoplasms.

Acknowledgements: This work was supported by grants M-820 from the Universidad de Buenos Aires and PIP-5975 from CONICET given to N.S. N.S. is a member of the Research Career of the Consejo Nacional de Investigaciones Científicas y Técnicas de la República Argentina (CONICET). S.S. was the recipient of a doctoral fellowship from the University of Buenos Aires.

Conflicts of interests: This work does not have conflicts of interests.

References

1. Benjamin TL. Polyoma Virus: Old findings and new challenges. *Virology* 2001; 289: 167-73.
2. zur Hausen H. Novel human polyomaviruses. Re-emergence of a well known virus family as possible human carcinogens. *Int J Cancer* 2008; 123: 247-50.
3. Cole CN. Poliomavirinae. In Fields BN, Knipe DM, Howley P, Chanock RM, Hirsch MS, Melnick JL, Monath TP, Roizman B (eds). Field's Virology 3rd Ed. Lippincott-Raven Press, New York. 1996, p 1997-2025.
4. Otero J, Sanjuan N. Telomerase activity in thymomas and mammary gland adenocarcinomas produced by murine polyomavirus. *Medicina (Buenos Aires)* 1998; 58: 497-500.
5. Courtneidge SA, Smith AE. Polyoma virus transforming protein associates with the product of the c-src cellular gene. *Science* 1983; 435-9.
6. Dilworth SM, Brewster CE, Jones MD, Lanfrancone L, Pellici G, Pellici PG. Transformation by Polyoma virus middle T-antigen involves the binding and tyrosine phosphorylation of Shc. *Nature* 1994; 367: 87-90.
7. Dahl J, Jurczak A, Cheng A, Baker DC, Benjamin TL. Evidence of a role for phosphatidylinositol 3-kinase activation in the blocking of apoptosis by polyomavirus middle T antigen. *J Virol* 1998; 72: 3221-6.
8. Cullere X, Rose P, Thathamangalam U, Chatterjee A *et al*. Serine 257 phosphorylation regulates association pf polyomavirus middle T antigen with 14-3-3 proteins. *J Virol* 1998; 72: 558-63.
9. Dawe C, Freund R, Mandel G, Ballmer-Hofer K, Talmage DA, Benjamin TL. Variations in polyoma virus genotype in relation to tumor induction in mice. Characterization of wild type strains with widely diuffering tumor profiles. *Am J Pathol* 1987; 127: 243-58.
10. Freund R, Dawe C, Carroll JP, Benjamin TL. Changes in frequency, morphology, and behavior of tumors induced in mice by a polyoma virus mutant with a specifically altered oncogene. *Am J Pathol* 1992; 141: 1409-25.
11. Storm CA, Seykora JT. Cutaneous adnexal neoplasms. *Am J Clin Pathol* 2002; 118: 33-49.
12. Leavitt AD, Roberts TM, Garcea RL. Polyoma virus major capsid protein, VP-1. Purification after high level expression in Escherichia coli. *J Biol Chem* 1985; 260: 12803-9.
13. Sanjuan N, Porrás A, Otero J, Perazzo S. Expression of Major Capsid Protein VP-1 in the absence of viral particles in thymomas induced by murine polyomavirus. *J Virol* 2001; 75: 2891-9.
14. Sanjuan N, Porrás A, Otero J. Microtubule-dependent intracellular transport of murine polyomavirus. *Virology* 2003; 313: 105-16.
15. Feng H, Shuda M, Chang Y, Moore PS. Clonal integration of a polyomavirus in human Merkel cell carcinoma. *Science* 2008; 319: 1096-100.

- - - -

Sócrates criticó el fetichismo del libro (Fedro). Dos siglos después, en otro pueblo del libro (el pueblo bíblico), dijo el Eclesiastés (12:12): "componer muchos libros es nunca acabar, y estudiar demasiado daña la salud. Basta de palabras. Todo está escrito". En el siglo I, Séneca le escribe a Lucilio: "La multitud de libros disipa el espíritu". En China, en el siglo IX, el poeta Po Chu Yi se burla de Lao-Tsé: "De sabios es callar, los que hablan nada saben" —dicen que dijo Lao-Tsé, en un librito de ochocientas páginas. En Argelia, en el siglo XIV, Ibn Jaldún: "Los demasiados libros sobre un tema hacen más difícil estudiarlo" (Al-muqaddi-mah VI 27). En Alemania, en el siglo XVI, Lutero: "La multitud de libros es una calamidad" (Charlas de sobremesa). Don Quijote, al enterarse de que se había escrito el Quijote: "Hay algunos que así componen y arrojan libros de sí como si fueran buñuelos" (II 3). Descartes: "abandoné el estudio de los libros, decidido a no buscar más ciencia que en mí mismo o en el gran libro del mundo" (Discurso del método). Samuel Johnson: "Para convencerse de la vanidad de las esperanzas humanas, no hay un lugar más impresionante que una biblioteca pública".

Gabriel Zaid

Los demasiados libros. La Jornada Semanal (Caracas), 28 de abril de 1996.
En: *http://www.analitica.com/bitBlioteca/zaid/libros.asp*; consultado el 7/12/2006.