[短報]Squamous metaplasia : Human papillomavirus DNA transfected to cultured adenocarcinoma cells causes squamous metaplasia

Squamous metaplasia: Human papillomavirus DNA transfected to cultured adenocarcinoma cells causes squamous metaplasia

Iwao Nakazato and Teruo Iwamasa

Department of Pathology, Ryukyu University School of Medicine
207 Uehara, Nishihara, Okinawa 903-01, Japan

(Received on July 29, 1997, accepted on September 16, 1997)

In Okinawa, there are a significant number of cases with squamous cell carcinoma and adenosquamous carcinoma of the lung which are positive for HPV DNA. In the normal tracheobronchial tree, however, there is no squamous epithelium. The squamous cell carcinoma and adenosquamous carcinoma of the lung is proceeded by progressive mucosal changes, squamous metaplasia and dysplasia. It has been considered that cigarette smoking and other environmental agents may cause such squamous metaplasia and dysplasia[1,2]. We postulate that HPV DNA causes squamous metaplasia and dysplasia of the bronchial epithelium. Then we transfected HPV genomes, HPV 6, 16 and 18 into cultured adenocarcinoma cells, DLD-1 (human intestinal adenocarcinoma, moderately differentiated) or PC-14 (human lung adenocarcinoma, poorly differentiated), obtained from the Japanese Cancer Research Resource Bank. The HPV 6 in plasmid pML and HPV 16 or 18 in plasmid pBR322 were also obtained from the Japanese Cancer Research Resource Bank with permission from Dr. Zur Hausen. Hygromycin B and neomycin genes were used as dominant selectable markers. Reconstructed plasimids[3] which have genes resistant to hygromycin B or neomycin, and HPV 6, 16, or 18 were transfected into the adenocarcinoma cells. The physical state of the HPV in the transfected cells were confirmed as both episomal and integrated by Southern blot analysis and also by in situ hybridization methods (data not shown). HPV mRNA was demonstrated by use of the RT-PCR method (deta not shown). The HPV transfected adenocarcinoma cells, both DLD-1 (Table 1) and PC-14 (Table 2), changed to large polygonal shaped cells with abundant cytoplasm and large irregular nuclei. The PC-14 cells which were originally weakly adherent, became adherent strongly to the dish after HPV transfection. Some of the transfected cells showed apoptosis showing a ladder DNA pattern by gel electrophoresis (data not shown). HPV transfected cells which had small to large numbers of virus copies were injected into SCID mice subcutaneously on the back. 3−4 weeks after injection of the cells (10^7cells), a tumor about 1×1cm in diameter was noted. Histologically, this tumor showed squamous differentiation (Fig. 1a, b). The tumor was embedded in the connective tissue stroma. Interaction between the tumor cells and stroma was expressed in the polar arrangement of the peripheral epithelial tumor cells (palisading). A large number of tumor cells appeared similar to the basal cells of squamous epithelium with round nuclei and slightly wide cytoplasms. Furthermore, some showed differentiation to prickle cells, and intercellular bridges were also observed. Immunohistochemically, involucrin, a marker of keratinocyte differentiation[5], was demonstrated (Fig. 2). There were no tubular structures, nor was mucin detected by Alcian blue or PAS staining. The squamous differentiation was more clearly demonstrated in the cases with tumor cells having large number of HPV (HPV 6 or 16 rather than HPV 18) copies.

From these tumor cells, cytokeratins were prepared according to the method of Franke et al.[6] and Katagata et al.[7]. The keratin molecules were analyzed by two dimensional electrophoresis and Western blot analysis using anti-keratin monoclonal antibody (DAKO Co., Carpinteria, CA. U.S.A.). Gel electrophoresis was performed essentially as described by O'Farrell[8]; the first step, isoelectric focusing and then SDS-PAGE. The keratin molecules observed in cornifing squamous cells (Moll's No. 1, 10 and 14)[9,10] were demonstrated. In addition, we also examined the changes of adenocarcinoma cells which was transfected with double or triple different types of HPV. In this case, to the adenocarcinoma cells transfected with one of either type 16 or 18 HPV, the other types of HPV was transfected. The HPV copy number of superimposed transfection was usually very small, although the superimposed transfection of HPV 6 or 16 accelerated the squamous metaplasia, frequently showing intercellular bridges.

We are now considering HPV as an important cause of squamous metaplasia. However, it is reported that the incidence of HPV DNA in squamous cell carcinoma varies significantly in different geographic regions[11]. In the United States of America and European countries, the incidence of HPV infection in squamous cell carcinoma has been reported to be low (about 30%). On the other hand, in Portugal, South Africa and Taiwan

Key words: Squamous metaplasia, HPV, Keratin molecule, Involucrin

Table 1 Using calcium phosphate method (4), human papillomavirus (HPV) genomes were transfected into cultured DLD-1 cells (moderately differentiated adenocarcinoma of the colon).

type of transfected HPV and dominant selectable marker gene	clone number	copies/cell
HPV 6 (neomycin resistant)	1*	(-)
	2	(-)
	3	(-)
	4	(-)
	5*	(-)
HPV 16 (neomycin resistant)	1*	275
	2*	27.5
	3	2.75
	4*	2.75
	5	(-)
HPV 18 (neomycin resistant)	1*	38.5
	2	38.5
	3	38.5
	4	3.85
	5	3.85
HPV 18 (hygromycin B resistant)	1*	3850
	2*	385
	3*	38.5
	4	38.5
	5*	3.85
	6	3.85
	7	3.85
	8	3.85
	9*	0.385
	10	0.385
	11	0.385
	12	0.000385

type of transfected HPV and dominant selectable marker gene	clone number	copies/cell	
		HPV 6	HPV 18
HPV 6 + HPV 18 (neomycin resistant) (hygromycin B resistant)	1*	42.5	0.385
	2*	42.5	0.00385
	3*	42.5	0.0128
	4	4.25	0.0385
	5	4.25	0.00385
	6	4.25	0.00385
	7	4.25	0.000385
	8	4.25	0.000385
	9	0.425	0.00385
	10*	(-)	3.85
	11*	0.00425	0.385
	12	(-)	0.0385
	13	(-)	0.0385
	14	(-)	0.0385
	15	(-)	0.00385
	16	(-)	0.00385
	17*	(-)	0.00128
	18	(-)	0.00128
	19	(-)	0.00128
HPV 16 + HPV 18 (neomycin resistant) (hygromycin B resistant)	1*	27.5	0.385
	2	27.5	0.385
	3	27.5	0.385
	4	2.75	0.0385
	5*	2.75	0.00385
	6	2.75	0.00385

*:HPV transfected cells which had small (less than 5 copies per cell) to large numbers of virus copies were injected into SCID mice subcutaneously. Hygromycin B or neomycin genes were used as dominant selectable markers.

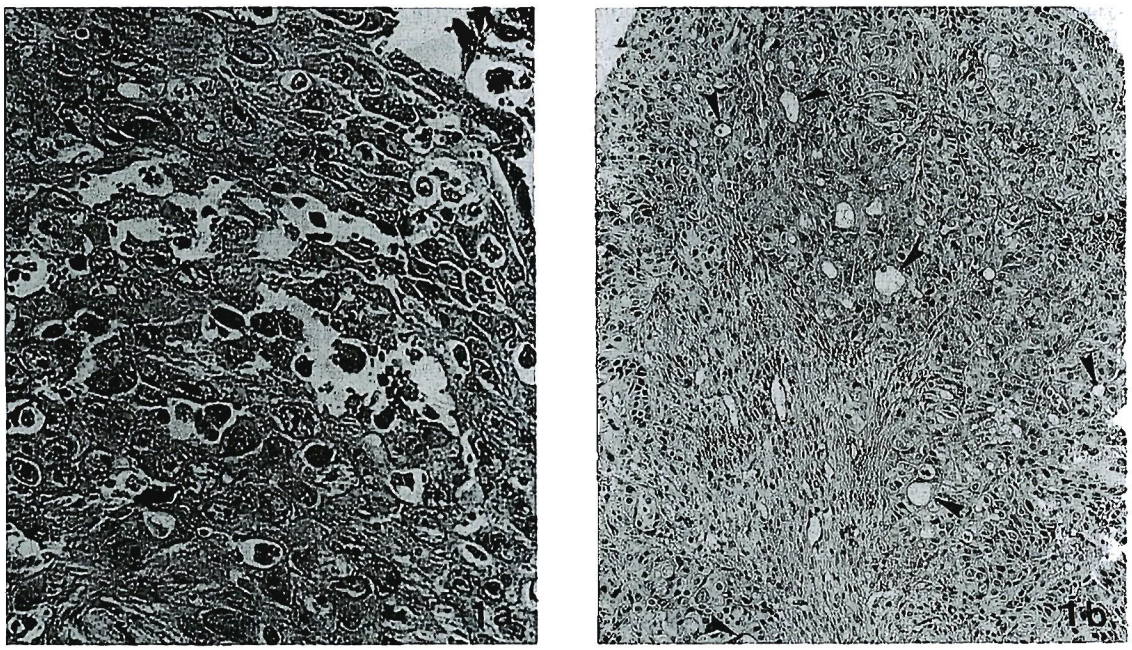

Fig. 1a. HPV 16 transfected DLD-1 cells injected into SCID mouse subcutaneously. Squamous metaplasia is clearly demonstrated. H&E staining. ×300.
b. Control DLD-1 cells injected into SCID mouse subcutaneously. Histology of adenocarcinoma is observed showing tubular structure (arrowheads). H&E staining. ×70.

Table 2 Using calcium phosphate method (4), human papillomavirus (HPV) genomes were transfected into cultured PC-14 cells (poorly differentiated adenocarcinoma of the lung).

type of transfected HPV and dominant selectable marker gene	clone number	copies/cell
HPV 6 (neomycin resistant)	1*	42.5
	2	4.25
	3	4.25
	4	0.425
	5	0.0425
	6	(-)
	7	(-)
	8	(-)
HPV 16 (neomycin resistant)	1*	275
	2	27.5
	3	27.5
	4	27.5
	5	27.5
	6	27.5
	7	2.75
	8	2.75
	9	2.75
	10	2.75
	11	2.75
	12	2.75
	13	2.75
	14	2.75
	15	2.75
	16	(-)
	17	(-)
	18	(-)
	19	(-)
	20	(-)
	21	(-)
	22	(-)
	23	(-)
HPV 18 (neomycin resistant)	1*	38.5
	2	38.5
	3	38.5
	4	38.5
	5	38.5
	6	3.85
	7	3.85
	8	3.85
	9	3.85
HPV 18 (hygromycin B resistant)	1	38.5
	2	0.385
	3	0.385
	4	0.385
	5	0.385
	6	0.385
	7	0.385
	8	0.385
	9	0.385
	10	0.0385

*: HPV transfected cells which had large number of HPV copies, HPV 6, 42.5/cells, HPV 16,275/cells and HPV 18, 38.5/cells, injected into SCID mice subcutaneously. Hygromycin B or neomycin genes were used as dominant selectable markers.

Fig. 2 Immunohistochemical demonstration of involucrin. HPV 16 transfected DLD-1 cells injected into SCID mouse. Arrows indicated the positive reaction. ×100.
Anti involucrin antibody was obtained from Sigma Chemical Co., St. Louis, MO. U.S.A.

etc., HPV infection is high (about 70-90%)[11]. In mainland Japan, HPV is only demonstrated in 10-30% of squamous cell carcinoma and adenosquamous carcinoma of the lung[12, 13]. Here, other factors, cigarette smoking and environmental agents, can be considered the dominant cause of squamous metaplasia.

Furthermore, which region or sequence in HPV genome is necessary for the squamous metaplasia has to be clarified.

REFERENCES

1) Peter E.J., Morice R., Benner S.E., Lippman S, Lukeman J., Lee J.S., Ro J.Y. and Hong W.K.: Squamous metaplasia of the bronchial mucosa and its relationship to smoking. Chest 103: 1429-1432, 1993.

2) Simosato Y. : Lung cancer: Histogenesis, differentiation, and prognostic factors. Tr. Soc. Pathol. Jpn. 72: 29-57, 1983.

3) Nakazato I., Hirayasu T., Kamada Y., Tsuhako K. and Iwamasa T.: Carcinoma of the lung in Okinawa:

With special reference to squamous cell carcinoma and squamous metaplasia. Pathol. Int. 47: 659-672,1997.
4) Okayama H. and Chen C.: Calcium phosphate mediated gene transfer into established cell lines. In Gene Transfer and Expression Protocols (Murray E.J.ed) Humana Press, Clifton, New Jersey, pp. 15-21, 1991.
5) Mitra R.S., Wrone-Smith T., Simonian P., Foreman K.E., Nunez G. and Nickolloff B.J.: Apoptosis in keratinocytes is not dependent on induction of differentiation. Lab. Invest. 76: 99-107, 1997.
6) Franke W.W., Scheller D.L., Moll R., Winter S., Schmid E. and Engelbrecht I. : Diversity of cytokertins, Differentiation of specific expression of cytokeratin polypeptides in epithelial cells and tissues. J.Mol. Biol. 153: 933-959, 1981.
7) Katagata Y., Aso K., Sato M. and Yoshida T. : Occurence of differentiated keratin peptide (K1) in cultured human squamous cell carcinoma. Biochem. Biophys. Res. Commun. 182: 1440-1445, 1992.
8) O'Farrell P.H. : High resolution two-dimentional electrophoresis of proteins. J. Biol. Chem. 250: 4007-4021, 1975.
9) Cooper D., Schermer A. and Sun T-T.: Biology of diseases. Classification of human epithelia and their neoplasma using monoclonal antibodies to keratins: stategies, applications, and limitations. Lab. Invest. 52: 243-256, 1985.
10) Moll R., Franke W.W., Schiller D.L., Geiger B. and Krepler R.: The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. Cell 31: 11-24, 1982.
11) Suzuk L., Noffsinger A.E., Hui Y.Z. and Fenoglio-Preise C.M.: Detection of human papillomavirus in esophageal squamous cell carcinoma. Cancer 78: 704-710. 1996.
12) Hirayasu T., Iwamasa T., Kamada Y., Koyanagi Y. and Genka K. : Human papillomavirus DNA in squamous cell carcinoma of the lung. J Clin Pathol. 49: 810-817, 1996.
13) Kinoshita I., Dosaka-Akita H., Shindoh M., Fujino M., Akie K., Kato M., Fujinaga K. and Kawakami Y.: Human papillomavirus type 18 DNA and E6-E7 mRNA are detected in squamous cell carcinoma and adenosquamous carcinoma of the lung. Br. J. Cancer 71: 344-349, 1995.