Virchows Arch A (1987) 411:73–77

Virchows Archiv A

Virus expression EGF and transferrin receptors in human papillomas *

J. Viac[1], Y. Chardonnet[1], V. Bouvard[1], J. Leval[2], A. Morgon[3], and J. Thivolet[1]
[1] INSERM U209, CNRS UA 601, Hopital Edouard Herriot, Pavillon R, 69437 Lyon Cedex 03, France
[2] Clinique ORL, Hopital La Croix Rousse, 69317 Lyon Cedex 04, France
[3] Clinique ORL, Hopital E. Herriot, Pavillon U, 69437 Lyon Cedex 03, France

Summary. Thirty five non regressing cutaneous and mucosal human papillomas were studied for the expression of EGF and transferrin receptors by indirect immunofluorescence on frozen sections. The lesions were also examined for the presence of human papillomavirus (HPV) DNA by in situ-hybridization with biotinylated probes and viral capsid antigen. The mapping of EGF and transferrin receptors was modified in cutaneous lesions with drastic viral cytopathic effects and was enhanced in mucosal lesions mainly in laryngeal papillomas, which are poor virus producers. The greatest increase in EGF and transferrin receptor reactivity was observed in the group of mucosal lesions in which viral DNA was more frequently detected than viral antigen. This suggests that viral DNA may play a role in basal cell stimulation. Moreover some of these lesions with dense inflammatory reactions showed DR antigen expression by epithelial cells. Our findings indicate that epithelial cell activation in papillomas might be modulated by other factors than HPV such as mediators of the local immune response.

Key words: Papillomas – Papillomavirus – EGF receptors – Transferrin receptors

Introduction

Human papillomas are epithelial proliferations induced by a wide range of HPV types. Nearly 40 different types have so far been identified (McCance 1986) some associated with benign papillomas and others found in lesions which may undergo malignant conversion (Zur Hausen 1977; Orth et al. 1980; Gissmann 1984; Pfister 1984). Type specific cytopathic effects have been described among lesions with high or low virus production, such as plantar warts type 1 and mucosal papillomas infected either with benign types 6 and 11 or with oncogenic types 16 and 18, respectively.

In our previous investigations most of non regressing cutaneous and mucosal papillomas showed a moderate local cellular immune response with a mild infiltration and disappearance of Langerhans cells from the epithelium (Chardonnet et al. 1983; Chardonnet et al. 1986). However, an intense inflammatory reaction with DR expression by epithelial cells was observed in some mucosal lesions (Viac et al. 1987), suggesting an enhanced cell activation. In the commonly accepted hypothesis, the basal layer cells are target for virus infection leading to the proliferation and to papilloma formation.

EGF and transferrin receptors are known to be associated with proliferating cells. They play an important role by mediating mitogenesis (Carpenter and Cohen 1979; Carpenter and Zendegui 1986) and cellular iron uptake (Hemmaplardh and Morgan 1974) respectively. Moreover a sequence homology between the cytoplasmic portion of EGF receptor and v-erb-B transforming oncogene (Downward et al. 1984) has been found emphasizing the potential role of the EGF receptors in the control of normal and abnormal cell differentiation. To date nothing has been published on the incidence of HPV components on EGF and transferrin receptors of epithelial cells. Our study was aimed at determining if the virus expression and DR antigen in epithelial cells in papillomas were related to cell activation through EGF and transferrin receptors.

* This work was supported by grants from Féderation Nationale des Centres de Lutte Centre le Cancer, 1985 and Fondation Mérieux, Lyon, France

Offprint requests to: J. Viac at the above address

Material and methods

Thirty five non regressing cutaneous and mucosal papillomas from various sites (Table 1) were taken from 31 patients aged from 2 years to 72 years. They were removed by surgery, immediately frozen in liquid nitrogen and stored at −20° C.

Four μm adjacent frozen sections were prepared on slides coated with histostik (Brigati et al. 1983), air dried and fixed 10 min in cold acetone for immunofluorescence or 3 min in methanol:acetic acid (3:1) for in situ hybridization.

Monoclonal antibodies directed against specific antigens were used: EGF-R1 (Oncor) specific for EGF receptors, OKT9 (Ortho Pharmaceuticals, Raritan, NJ, USA) specific for transferrin receptors, BL2 (Immunotech) specific for monomorphic HLA-DR antigen.

Viral antigen was detected with a human papillomavirus antiserum raised in rabbit against SDS-dissociated purified virus, which recognizes HPV genous specific (common) antigen (Jenson et al. 1980).

A biotin streptavidin system (Amersham) was used with EGF-R1 and OKT9 monoclonal antibodies which require a high sensitivity. Sections were incubated with the appropriate diluted monoclonal antibody at room temperature for 45 min. After washing in PBS, sections were subsequently incubated for 45 min with a biotinylated goat antimouse immunoglobulin (1/50 dilution) and the fluorescein streptavidin (1/40 dilution). Viral antigen was detected by a standard technique of indirect immunofluorescence as previously reported (Chardonnet et al. 1983).

The specificity of the immunofluorescent tests was shown by the absence of staining in sections incubated with PBS or PBS containing 0.5% bovine serum albumin instead of primary antibody. After mounting in polyvinyl alcohol, slides were examined under a fluorescence microscope.

In situ hybridization with biotinylated probes was assessed with the method described by Beckmann et al. (1985), with minor modifications (Bouvard et al. 1986). Cloned DNA type 1a provided by G. Orth (Paris) was used to prepare biotinylated DNA probes. The DNA plasmid was nick translated using biotinylated deoxyuridine triphosphate, biotin-11-dUTP (BRL). The DNA of sections was denatured in boiling PBS for 30 s. The hybridizations were performed in conditions of low stringency with 20% formamide with an equivalent Tm of −33° C. The mixtures containing the biotinylated probes were denatured in a boiling water bath for 10 min. After 16 h incubation at 42° C the DNA-DNA hybrids were visualized using immunocytochemical reactions. The hybrids were detected as brown precipitates in infected nuclei from various papilloma lesions with sequential treatments with avidin D, biotinylated rabbit goat antiIgG and avidin-biotinylated peroxidase complex (Vector Laboratories). Adjacent papilloma sections acted as negative controls in the absence of HPV DNA probe or when the pBR322 DNA probe was used. Normal skin and clinically non HPV related pathological specimens were negative with HPV DNA probes.

Table 1. Expression of EGF and transferrin receptors, DR antigen and HPV components in human papillomas

Tissue	Receptors of		DR in Epithelial cells	HPV	
	EGF	Transferrin		Antigen	DNA
Plantar warts	↘9/9	↗9/9	0/9	6/9	4/4
Hand warts	↘8/8	↘8/8	0/8	2/8	5/5
Condyloma acuminata	↗4/4	↗3/3	0/4	0/4	4/4
Juvenile LP	↗6/9	↗6/9	1/9	1/9	5/8
Adult LP	↗3/5	↗3/5	3/5	1/5	4/4
Normal epithelia	5/5	5/5	0/5	0/5	0/5

LP = Laryngeal papilloma: ↗ increased or ↘ decreased intensity and distribution of immunofluorescence on epithelial cells

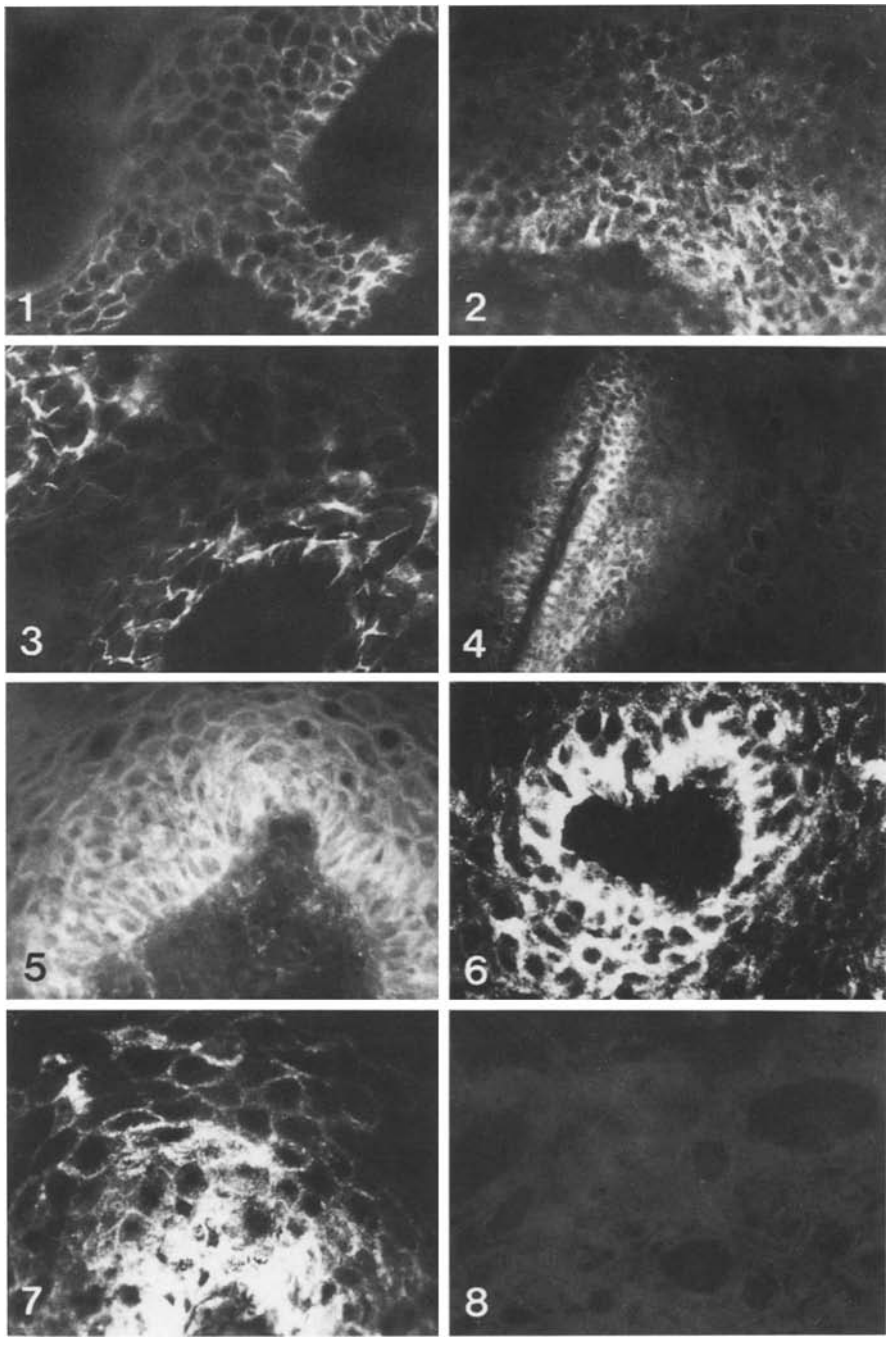
Results

These are summarized in Table 1. In normal tissues, EGF receptors were detected on the cell margins of viable layers of the epidermis, basal cells were more intensely stained than upper cell layers (Fig. 1). Transferrin receptors were barely detectable in the basal cell layer and to a variable degree in the spinous layers (Fig. 2). Both staining patterns were similar to those previously reported for human epidermis (Nanney et al. 1984; Green et al. 1985; Soyer et al. 1986). DR antigen was expressed only by Langerhans cells through the epithelium.

In cutaneous warts, the mapping of EGF receptors was reduced; only cells from the germinative layers were positive (Fig. 3). Transferrin receptors were expressed in basal layer cells of plantar warts (Fig. 4) and unevenly detected in the upper layer cells; they were decreased in hand warts. DR antigen was expressed both by infiltrating cells of the dermis and the rare Langerhans cells in the epithelium of plantar and hand warts as previously shown (Chardonnet et al. 1986).

In contrast, the reactivity pattern (intensity and distribution) of both EGF and transferrin receptors was enhanced in mucosal lesion epithelia (condyloma acuminata and laryngeal papillomas) (Figs. 5 and 6). Strong staining was associated with laryngeal papillomas with a dense infiltrate. Moreover, in 4 of 14 cases epithelial cells of these lesions expressed DR antigen strongly, the labelling was cytoplasmic in basal cells and intercellular in the upper layers (Fig. 7). In presence of non related antibody or of PBS, control sections were negative (Fig. 8).

The direct effect of HPV in these lesions was shown by the presence of virus capsid antigen and viral DNA. The HPV antiserum detected the common viral antigen of any papilloma lesions. Capsid antigen was present in large amounts in upper layer cells of most plantar warts (Fig. 9) whereas in mucosal lesions such as in laryngeal papillomas few isolated cells from intermediate layers were positive (Fig. 10). In most papillomas, viral DNA was visualized as brown precipitate in a larger number of nuclei than viral antigen (Figs. 11 and 12). In

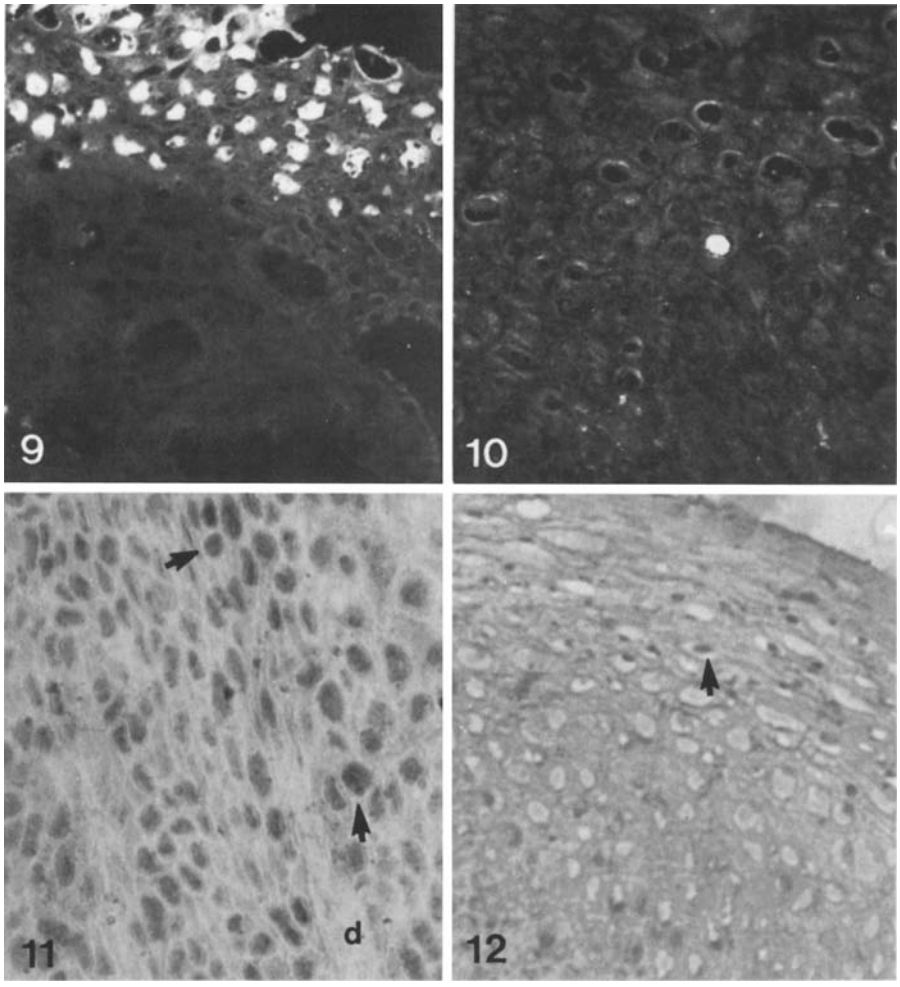


Figs. 1, 2. Immunofluorescent staining of normal human epidermis with EGF-R1 (**1**) and OKT9 (**2**) monoclonal antibodies: EGF and transferrin receptors are mainly located on basal layer keratinocytes. ×250

Figs. 3–8. Mapping of EGF and transferrin receptors in cutaneous and mucosal papillomas. **3** EGF receptors on hand warts. ×250. **4** Transferrin receptors on plantar warts. ×250. Only germinative layers are intensely stained. **5** EGF receptors in laryngeal papillomas. ×250. **6** Transferrin receptors in laryngeal papillomas. ×250. Note the increased expression of EGF and transferrin receptors in cells of germinative and inner layers of the epithelium. **7** DR expression by epithelial cells in a laryngeal papilloma with a dense infiltrate. ×250. **8** Control papilloma section stained only with fluorescein conjugate. ×250

non stringent conditions, the HPV type 1 DNA probe hybridized sequences of any HPV type present in lesions. On the contrary, only plantar warts infected with HPV type 1 reacted with HPV type 1 DNA probe under stringent conditions with an equivalent Tm of −12° C. In cutaneous lesions viral DNA was detected in granular layer cells and in mucosal lesions only foci of cells exhibited viral DNA in upper cell layers. Occasionally basal or parabasal cells were positive. Viral DNA was found in lesions lacking of virus capsid antigen. Three out of 7 juvenile laryngeal papillomas were negative both for viral antigen and viral DNA; there was no major difference between their receptor mappings and the other specimens.

Skin lesions with severe cytopathic effects and large amounts of viral antigen and DNA showed the lowest intensity of EGF receptors and an abnormal expression of transferrin receptors in upper layer cells of plantar warts. Conversely, in mucosal



Figs. 9, 10. Immunofluorescent staining of specific common viral antigen. **9** Large number of positive nuclei in upper layer cells of a plantar wart. ×250. **10** An isolated positive nuclei in a laryngeal papilloma

Figs. 11, 12. Detection of viral DNA by in situ hybridization with biotinylated probes and immunoperoxidase. **11** Hand wart ×170. **12** Condyloma acuminata. ×170. *d*=dermis ↗ positive nuclei

lesions with minor amounts of viral antigen and DNA, the highest reactivity of both EGF and transferrin receptors was observed.

Discussion

An enhanced proliferation of epidermal cells is a typical feature of papillomas. The increased expression of some epithelial antigens is suggestive of an epithelial cell activation induced by viral infection. The staining patterns of EGF and transferrin receptors in papilloma lesions were modified in intensity and distribution according to their clinical type and their viral expression.

The use of a biotin streptavidin system allowed the detection of variations in the expression of EGF and transferrin receptors on frozen sections of human papillomas. The most drastic alterations of EGF and transferrin receptors have been observed in lesions with an intense cytopathic effect as previously observed with other antigens (Viac et al. 1985). Similarly, we have found that HPV infection induced modifications of epithelial cell antigens such as keratins, involucrin, desmosome related antigen, B2 microglobulin.

The loss of EGF receptors and the unexpected reactivity of transferrin receptors in the upper layer cells of plantar warts suggests that these receptors are abnormally regulated under viral infection. The presence of transferrin receptors in plantar warts which are more vigorous producers of virus than hand warts may indicate that infected cells are in a metabolically active state, as suggested for other tissues (Walker and Day 1986). Their location in upper layer cells of plantar warts, may have significance other than that related to proliferation.

Conversely mucosal lesions which are poor virus producers exhibited an increased expression of both EGF and transferrin receptors. In most cases in our series, their mapping showed similar and correlative distribution. This suggests that the cycling of the transferrin receptors might be regulated by EGF as previously reported (Davis and Czech 1986). It remains to be determined whether the immunologically active EGF receptors possess

binding capacity (Nanney et al. 1984) as reported in condyloma acuminata and in other skin tumors (Bauknecht et al. 1985). Their relation with v-erb-B transforming oncogene remains to be elucidated in papillomas which tend to malignant conversion or to recur.

The intense local immune response with high densities infiltrating DR positive cells and presence of epithelial cells expressing DR antigen (Viac et al. 1987) might influence the expression of EGF and transferrin receptors in some papillomas. It has already been described that in some disease processes of the skin with a hyperimmune state, activated T lymphocytes produce quantities of γ-interferon sufficient to induce DR antigen by epithelial cells (Volc-Platzer et al. 1984). Our findings favor the hypothesis that DR expression in papillomas occur as a consequence of epithelial cell activation through EGF and transferrin receptors. However some other unknown factors are probably implied in such a process since only mucosal papillomas exhibited DR antigen. As HPV DNA was detected in a large proportion of lesions (9/9 in cutaneous warts and 13/16 in mucosal warts) the virus production might be related to variations in the receptor distribution among different papillomas. The presence of viral DNA in some parabasal cells might explain cell activation in laryngeal papillomas, whereas the virus particles or antigen could restrict this phenomenon in cutaneous warts.

In conclusion, an enhanced expression of EGF and transferrin receptors were signs of cellular stimulation, mainly in lesions in which epithelial cells expressed DR antigen. It remains to be determined whether this may contribute to restriction of virus infection and persistence of the lesion. It is possible that the nature of the local immune response is one factor in epithelial cell activation. This hypothesis is currently under investigation in other skin diseases.

Acknowledgements. We thank the clinicians who provided the papilloma specimens.

References

Bauknecht T, Gross G, Hagedorn M (1985) Epidermal growth factor receptors in different skin tumors. Dermatologica 171:16–20

Beckmann AM, Myerson D, Daling JR, Kiviat NB, Fenoglio CM, McDougall JK (1985) Detection and localization of human genital condylomas by in situ hybridization with biotinylated probes. J Med Virology 16:265–273

Bouvard V, Chardonnet Y, Viac J (1986) The use of in situ hybridization technique for papillomavirus DNA detection in human pathology. Microbios Letters 32:147–155

Brigati DJ, Myerson D, Leary JJ, Spalholz B, Travis SZ, Fong CKY, Hsiung GD, Ward DC (1983) Detection fo viral genomes in cultured cells and paraffin-embedded tissue sections using biotin-labeled hybridization probes. Virology 126:32–50

Carpenter G, Cohen S (1979) Epidermal growth factor. Ann Rev Biochem 48:193–216

Carpenter G, Zendegui JG (1986) Epidermal growth factor, its receptor, and related proteins. Exp Cell Res 164:1–10

Chardonnet Y, Beauve P, Viac J, Schmitt D (1983) T cell subsets and Langerhans cells in wart lesions. Immunol Lett 6:191–196

Chardonnet Y, Viac J, Thivolet J (1986) Langerhans cells in human warts. Br J Dermatol 115:669–675

Davis RJ, Czech MP (1986) Regulation of transferrin receptor expression at the cell surface by insulin-like growth factors, epidermal growth factor and platelet derived growth factor. EMBO J 5:653–658

Downward J, Yarden Y, Mayes E, Scarce G, Totty N, Stockwell P, Ullrich A, Schlessinger J, Waterfield MD (1984) Close similarity of epidermal growth factor and v-erb B oncogene protein sequences. Nature 307:521–527

Gissmann L (1984) Papillomaviruses and their association with cancer in animals and in man. Cancer Surveys 3:161–181

Green MR, Phil D, Couchman JR (1985) Differences in human skin between the epidermal growth factor receptor distribution detected by EGF binding and monoclonal antibody recognition. J Invest Dermatol 85:239–245

Hemmaplardh D, Morgan EH (1974) Transferrin and iron uptake by human cells in culture. Exp Cell Res 87:207–212

Jenson AB, Rosenthal JD, Olson C, Pass F, Lancaster WD, Shah K (1980) Immunologic relatedness of papillomaviruses from different species. J Nat Cancer Inst 64:495–500

McCance DJ (1986) Human papillomaviruses and cancer. Biochim Biophys Acta 823:195–205

Nanney LB, Magid M, Stoscheck CM, King LE (1984) Comparison of epidermal growth factor binding and receptor distribution in normal human epidermis and epidermal appendages. J Invest Dermatol 83:385–393

Orth G, Favre M, Breitburd F, Croissant O, Jablonska S, Obalek S, Jarzabek-Chorzelska M, Rzesa G (1980) Epidermodysplasia verruciformis: a model for the role of papillomaviruses inhuman cancer. In "Viruses naturally occurring cancer". Cold Spring Harbor Conference on Cell Proliferation 7:259–282

Pfister H (1984) Biology and Biochemistry of Papillomaviruses. Rev Physiol Biochem Pharmacol 99:112–181

Soyer HP, Smolle J, Torne R, Kerl H (1986) Transferrin receptor expression in normal skin and in various skin tumors. J Invest Dermatol 86:335–336

Viac J, Staquet MJ, Chomel G, Thivolet J (1985) Alterations in the expression of two epidermal differentiation antigens in human epidermal disorders. Pathol Res Pract 180:577–583

Viac J, Chardonnet Y, Leval J, Morgon A, Thivolet J (1987) Epithelial expression of HLA-DR antigens in laryngeal papillomas with dense inflammatory reaction. Pathol Res Pract 657 (in press)

Volc-Platzer B, Majdic C, Knapp N, Wolff K, Hinterberger W, Lechner K, Stingl G (1984) Evidence of HLA-DR antigen biosynthesis by human keratinocytes in disease. J Exp Med 159:1784–1789

Walker RA, Day SJ (1986) Transferrin receptor expression in non-malignant human breast tissue. J Pathol 148:217–224

Zur Hausen H (1977) Papillomaviruses and their possible role in squamous cell carcinomas. Curr Top Microbiol Immunol 78:1–30

Accepted January 5, 1987