International Journal of Oral Biology, Vol. 32, No. 3, September 30 2007, p. 93~101


**International Journal of Oral Biology**

# Cell Behavior of Human Papillomavirus-immortalized and Tumorigenic Human Oral Keratinocytes Does Not Depend on the Integrin Expression

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*(Received September 10, 2007 ; Accepted September 17, 2007)*

**Cell behavior of the transformed cells is known to affect by interaction with extracellular matrix (ECM) proteins and integrin. To investigate the alterations of both integrin expression and cell-matrix interaction during neoplastic conversion of human oral kerationcytes, we studied expression levels of integrin subunits by flow cytometry and cellular responses to the ECM proteins in normal human oral keratinocytes (NHOKs), HPV-immortalized HOK-16B line, and three oral cancer cell lines established from HOK-16B line, CTHOK-16B-BaP, CTHOK-16B-DMBA, and CTHOK-16B-Dexa lines. The expression levels of α and β integrin subunits were shown decreased tendency in human oral keratinocytes undergoing immortalization and tumorigenic transformation except CTHOK-16B-DMBA line tested. Although αvβ6 integrin is known to be highly expressed in squamous cell carcinomas, and the altered integrin expression is suspected to be associated with cellular carcinogenesis, αv integrin subunit and αvβ6 integrin did not express in oral cancer cell lines tested. Cell behavior to the ECM proteins in HOK-16B line was generally similar to that of exponentially proliferating NHOKs. The adhesion activity profiles of type I collagen were very similar to that of its laminin counterparts, but fibronectin showed minimal adhesion activity under our conditions compared to the BSA control. The ability of the CTHOK-16B-BaP line to spread upon type I collagen and laminin markedly decreased, but migration was notably increased on type I collagen. In contrast, CTHOK-16B-DMBA and CTHOK-16B-Dexa lines spread less but migrated more upon type I collagen than immortalized HOK-16B line. These data indicate that downregulation of integrin subunits causes the changes of cellular responses to the ECM proteins during neoplastic conversion of human oral keratinocytes, and that cellular responses to the ECM proteins in oral cancer cell lines established by exposing different carcinogens are variable according to chemical carcinogens treatment.**

**Key words: Cell behavior, Integrin, Extracellular matrix proteins, Oral cancer cells**

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## Introduction

Integrins are heterodimeric (α/β subunits) cell surface glycoproteins that bind to extracellular matrix (ECM) proteins outside of the cell and connect via their cytoplasmic domains to components of the actin cytoskelecton within the cell. There are at least 18α subunits and 8β subunits, giving rise to more than 24 distinct integrins in mammalian cells (Hynes, 1999). Integrins, which are widely expressed in various cell types function in cell-to-cell and cell-to-ECM adhesive interactions and transduce signals from or to the ECM. Integrins can transduce two types of signals: receptor conformation, affinity, and clustering are regulated by intracellular events (inside-out signaling), whereas ligand binding triggers a variety of cellular responses (outside-in signaling), including actin polymerization and cell spreading, induction of gene expression, initiation of differentiation, and suppression of apoptosis (Hynes, 1992; Juliano and Haskill, 1993; Williams *et al.*, 1994; Hughes and Pfaff, 1998). The most abundant, constitutive integrins in the epidermis are α2β1 (collagen receptor), α3β1 (predominantly a laminin-5

receptor), and α6β4 (laminin receptor) (Watt and Hertle, 1994).

There are various ECM proteins, such as fibronectin, laminin, vitronectin, collagen, thrombospondin, entactin, fibrinogen, intracellular adhesion molecule (ICAM), and vascular cell adhesion molecule (VCAM) (Albelda and Buck, 1990; Cheresh, 1992), which are mediated through the integrins. Integrins and ECM are not only involved in physiological functions, but also in tumorigenesis. They can modulate tumor cell proliferation and survival (Varner and Cheresh, 1996; Giancotti and Ruoslahti, 1999; Sethi *et al.*, 1999). Transformed cells are generally characterized by decreased expression of ECM proteins, ECM receptors, and cytoplasmic components of adhesion molecules. The down-regulation of these molecules plays an important role in tumorigenic phenotype, including abnormal cell morphology, anchorage-independent growth, and ability to form tumors in transplantation models (Lukashev and Werb, 1998). Similarly, overexpression of integrin also promotes tumorigenic and metastatic potential. For example, αvβ6 is not expressed on normal epithelial cells, however, it becomes highly expressed during morphogenesis, wound healing, and tumorigenesis (Sheppard *et al.*, 1990; Breuss *et al.*, 1995). The altered integrin expression is suspected to be associated with cellular carcinogenesis through the Ras-extracellular signal-regulated kinase (ERK) (Mainiero *et al.*, 1997) or via the focal adhesion kinase (FAK)-Jun $NH_2$-terminal kinase (JNK) pathway (Oktay *et al.*, 1999). Reports of altered integrin expression in bladder (Liebert *et al.*, 1993), prostate (Bonkhoff *et al.*, 1993), skin (Savoia *et al.*, 1993), colorectal (Pignatelli *et al.*, 1990; Koretz *et al.*, 1991), breast (Pignatelli *et al.*, 1990; Zutter *et al.*, 1990; D'Ardenne *et al.*, 1991; Pignatelli *et al.*, 1991; Koukolis *et al.*, 1991; Friederichs *et al.*, 1995), pancreas (Hall *et al.*, 1991; Weinel *et al.*, 1995), thyroid cancers (Serini *et al.*, 1996), and oral squamous cell carcinomas (Maragou *et al.*, 1999) seem to support this hypothesis.

In this study, we determined the expression levels of α2, α3, α5, α6, αv, β1, and β4 integrin subunits and αvβ6 integrin in normal human oral keratinocytes (NHOKs), human papillomavirus (HPV)-immortalized oral keratinocytes, and human oral cancer cell lines established from HPV-immortalized cells. We also examined the effects of ECM proteins on cell adhesion, spreading, and migration to investigate the association between the expression of integrins and the changes of cellular responses to ECM proteins during the neoplastic conversion of oral keratinocytes.

## Materials and Methods

### Cell culture

Primary NHOKs were prepared and maintained as described previously (Oh *et al.*, 2005). Primary cultures were established in keratinocyte growth medium containing 0.15 mM calcium and a supplementary growth-factor bullet kit (KGM; Clonetics, San Diego, CA). HOK-16B cells, human oral keratinocytes immortalized by transfection with cloned HPV type 16 genome (Park *et al.*, 1991), were also cultured in KGM. Three human oral cancer cell lines, CTHOK-16B-BaP, CTHOK-16B-DMBA, and CTHOK-16B-Dexa, were established by exposing the HOK-16B cells to benzo(*a*)pyrene, 7,12-dimethylbenz(*a*)anthracene, or dexamethasone, respectively (Min *et al.*, 1995). The cancer cell lines SCC-4, SCC-9, HEp-2, and FaDu were purchased from the American Type Culture Collection (Rockville, MD). Human oral squamous cell carcinoma (OSCC) cell lines (KOSCC-11, -25A, -25B, -25C, -25D, -25E, -33A, and -33B) were established by explantation culture from human OSCCs (Lee *et al.*, 2002). All oral cancer cell lines except the SCC-4 and SCC-9 lines were cultured in Dulbecco's modified minimum essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 0.4 μg/ml hydrocortisone. On the other hand, the SCC-4 and SCC-9 lines were grown in DMEM/Ham's F12 (Invitrogen, Carlsbad, CA) supplemented with 10% FBS and 0.4 μg/ml hydrocortisone.

### Antibodies and ECM proteins

Monoclonal antibodies (mAbs) against the human integrin α2 (P1E6), α3 (P1B5), α5 (P1D6), α6 (NKI-GoH3; Oncogene, Cambridge, MA), β1 (4B7), αv (AV1), αvβ6 (E7P6; Chemicon, Temecula, CA), β4 (3E1) subunits (Santa Cruz, Santa Cruz, CA) were used for flow cytometry assay. Human placental laminin, human plasma fibronectin, and rat-tail type I collagen were supplied by Sigma (St. Louis, MO).

### Flow cytometric analysis

Flow cytometric analysis of the cell surface integrin expression level was performed as described previously (Rodeck *et al.*, 1997). Briefly, cells were detached using a trypsin/EDTA solution, and trypsin was inactivated by washing cells in 15% FBS in PBS followed by 2 washes in reaction buffer [PBS without $Ca^{2+}$ and $Mg^{2+}$ containing 1% bovine serum albumin (BSA)]. Incubation with primary Abs was for 45 min followed by 2 washes in reaction buffer and incubation with secondary Abs, i.e. FITC-conjugated goat anti-mouse Ig (Becton-Dickinson, San Jose, CA) or goat anti-rat IgG (H+L) (Southern Biotechnology Associates, Birmingham, AL) for 45 min. After 2 additional washes in reaction buffer, cells were analyzed on a FACSCalibur flow cytometer. All incubations and washes were performed at 4°C.

### Centrifugal cell adhesion assay

Centrifugal cell adhesion was assessed using the method of Hashimoto-Uoshima *et al.* (1997). Briefly, 96-well U-bottomed polyvinylchloride plastic microtiter plates (Falcon, Lincoln Park, NJ) were coated with 100 μl/well of ECM components, in this case type I collagen (50 μg/ml), fibronectin (1 μg/ml), or laminin (10 μg/ml) in PBS by overnight adsorption at 4°C. The wells were then washed and unbound sites blocked with 10 mg/ml of BSA in PBS.

Cells were detached with 10 mM EDTA (Invitrogen), 100 μl of a cell suspension containing $5\times10^3$ cells was placed in each well, and the cells were allowed to adhere for 15 min at 37°C in an atmosphere of 5% $CO_2$. Following this incubation period, the plate was centrifuged at 1,000 g for 5 min at 4°C in a Sorval RT6000B centrifuge. The medium in the wells was gently removed and replaced with 17.5% formaldehyde containing 0.005% crystal violet in PBS for 2 h for fixation. The effect of centrifugation on cell adhesion to the previously coated ECM proteins was determined by examining the cells in the round bottom wells and scoring as previously described (Hashimoto-Uoshima *et al.*, 1997). In addition, the center of each well was photographed.

### Cell spreading assay

24-well culture plates were coated at 200 μl/well with the ECM proteins, in this case type I collagen (50 μg/ml), fibronectin (1 μg/ml), or laminin (10 μg/ml) in PBS by overnight adsorption at 4°C. The wells were then washed and unbound sites blocked with 10 mg/ml of BSA in PBS. Cells were detached with 10 mM EDTA. 200 μl of a cell suspension containing $1\times10^5$ cells was placed in each well, and the cells were allowed to adhere for 1 h at 37°C in an atmosphere of 5% $CO_2$. The cells were then fixed in 17.5% formaldehyde containing 0.005% crystal violet in PBS for 1 h. The fixative was then removed, and the wells were washed gently in PBS. To ensure a representative count, each well was divided into quarters and two fields per each quarter were photographed with an Olympus BX51 microscope at 300X. The cells that adopted a flattened, polygonal shape, with filopodia- and lamellipodia-like extensions were regarded as spreading cells. In contrast, the cells that resisted washing and remained tethered to the plate surface were regarded as non-spreading cells. A minimum of 200 cells was counted on each occasion.

### Migration assay

A cell migration assay was performed as previously described (Woodley *et al.*, 1988). Briefly, colloidal gold salts were immobilized on BSA coated coverslips and covered with ECM proteins, in this case type I collagen (50 μg/ml), fibronectin (25 μg/ml), or laminin (10 μg/ml) in PBS. Cells were suspended and plated ($1\times10^4$ cells) on coverslips and allowed to migrate for 48 h. Cells were then fixed in 4% formaldehyde in PBS and photographed under an Olympus BX51 microscope at 200X. The area of the migration track of each cell and the area of cell itself were determined using a computer equipped with Image Access-Analysis software (Bildanalysissystem AB, Sundbyberg, Sweden). The cell migration area was calculated by subtracting the area of the cell from the area of its migration track. A minimum of 100 cells was examined on each occasion. The relative migration levels were normalized by setting the mean migration area of proliferating NHOKs in the gold only-coverslip to one.

## Results

### Expression of α2, α3, α5, α6, β1, and β4 integrin subunits and αvβ6 integrin in NHOKs, HPV-immortalized HOK-16B line, and oral cancer cell lines

To study the levels of integrin expression during the neoplastic conversion of human oral keratinocytes, we analyzed the surface expressions of α2, α3, α5, α6, αv, β1, and β4 integrin subunits and αvβ6 integrin by flow cytometry. The results are presented in Figs. 1 and 2. The levels of α and β integrin subunits were showed decreased tendency during the neoplastic conversion of oral keratinocytes (Fig. 1A). Although the α5 integrin subunit was detected in these cells, its level was extremely low. The immortalized HOK-16B cells showed notably lower surface

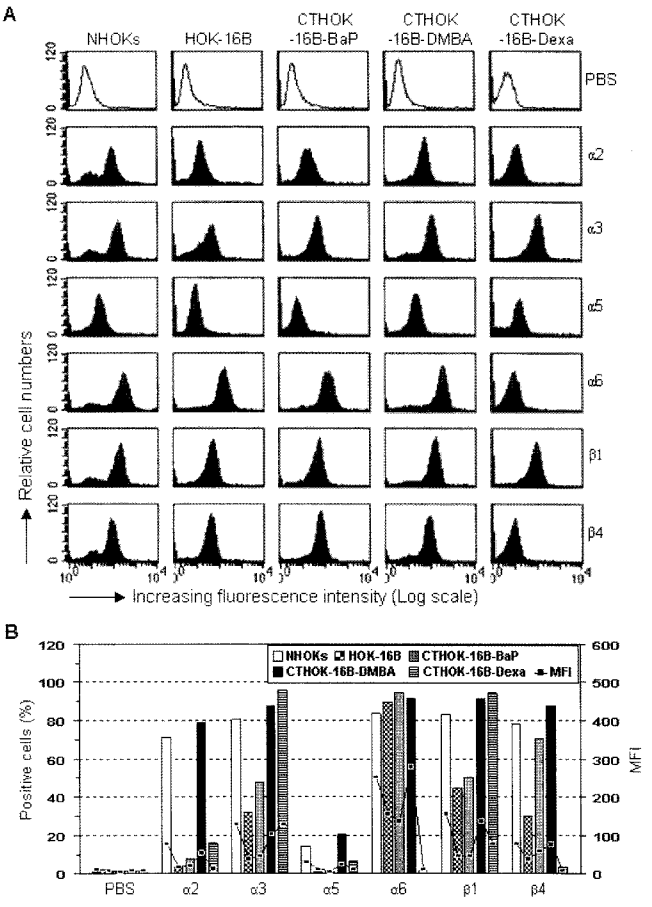


**Fig. 1.** Expression of a2, a3, a5, a6, b1, and b4 integrin subunits in NHOKs, HPV-immortalized HOK-16B cells, and three oral cancer cell lines. (A) Analysis of cell surface integrin subunit expression was carried out by flow cytometry. Cells were reacted with PBS, anti-a2 mAb P1E6 (a2 subunit), anti-a3 mAb P1B5 (a3 subunit), anti-a5 mAb P1D6 (a5 subunit), anti-a6 mAb NKI-GoH3 (a6 subunit), anti-b1 mAb 4B7 (b1 subunit), and anti-b4 mAb 3E1 (b4 subunit) and were subjected to flow cytometry. (B) The amount of integrin subunit expression was quantified the percentage of cells expressing integrin on their surface and their mean fluorescence intensity (MFI). Results of one representative experiment of three are shown.

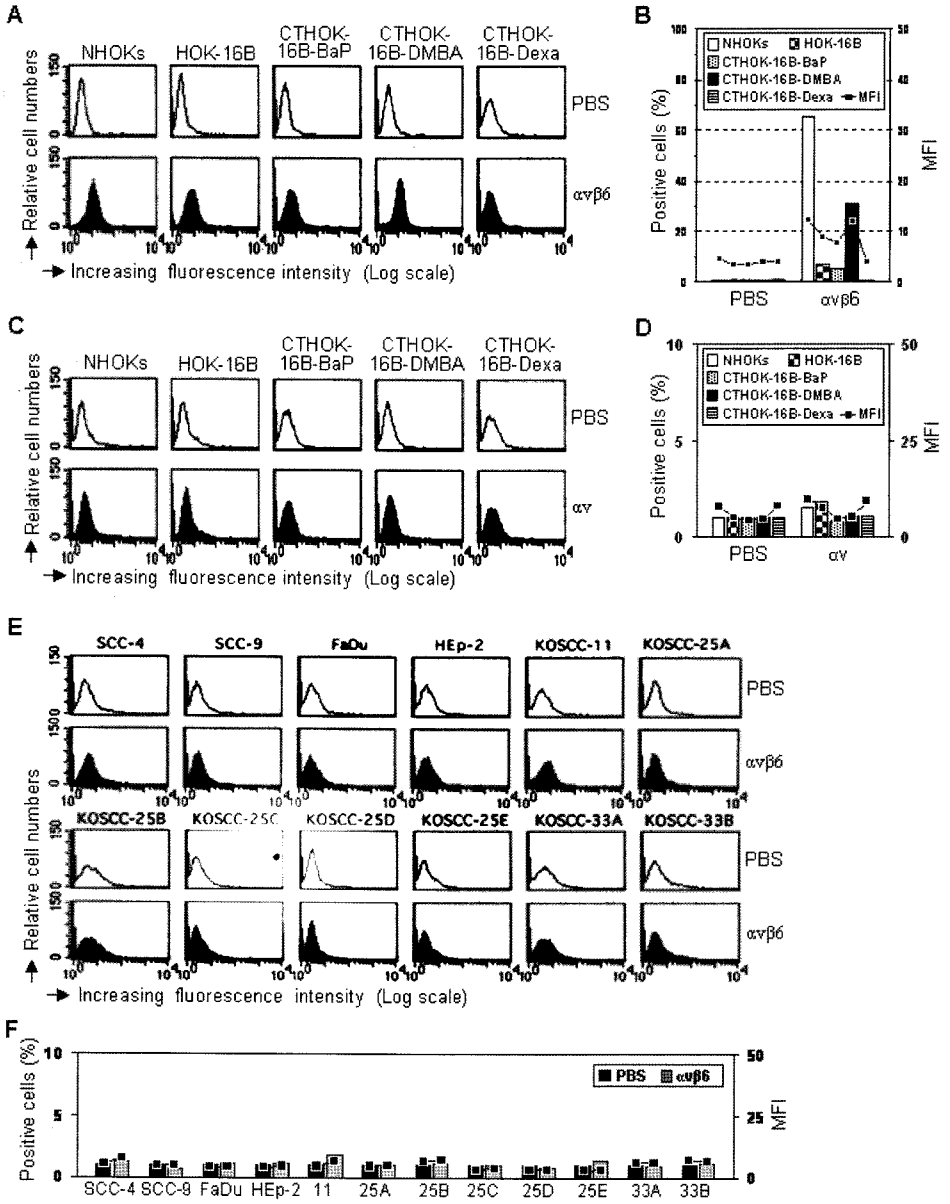


**Fig. 2.** Expression of αvβ6 integrin and αv integrin subunit in NHOKs, HPV-immortalized HOK-16B cells, and oral cancer cell lines. (A, C, and E) Cell surface integrin expression was carried out by flow cytometry. Cells were reacted with PBS, anti-αvβ6 mAb E7P6 (αvβ6 integrin) and anti-αv mAb (αv integrin subunit), and were subjected to flow cytometry. (B, D, and F) The amount of integrin expression was quantified the percentage of cells expressing integrin on their surface and their mean fluorescence intensity (MFI). Results of one representative experiment of three are shown. 11, KOSCC-11; 25A, KOSCC-25A; 25B, KOSCC-25B; 25C, KOSCC-25C; 25D, KOSCC-25D; 25E, KOSCC-25E; 33A, KOSCC-33A; 33B, KOSCC-33B.

expression of α and β integrin subunits than on NHOKs. In oral cancer cell lines, there was different expression of integrin as malignant transformed cells that were treated different kinds of chemical carcinogen. The CTHOK-16B-BaP line, similar to HPV-immortalized cells, expressed lower levels of integrin subunits tested. In the CTHOK-16B-Dexa line, the levels of α2, α6, and β4 integrin subunits were little expressed, and β1 integrin subunit was also downregulated. In contrast, the expressions of integrin subunits in the CTHOK-16B-DMBA were similar to that of NHOKs (Fig. 1B). Fig. 2 shows flow-cytometric analysis of αvβ6 and αv integrin in NHOKs, HPV-immortalized cells, oral cancer cell lines established from HPV-immortalized cells, and other human squamous carcinoma cell lines. There was no expression of αvβ6 integrin in NHOKs, immortalized cells, and oral cancer cell lines (Figs. 2A and B). It caused αv integrin subunit does not express in these cells (Figs. 2C and D). In the previous study, αvβ6 integrin is often expressed in squamous cell carcinomas (Jones *et al.*, 1997). Therefore, we examined the expression of αvβ6 integrin in other oral cancer cell lines, but it was not detected (Figs. 2E and F).

**Effect of ECM proteins on cell adhesion, spreading, and migration in NHOKs, HPV-immortalized HOK-16B line, and oral cancer cell lines**

The adhesion of cultured oral keratinocytes was evaluated using the centrifugal cell adhesion assay in serum-free medium, using as laminin (10 μg/ml), type I collagen (50 μg/ml), or fibronectin (1 μg/ml), which were absorbed onto the microtiter wells, as substrates. This assay was designed as an indicator of the relative strengths of cell adhesion to the tested ECM molecules (Hashimoto-Uoshima *et al.*, 1997). NHOKs, HPV-immortalized oral keratinocytes, and oral cancer cell lines mostly resisted to centrifugal detaching force on type I collagen and laminin, but fibronectin counterpart showed minimal adhesion activity under our conditions compared to BSA control. In oral cancer cell lines, the resistance to fibronectin was higher than NHOKs and HPV-immortalized oral keratinocytes (Figs. 3B and C). These results indicate that type I collagen and laminin are functionally active in terms of cell adhesion in human oral keratinocytes.

To further evaluate the adhesion of laminin, type I collagen, and fibronectin, we determined whether adherent cells were tethered to the substrate or spreading over the

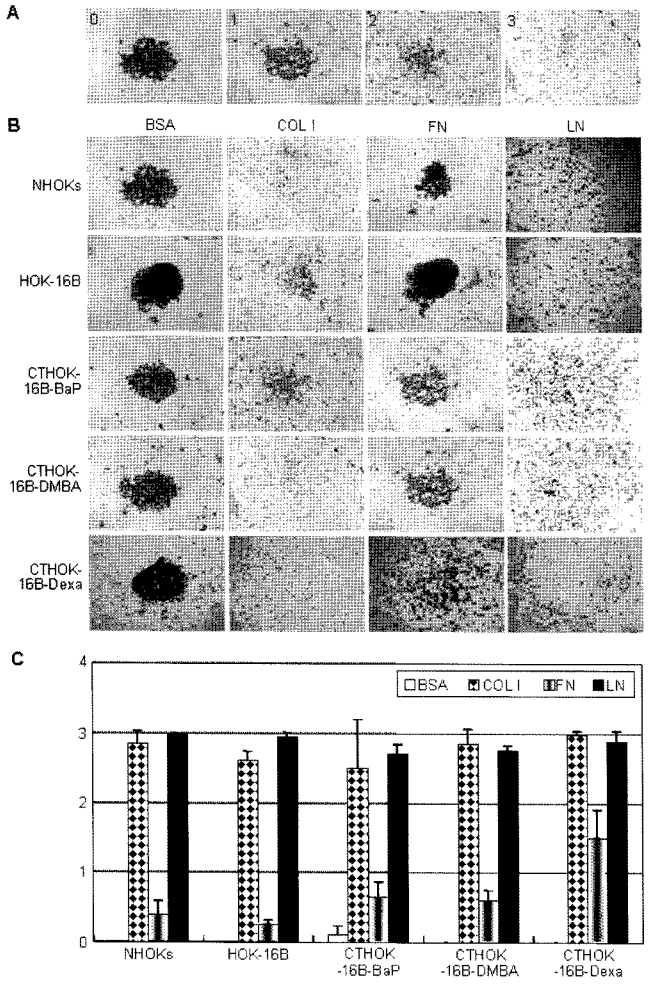


**Fig. 3.** The relative strength of cell adhesion to type I collagen, fibronectin, and laminin in NHOKs, HPV-immortalized HOK-16B cells, and three oral cancer cell lines. (A) Examples of scoring criteria are 0 for complete pelleting, 1 for mostly pelleting in the center with a few cells resisting detachment around the periphery, 2 for cells that resist detachment, with the exception of a few clusters at the center, and 3 for cells that have resisted detachment without pelleting or clusters in center or in the periphery of 96-well U-bottomed plates. (B) Examples of centrifugal cell adhesion assay on type I collagen (50 mg/ml), fibronectin (1 mg/ml), and laminin (10 mg/ml). BSA was used as a negative control giving 0 scores. (C) Level of cell adhesion of NHOKs, HPV-immortalized HOK-16B cells, and three oral cancer cell lines on ECM proteins. Values are expressed as the mean °æS.D. (n = 6). COL I, type I collagen; FN, fibronectin; LN, laminin.

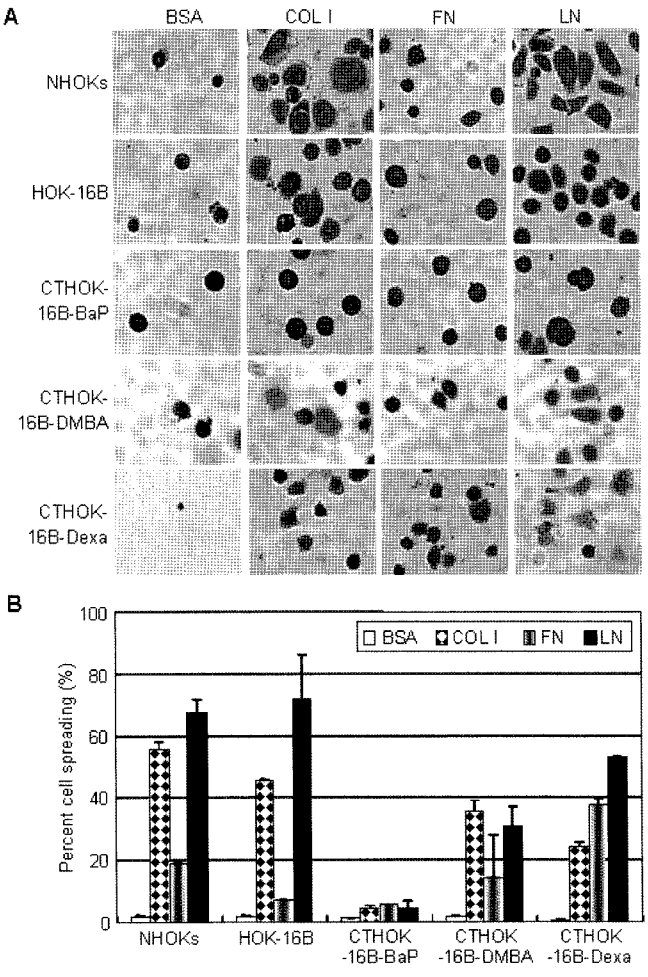


**Fig. 4.** Cell spreading on type I collagen, fibronectin, and laminin in NHOKs, HPV-immortalized HOK-16B cells, and oral cancer cell lines. (A) Examples of cell spreading on type I collagen (50 mg/ml), fibronectin (1 mg/ml), and laminin (10 mg/ml) in NHOKs, HPV-immortalized HOK-16B cells, and three oral cancer cell lines. BSA was used as a negative control. (B) Incidence of cell spreading to ECM proteins. The percentage of cells displaying a spread morphology was quantified by dividing the number of spread cells by the total number of bound cells. Values are expressed as the mean °æ S.D. (n = 4). X 300 magnification. COL I, type I collagen; FN, fibronectin; LN, laminin.

substrate. NHOKs, HPV-immortalized oral keratinocytes, and oral cancer cell lines adherent to integrin ligands were micro-photographed during the adhesion assay. On type I collagen, 55% of NHOKs and 45% of HPV-immortalized cells showed a spreading morphology, i.e., they adopted a flattened, polygonal shape, with filopodia- and lamellipodia-like extensions (Figs. 4A and B). The remaining non-spreading cells on integrin ligands resisted washing and remained tethered to the plate surface (Fig. 4A). In oral cancer cell lines, the ability of spreading on type I collagen was less than that of NHOKs or HPV-immortalized cells, because it demonstrated 5% of CTHOK-16B-BaP line, 35% of CTHOK-16B-DMBA line, and 24% of CTHOK-16B-Dexa line displayed a spreading morphology. Laminin displayed functional properties similar to type I collagen. On laminin, 67% of NHOKs and 71% of immortalized cells showed a spreading morphology, but oral cancer cell lines were not so much as that of NHOKs or immortalized cells. Extremely low cell spreading was observed on either BSA- or fibronectin-coated plates for tested cells except CTHOK-16B-Dexa line (Fig. 4A and B). These results indicate that type I collagen and laminin support cell spreading in NHOKs and HPV-immortalized cells, and that oral cancer cell lines are functionally inactive in terms of cell spreading over integrin ligands, such as type I collagen and laminin.

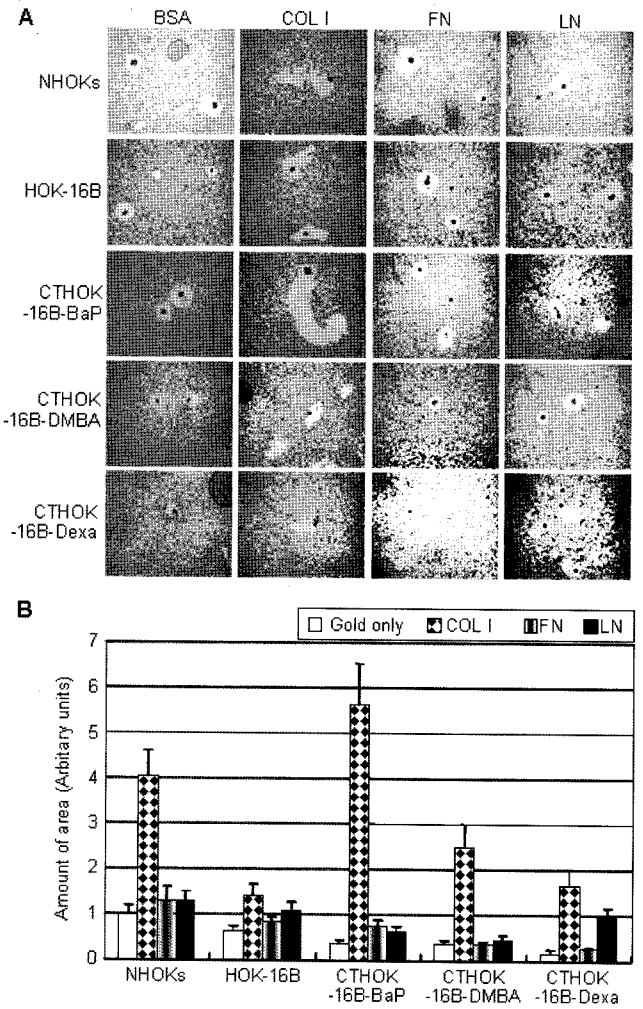


**Fig. 5.** Cell migration on type I collagen, fibronectin, and laminin in NHOKs, HPV-immortalized HOK-16B cells, and oral cancer cell lines. (A) Migration of human oral keratinocytes on ECM proteins after 48 h of culture by the gold colloidal migration assay. BSA-coated coverslips were immobilized with colloidal gold and then coated with or without type I collagen (50 mg/ml), fibronectin (25 mg/ml), or laminin (10 mg/ml), and oral keratinocytes were plated and cultured for 48 h. Representative fields were photographed with an Olympus BX51 microscope at 200X. (B) Levels of cell migration on ECM proteins. The areas covered by human oral keratinocytes when migrating on coated ECM proteins were determined using the track assay. The relative migration areas were normalized by setting the mean migration area of NHOKs in the gold only-coverslip to one. COL I, type I collagen; FN, fibronectin; LN, laminin.

Since integrin ligands are a migratory as well as an adhesive substrate (Miyazaki *et al.*, 1993; Giannelli *et al.*, 1997), we evaluated cell migration on ECM proteins using a phagokinesis assay. Fig. 5A shows representative microscopic fields for keratinocytes migrating on immobilized type I collagen, fibronectin, and laminin, which are covering the colloidal gold. Human oral keratinocytes plated on the immobilized gold alone made very small circular tracks after 48 h migration (Fig. 5A). Coating the slides with type I collagen promoted cell migration of the NHOKs, whereas fibronectin- and laminin-coated slides showed negligible cell migration compared to that observed on gold only (Figs. 5A and B). NHOKs migrated approximately 4-fold more on type I collagen-coated slides than that on immobilized gold only. In addition, the migration patterns of oral cancer cell lines were generally similar to that of NHOKs in ECM protein-coated slides. However, the CTHOK-16B-BaP line migrated more than NHOKs on type I collagen-coated slides. The CTHOK-16B-DMBA and CTHOK-16B-Dexa lines migrated less than NHOKs but more than HPV-immortalized cells. These results indicate that laminin and type I collagen promote cell adhesion of NHOKs, immortalized cells, and oral cancer cell lines, but functionally inactivate cell spreading during neoplastic conversion. In addition, only type I collagen among the ECM proteins tested functions as a migration stimulus in oral keratinocytes, and oral cancer cell lines migrate more than HPV-immortalized cells.

## Discussion

Since the first observation alters integrin expression with transformation (Plantefaber and Hynes, 1989), several *in vitro* and *in vivo* studies have demonstrated the association between the regulation of integrin expression and cancer. The transformed cells are greatly affected their behavior by interaction with the surrounding ECM. Different classes of cell adhesion receptors mediate many of those interactions. Integrins are one of them and play a critical role in physio-

logical events like adhesion, migration, proliferation, and cell survival. Moreover, integrins mediated pathological conditions, such as inflammation and tumor progression and influenced several important aspects of cancer cell behavior, including motility and invasiveness, cell growth, and cell survival. Three major integrins α2β1, α3β1, and α6β4 are expressed in normal basal keratinocyte layer (Adams and Watt, 1993), and αvβ6 is often expressed in squamous cell carcinomas (Jones *et al.*, 1997). The malignant properties are contributed by positive or negative expression of integrin subunits. We thus determined the expression levels of α2, α3, α5, α6, αv, β1, and β4 integrin subunits and αvβ6 integrin in NHOKs, HPV-immortalized HOK-16B line, and oral cancer cell lines to investigate the association between the expression levels of integrins and the change of cellular responses to ECM proteins during neoplastic conversion of oral keratinocytes.

In the present study, the expression of α2 and β1 integrin subunits and the ability of spreading on type I collagen were decreased both in HPV-immortalized HOK-16B cells and in oral cancer cell lines. α2β1 integrin, a receptor for collagen and laminin, is considered to play a role in keratinocyte intercellular adhesion (Carter *et al.*, 1990; Symington *et al.*, 1993), and a reduction of α2β1 integrin was observed in HPV-immortalized keratinocytes (Hodivalva *et al.*, 1994). These results also support a previous study that α2 integrin reduction may serve as a step in the progression toward malignancy (Sashiyama *et al.*, 2002). In addition, the expression of α6β4 integrin and the ability of spreading to laminin in CTHOK-16B-BaP and CTHOK-16B-Dexa lines were decreased. The α6β4 integrin is polarized on the basal lateral membrane in normal epithelial cells (Sonnenberg *et al.*, 1991; Quaranta, 1990). Epithelial malignancies, such as invasive human prostate, bladder, basal cell cancers, and oral squamous cell carcinomas, have lost expression of the α6β4 integrin (Downer *et al.*, 1993; Cress *et al.*, 1995; Liebert and Seigne, 1996; Allen *et al.*, 1998; Schofield *et al.*, 1998), while in other malignancies, such as human colon cancer, expression persists and in human breast cancer expression is variable (Natali *et al.*, 1992; Hanby *et al.*, 1993; Gui *et al.*, 1995; Rabinovitz and Mercurio, 1996). α6β4 integrin on the carcinoma cells does not distribute or polarize as does the integrin on the keratinocyte and papilloma cells, and the expression of this integrin is decreased (Witkowski *et al.*, 2000). Although we detected little expression of α6β4 integrin in the CTHOK-16B-Dexa line, the abilities of adhesion and spreading were not completely blocked. It may be due to another laminin receptor α3β1, which expressed as much as NHOKs in CTHOK-16B-Dexa line.

We have demonstrated that the expression of α5 integrin subunit was almost negligible. α5β1 integrin is a receptor for fibronectin, but in oral cancer cell lines the effect of fibronectin was upregulated in centifugal cell adhesion assay. Another fibronectin receptor αvβ6 integrin did not express on normal epithelial cells; however, it becomes highly expressed during morphogenesis, wound healing, and tumorigenesis (Sheppard *et al.*, 1990; Breuss *et al.*, 1995), and *de novo* expression of this epithelium-restricted integrin has been observed in cancers of the colon and oropharynx (Agrez *et al.*, 1996; Thomas *et al.*, 1997). However, we could not detect the different expression of αvβ6 integrin between oral cancer cell lines and NHOKs. In addition, we examined the expression of αvβ6 integrin in other oral cancer cell lines, but we could not detect any alteration compare with NHOKs. It may be caused by no expression of αv integrin subunit in other oral cancer cell lines, including SCC-4, SCC-9, FaDu, HEp-2, KOSCC-11, KOSCC-25A, KOSCC-25B, KOSCC-25C, KOSCC-25D, KOSCC-25E, KOSCC-33A, and KOSCC-33B (data not shown) as well as NHOKs, HPV-immortalized cells, and oral cancer cell lines. Therefore, fironectin receptors expressed on keratinocytes, α5β1, αvβ1, αvβ5 and αvβ6, were not expressed in oral keratinocytes tested.

In this study, type I collagen functioned as a migration stimuli in keratinocytes. In previous studies, type I and type IV collagens support human keratinocyte migration (Kim *et al.*, 1992; 1994), and that laminin-1 and -5 inhibit keratinocyte migration and keratinocyte motility driven by type I and type IV collagens in dose-dependent manner (O'Toole *et al.*, 1997). Although there were some differences among oral cancer cell lines, all of them migrated more than HPV-immortalized cells. Especially, the CTHOK-16B-BaP line migrated approximately 4-fold more on type I collagen-coated slides than that of HPV-immortalized cells. In contrast, low cell spreading was observed on type I collagen for the oral cancer cell lines. These results suggest that the ability of migration might be increased during tumorigenic transformation of human oral keratinocytes.

In summary, our results indicate that downregulation of integrin subunits causes the changes of cellular responses to ECM proteins during neoplastic conversion of human oral keratinocytes, and that cellular responses to ECM proteins in oral cancer cell lines established by exposing different carcinogens are variable according to chemical carcinogens treatment.

## Acknowledgements

This work was supported by a grant from the KOSEF through the Intellectual Biointerface Engineering Center at Scoul National University(to B.-M. M.).

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