Journal of Cellular Biochemistry 78:141–150 (2000)

Vimentin Expression in Human Squamous Carcinoma Cells: Relationship with Phenotypic Changes and Cadherin-Based Cell Adhesion

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Abstract Phenotypic changes resembling an epithelial-to-mesenchymal transition often occur as epithelial cells become tumorigenic. Two proteins that have been implicated in this process are vimentin and N-cadherin. In this study, we sought to establish a link between expression of vimentin and N-cadherin as oral squamous epithelial cells undergo a morphologic change resembling an epithelial-to-mesenchymal transition. We found that N-cadherin and vimentin did not influence the expression of one another. J. Cell. Biochem. 78:141–150, 2000.

Key words: squamous cell carcinoma; N-cadherin; vimentin; epithelial to mesenchymal transition

Intermediate filaments are complex cytoskeletal arrays that are prevalent in the perinuclear region where they form a cage that surrounds and appears to position the nucleus. Continuous with this perinuclear array, intermediate filaments extend radially through the cytoplasm, eventually forming close associations with the cell surface. Frequently these membrane associations are concentrated in regions containing desmosomes, hemidesmosomes, and other types of cell-adhesion structures [Reviewed in Schwarz et al., 1990; Fuchs and Weber, 1994; Heins and Aebi, 1994; Klymkowsky, 1995].

Intermediate filaments are composed of highly elongated fibrous proteins that have an amino-terminal head domain, a carboxyl-terminal tail domain, and a central rod structure. Intermediate filament proteins are highly diverse, ranging in molecular weight from 40,000 to 200,000 Da. Intermediate filament proteins are expressed in a tissue- and cell-type–specific manner with epithelial cells expressing keratins, mesenchymal cells expressing vimentin, and neuronal cells expressing proteins of the neurofilament family.

The organization of the cytoskeletal network suggests that intermediate filaments are involved in numerous cellular functions, including maintenance of cell shape. This is supported by several studies. For example, point mutations in keratin genes result in blistering skin diseases, which are accompanied by phenotypic alterations in individual keratinocytes [reviewed in Fuchs, 1995]. The classic epithelial-to-mesenchymal transition that is essential to normal mammalian development is hallmarked by a change from cytokeratin expression to vimentin expression and is accompanied by loss of tight cell-cell adhesion and acquisition of a fibroblastic morphology [Hay and Zuk, 1995]. Recently it was shown that exogenous expression of vimentin in human breast cancer cells resulted in conversion from an epithelial to mesenchymal phenotype, reminiscent of the interconversions that occur during development [Hendrix et al., 1997].

Numerous studies have demonstrated that increased expression of vimentin in carcinomas correlates with parameters of malignant potential such as tumor grade and survival incidence [reviewed in Hendrix et al., 1996]. Carcinomas reported to consistently express vimentin include those of the kidney, endometrium, thy-

Grant sponsor: National Institutes of Health; Grant numbers: GM51188 and DE12308.

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Received 10 December 1999; Accepted 28 January 2000

This article published online in Wiley InterScience, April 2000.

roid, lung, ovary, salivary gland, breast, and prostate [Iyer and Leong, 1992]. Unlike the polarized epithelial cells of glandular-derived tumors, squamous carcinoma cells have been thought to express only keratin. However, clinical studies have shown that vimentin is expressed in these tumors in vivo, and that vimentin expression correlates with poor prognosis [Raymond and Leong, 1989; Domagala et al., 1990; Iyer and Leong, 1992]. A recent study by Tomson et al. [1996] has shown that expression of vimentin by squamous carcinoma cells coincides with loss of both anchorage-dependent growth and the cell-cell adhesion molecule E-cadherin.

In a previous study, we showed that expression of N-cadherin by oral squamous epithelial cells resulted in down-regulation of E-cadherin and an apparent epithelial-to-mesenchymal transition [Islam et al., 1996]. Because N-cadherin is a cadherin family member frequently expressed by mesenchymal cells, we hypothesized that in squamous epithelial cells expression of vimentin would correlate with expression of N-cadherin.

Human squamous cell carcinoma cell line SCC1 cells had an epithelial morphology, did not express vimentin and had low expression of N-cadherin. On the other hand, SCC11B cells had a scattered, fibroblastic morphology and expressed both vimentin and N-cadherin. We previously showed that transfection of N-cadherin into SCC1 cells resulted in transition from cells with an epithelial morphology to cells with a more scattered, fibroblastic morphology [Islam et al., 1996]. Therefore we aimed to determine if vimentin or N-cadherin regulated the expression of the other protein. First we isolated clones of SCC11B cells that were negative for vimentin. Transfection of vimentin into these cells did not result in increased expression of N-cadherin. Likewise, transfection of N-cadherin into SCC1 cells did not result in increased expression of vimentin. Thus, although both vimentin and N-cadherin are associated with an epithelial-to-mesenchymal transition, their expression is not codependent in oral squamous epithelial cells.

MATERIALS AND METHODS

Cell Culture

The human squamous cell carcinoma cell lines SCC1 and SCC11B (kind gifts of Dr. Thomas Carey, University of Michigan, Ann Arbor, MI) were cultured in minimal essential medium supplemented with 10% fetal calf serum (Hyclone Laboratories, Logan, UT) as described previously [Islam et al., 1996].

Antibodies and Reagents

Rabbit polyclonal antibodies to human E-cadherin [Wheelock et al., 1987] and mouse monoclonal antibodies to N-cadherin [13A9; Johnson et al., 1993] have been described. Mouse monoclonal anti-vimentin antibodies (V9 and LN-6) were purchased from Sigma Chemical Company, St. Louis, MO. Antibody V9 recognizes human vimentin but not mouse vimentin; antibody LN-6 recognizes both human and mouse vimentin. Antibody LN-6 to vimentin is an IgM, making it possible to do immunofluorescence colocalization for vimentin using LN-6 and N-cadherin using 13A9, which is an IgG.

Generation of Vimentin-Positive and Vimentin-Negative Clones of 11B cells.

Vimentin-positive and vimentin-negative cells were isolated through dilution cloning by plating 1 cell/well in 96-well plates. Clones were screened for vimentin-expressing and nonexpressing cells using immunofluorescence microscopy and western blot analysis. Several clones of each type were chosen for further studies.

Molecular Constructs and Transfections

The mouse vimentin cDNA in the pSP64 plasmid was a kind gift from Dr. Katrina Trevor, St. Luke's Medical Center, Milwaukee, WI [Hendrix et al., 1997]. A 1.8-kb BamHI fragment was excised and moved into the expression vector pLKhygro. pLKhygro was constructed from pLKneo using the hygromycin resistance gene from pG1-h (a kind gift from Dr. David Berg, Eli Lilly, Indianapolis, IN) [Berg et al., 1993] to replace the neomycin resistance gene in pLKneo (a kind gift from Dr. Nicholas Fasel, University of Lausanne, Lausanne, Switzerland) [Hirt et al, 1992]. Cell cultures were transfected using a calcium phosphate kit (Stratagene, La Jolla, CA) according to the manufacturer's instructions and selected in hygromycin (Gibco Life Technologies). Colonies of hygromycin-resistant cells were isolated, induced with dexamethasone as described [Islam et al., 1996], and screened for

expression of the transfected gene by immunofluorescence analysis with anti-vimentin antibody LN-6. Positive clones were stained with anti-vimentin clone V9 to verify the cells were expressing the mouse transgene and not endogenous human vimentin. Clones of N-cadherin–transfected SCC1 cells have been described [Islam et al., 1996].

Microscopy

Cells were grown on glass cover-slips, fixed for 30 min with Histochoice tissue fixative (Amresco, Solon, OH) or methanol at 0° for 1 min. Histochoice was used when possible to best preserve cellular morphology. The epitope recognized by antibody LN-6 is not preserved in cells fixed with Histochoice, thus cells to be stained with this antibody were fixed in methanol. Cover-slips were blocked with 10% goat serum in phosphate buffered saline for 1 h and exposed to primary antibodies for 1 h, followed by species-specific secondary antibodies conjugated to fluorescein (Jackson ImmunoResearch Laboratories, West Grove, PA). For colocalization of N-cadherin and vimentin, cells were stained with both primary antibodies followed by fluorescein-conjugated anti-mouse IgG and rhodamine-conjugated anti-mouse IgM. Fluorescence was detected using a Zeiss Axiophot microscope (Carl Zeiss Inc., Thornwood, NY) equipped with epifluorescence. All pictures were taken using a 40× objective and Kodak T-Max 3200 film.

Detergent Extraction of Cells

Monolayers of cells were washed with phosphate-buffered saline at room temperature and extracted on ice with 2 ml/75 cm^2 flask 10 mM Tris-acetate, pH 8.0, 0.5% Nonidet P-40 (BDH Chemicals Ltd., Poole, United Kingdom), 1 mM EDTA, and 2 mM phenylmethylsulfonyl fluoride. Insoluble material was removed by centrifugation at 15,000*g* for 15 min at 4°C.

Electrophoresis and Immunoblotting

Polyacrylamide slab gel electrophoresis in the presence of sodium dodecylsulfate (SDS-PAGE) was done as previously described [Johnson et al., 1993]. SDS-PAGE resolved proteins were transferred to nitrocellulose and immunoblotted as described [Knudsen and Wheelock, 1992]. Protein quantitation was done using the Bio-Rad assay (Bio-Rad Laboratories, Richmond, CA) according to the manufacturer's protocol. Equal amounts of protein were loaded into each lane for SDS-PAGE.

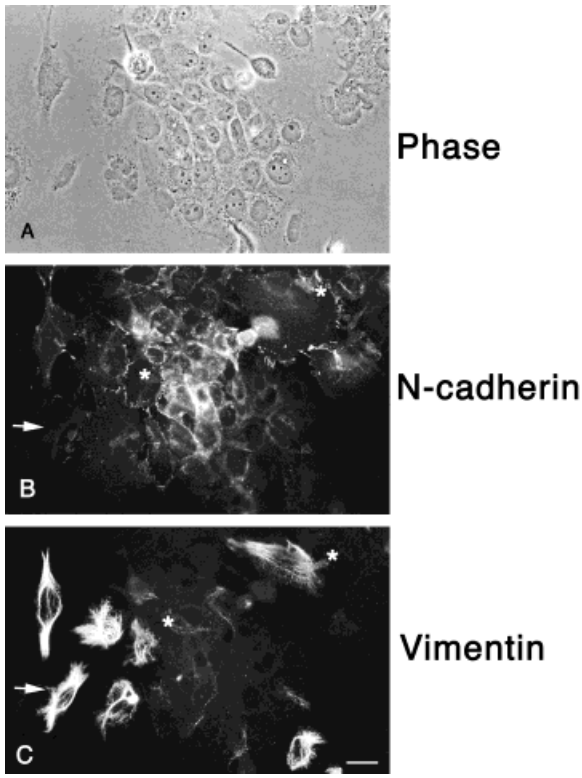

Fig. 1. Localization of N-cadherin and vimentin in SCC11B oral squamous carcinoma cells. Cells were grown on glass cover-slips and processed for double-label immunofluorescence with the monoclonal IgM antibody LN-6 to vimentin (**Panel C**) and the monoclonal IgG antibody 13A9 to N-cadherin (**Panel B**). The secondary antibodies were rhodamine-conjugated anti-mouse IgM and FITC-conjugated anti-mouse IgG. **Panel A** is a phase micrograph of the cells. Some vimentin-positive cells express high levels of N-cadherin (*) whereas other vimentin-positive cells express low levels of N-cadherin (arrowheads). Bar equals 10 μm.

RESULTS

Expression of Vimentin by Squamous Carcinoma Cells

We began our study with SCC11B cells, which were derived from an oral squamous cell carcinoma of the larynx [Baker, 1985]. We previously showed that this cell line expresses high levels of N-cadherin and low levels of E-cadherin [Islam et al., 1996]. Morphologically, SCC11B cells appeared more like fibroblasts than epithelial cells and displayed tenuous cell-cell contacts (Fig. 1A). When examined by immunofluorescence light micros-

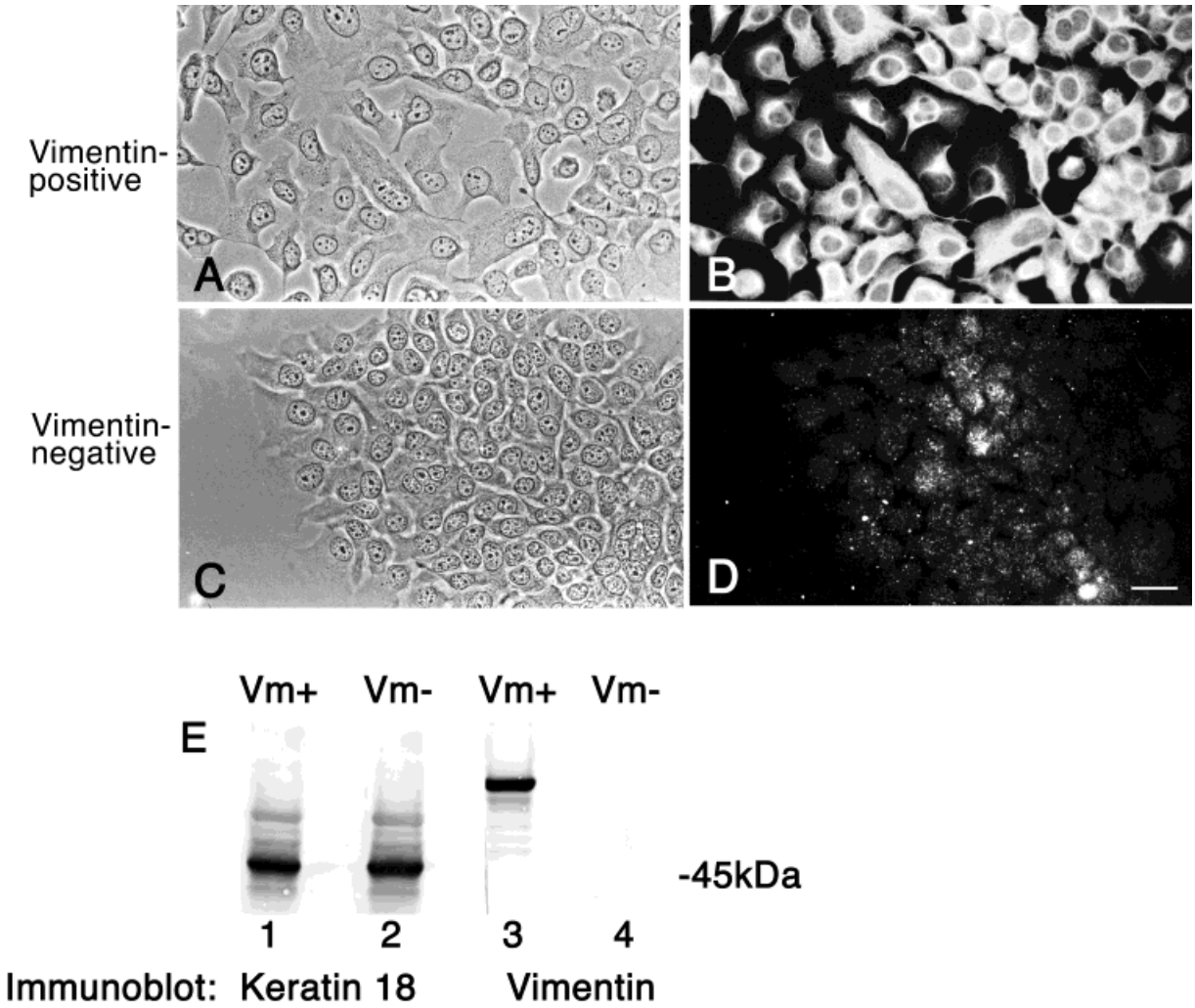

Fig. 2. Analysis of vimentin-positive and vimentin-negative clones of SCC11B cells. Cells were grown on glass cover-slips and processed for immunofluorescence with the monoclonal IgM antibody LN-6 to vimentin. **Panels A and C** show the morphology of vimentin-positive and vimentin-negative cells, respectively. **Panels B and D** show the localization of vimentin. Bar equals 10 μm. **Panel E** is an immunoblot of these cells for keratin (lanes 1 and 2) and vimentin (lanes 3 and 4). The 45-kDa marker is indicated.

copy, the population was heterogeneous with respect to N-cadherin expression. Some cells expressed high levels of N-cadherin and others expressed low levels (Fig. 1B) [Islam et al., 1996]. Figure 1C shows that these cells also were heterogeneous with regard to vimentin expression. Although vimentin and N-cadherin are both expressed in tissues of mesenchymal origin, we found that cells with high levels of N-cadherin were not necessarily those with high levels of vimentin. Asterisks in Fig. 1 point out vimentin-positive cells that express high levels of N-cadherin, whereas arrowheads point out vimentin-positive cells with low levels of N-cadherin.

To determine if there was a relationship among fibroblastic morphology, N-cadherin expression, and vimentin expression, we cloned vimentin-positive and vimentin-negative sublines of SCC11B by limiting dilution. Several positive clones and several negative clones were chosen for our studies. Figure 2 shows phase microscopy and immunofluorescence staining for vimentin in two representative clones, one positive for vimentin and one negative for vimentin. The expression of vimentin in the positive clones was homogeneous, and the staining pattern was consistent with that seen in mesenchymal cells. Likewise, the vimentin-negative

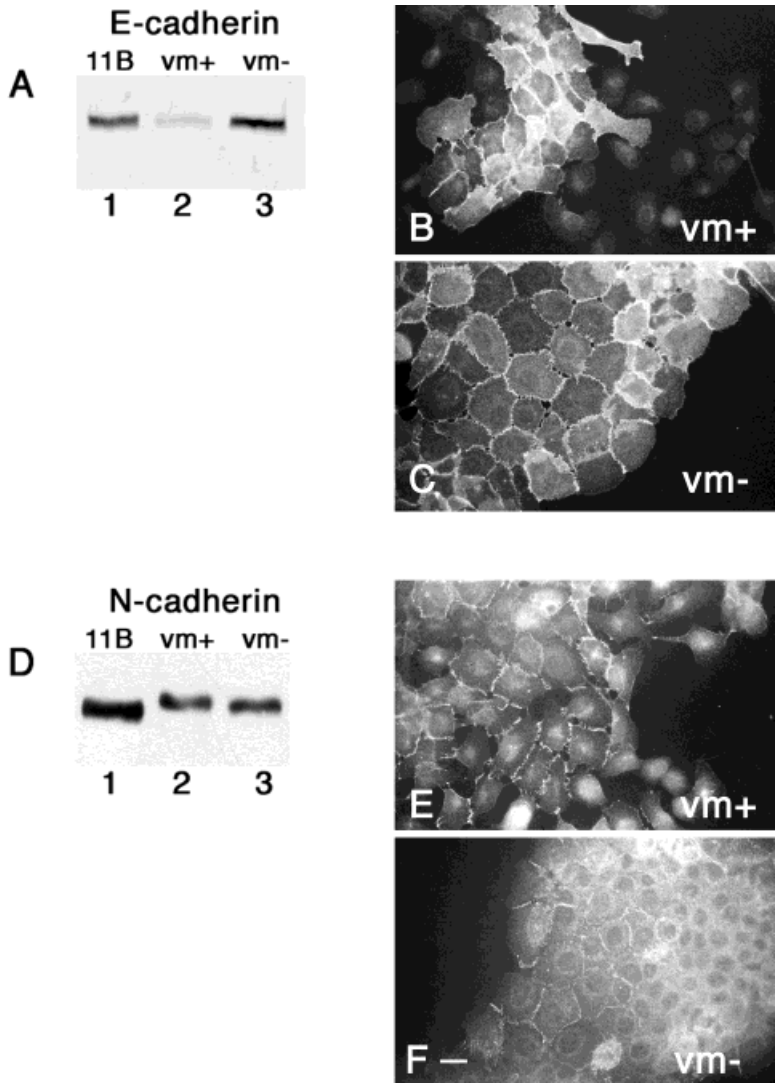

Fig. 3. Expression of cadherins by vimentin-positive and vimentin-negative clones of SCC11B cells. **Panels A–C:** Cells were grown to confluence and processed for immunoblot analysis using rabbit polyclonal anti-E-cadherin (Panel A) or grown on glass cover-slips and processed for immunofluorescence with the same antibody (Panels B and C). **Panels D–F:** Cells were grown to confluence and processed for immunoblot analysis using mouse monoclonal anti-N-cadherin (Panel D) or grown on glass cover-slips and processed for immunofluorescence with the same antibody (Panels E and F). Bar equals 10 μm.

clones were homogeneously negative for the expression of vimentin. The diffuse staining seen in Fig. 2D was background staining due to the secondary antibody. This is confirmed by the Western blot that is completely negative for vimentin (Fig. 2E, lane 4). Interestingly, keratin 18, which is normally expressed by epithelial cells, was expressed at similar levels in both the vimentin-positive and vimentin-negative clones (Fig. 2E, lanes 1 and 2). Vimentin-negative cells exhibited an epithelial morphology with tight cell-to-cell adhesion and grew as a sheet of cells in confluent cultures (Fig. 2C). In contrast, vimentin-positive cells had an elongated fibroblastic morphology and very little cell-to-cell contact (Fig. 2A).

Expression of Cadherins in Vimentin-Positive and Vimentin-Negative Clones of SCC11B Cells

Our previous studies showed that transfection of oral squamous cell carcinoma cells with N-cadherin resulted in a morphologic change resembling an epithelial-to-mesenchymal transition accompanied by decreased expression of E-cadherin. Thus, we were interested in whether or not selecting for vimentin-positive cells also selected for cells with increased N-cadherin and decreased E-cadherin expression. A number of clones of vimentin-positive and vimentin-negative cells were isolated by limiting dilution. Representative clones are shown in Fig. 3. Figure 3A shows that, indeed, the expression of E-cadherin was lower in

vimentin-positive clones (lane 2) than in vimentin-negative clones (lane 3), suggesting that vimentin up-regulation may be related to E-cadherin down-regulation. Immunofluorescence localization showed that the level of E-cadherin expression in the vimentin-positive cells was heterogeneous, and that much of the cadherin was diffusely localized rather than being characteristically concentrated at regions of cell-cell contact. There were also cells that did not express E-cadherin. In contrast, the expression of E-cadherin in vimentin-negative cells was more homogeneous and was localized to regions of cell-cell contact (Fig. 3C).

We predicted that N-cadherin would be up-regulated in the vimentin-positive clones of cells. To confirm this, we quantified the expression of N-cadherin in vimentin-positive and vimentin-negative clones by immunoblot analysis (Fig. 3D). N-cadherin expression levels did not appear to change. Indeed, the difference in expression levels was not sufficient to convince us that selecting clones of cells on the basis of vimentin expression also selected for cells expressing N-cadherin. The slight differences in apparent molecular weight of N-cadherin in clones of SCC11B cells is due to glycosylation variations in these cells [Islam et al., 1996]. Examination of these two cell populations using immunofluorescence microscopy showed that N-cadherin localization in vimentin-positive cells was typical of that found in fibroblasts [Sacco et al., 1995] and other mesenchymal cells [Wheelock and Knudsen, 1991]. That is, the protein was localized in regions where one cell made contact with one another (Fig. 3E). The expression of N-cadherin in vimentin-negative cells was more diffuse than in vimentin-positive cells and appeared not to be restricted to regions of cell-cell contact. These observations raise the interesting possibility that vimentin expression might be correlated with the distribution of N-cadherin when cells undergo an epithelial-to-mesenchymal transition.

Relationship Between N-cadherin and Vimentin Expression

Because both N-cadherin and vimentin have been correlated with a scattered fibroblastic phenotype, we wished to determine whether or not expression of one of these proteins would influence expression of the other. To address this question, we first transfected a full-length mouse vimentin cDNA under control of the dexamethasone-inducible mouse mammary tumor virus promoter into a clone of vimentin-negative cells. Hygromycin-resistant clones were selected and propagated. Mouse vimentin expression was not stable over time and the expanded population of cells quickly became heterogeneous for expression of mouse vimentin. The cells morphologically resembled epithelial cells rather than fibroblasts, even though many cells expressed high levels of both N-cadherin and vimentin (Fig. 4). Significantly, vimentin expression did not correlate with N-cadherin expression. That is, the whole population of cells expressed N-cadherin, whereas only about 50% of the cells expressed mouse vimentin.

Also of interest was whether or not forced expression of N-cadherin influenced the expression of vimentin in an N-cadherin–negative oral squamous epithelial cell. For these studies, we switched to the N-cadherin–negative, vimentin-negative SCC1 oral squamous epithelial cell line. We had previously shown that transfection of N-cadherin into these cells resulted in a more fibroblastic phenotype [Islam et al., 1996]. Fig. 5A–C show that the parental SCC1 cells express neither N-cadherin (Fig. 5B) nor vimentin (Fig. 5C). The transfectant is uniformly positive for N-cadherin (Fig. 5E). However, it remains negative for vimentin (Fig. 5F). Thus, we can conclude that expression of two proteins correlated with epithelial-to-mesenchymal transitions, namely vimentin and N-cadherin, do not influence the expression of one another.

DISCUSSION

N-cadherin is expressed by a wide variety of cells including neurons, mesothelial cells, cardiac and skeletal muscle, lens epithelial cells, and fibroblasts. N-cadherin can promote strong cell-cell interactions. For example, in cardiac muscle it plays an essential role in the morphogenesis of the myocardium [Radice et al., 1997] and in the formation of cell-cell contacts that allow cardiomyocytes to beat coordinately [Volk and Geiger, 1984; Soler and Knudsen, 1994]. In addition, tissues such as the mesothelium and the lens use N-cadherin to form junctions that very much resemble the E-cadherin–mediated contacts of epithelial cells [Volk and Geiger, 1984; Peralta Soler et al., 1995]. However, N-cadherin is also expressed by cells such

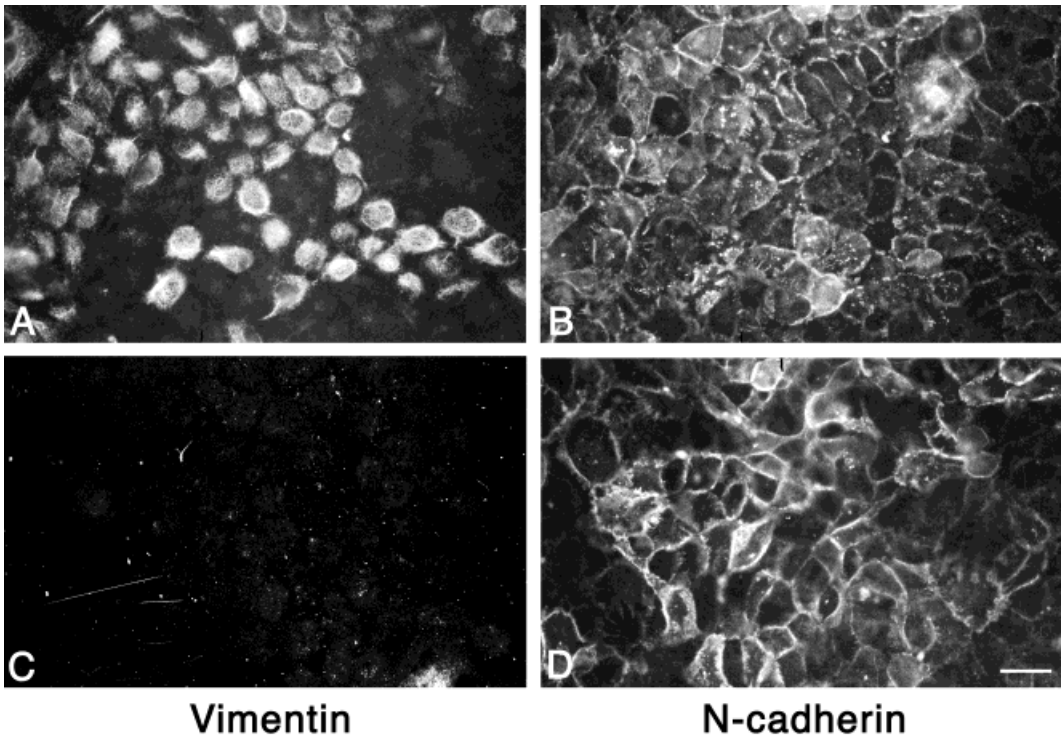

Fig. 4. Expression of exogenous vimentin in oral squamous epithelial cells does not influence the expression of N-cadherin. Clones of SCC11B cells expressing mouse vimentin were grown on glass cover-slips and processed for double-label immunofluorescence with the monoclonal IgM antibody LN-6 to vimentin (**Panel A**) and the monoclonal IgG antibody 13A9 to N-cadherin (**Panel B**). Control non-transfected cells are shown in **Panels C** (vimentin) and **D** (N-cadherin). The secondary antibodies were rhodamine-conjugated anti-mouse IgM and FITC-conjugated anti-mouse IgG. Bar equals 10 μm.

as fibroblasts that do not typically display close cell-cell contacts, but rather, act as solitary cells. We previously reported that inappropriate expression of N-cadherin in epithelial squamous carcinoma cells resulted in a scattered, more fibroblastic phenotype [Islam et al., 1996], suggesting that expression of N-cadherin may serve as a marker for epithelial-to-mesenchymal transition in these cells.

It has been shown by others that transfection of vimentin into polarized epithelial cells results in an epithelial-to-mesenchymal transition. Therefore, we sought to demonstrate a correlation between vimentin and N-cadherin expression in squamous epithelial cells. Interestingly, we found that N-cadherin was expressed at a similar level in both vimentin-positive and vimentin-negative cells. However, the localization pattern of N-cadherin was quite different between these two populations. N-cadherin was localized at cell-cell contacts in vimentin-positive cells, but was diffuse and less localized at regions of cell-cell contact in vimentin-negative cells. These data raise the possibility that N-cadherin may promote more effective cell-cell interactions in vimentin-positive cells. Thus, there may be an additive effect on morphology in cells that are positive for both N-cadherin and vimentin.

In a previous study, we used N-cadherin antisense technology to knock out N-cadherin in an aggressive, N-cadherin–positive squamous epithelial tumor cell line. The loss of N-cadherin resulted in a more epithelial morphology, suggesting that the N-cadherin–negative cells were less tumorigenic than N-cadherin–positive cells [Islam et al., 1996]. Because vimentin expression is associated with poorly differentiated tumors, we tested to see if there was any change in the expression of vimentin in the N-cadherin antisense-transfected cells versus the N-cadherin–positive parental cell line. Immunofluorescence light microscopic analysis suggested that a higher percentage of the parental cells expressed vimentin than did the antisense-transfected cells, raising the possibility that expression of N-cadherin may

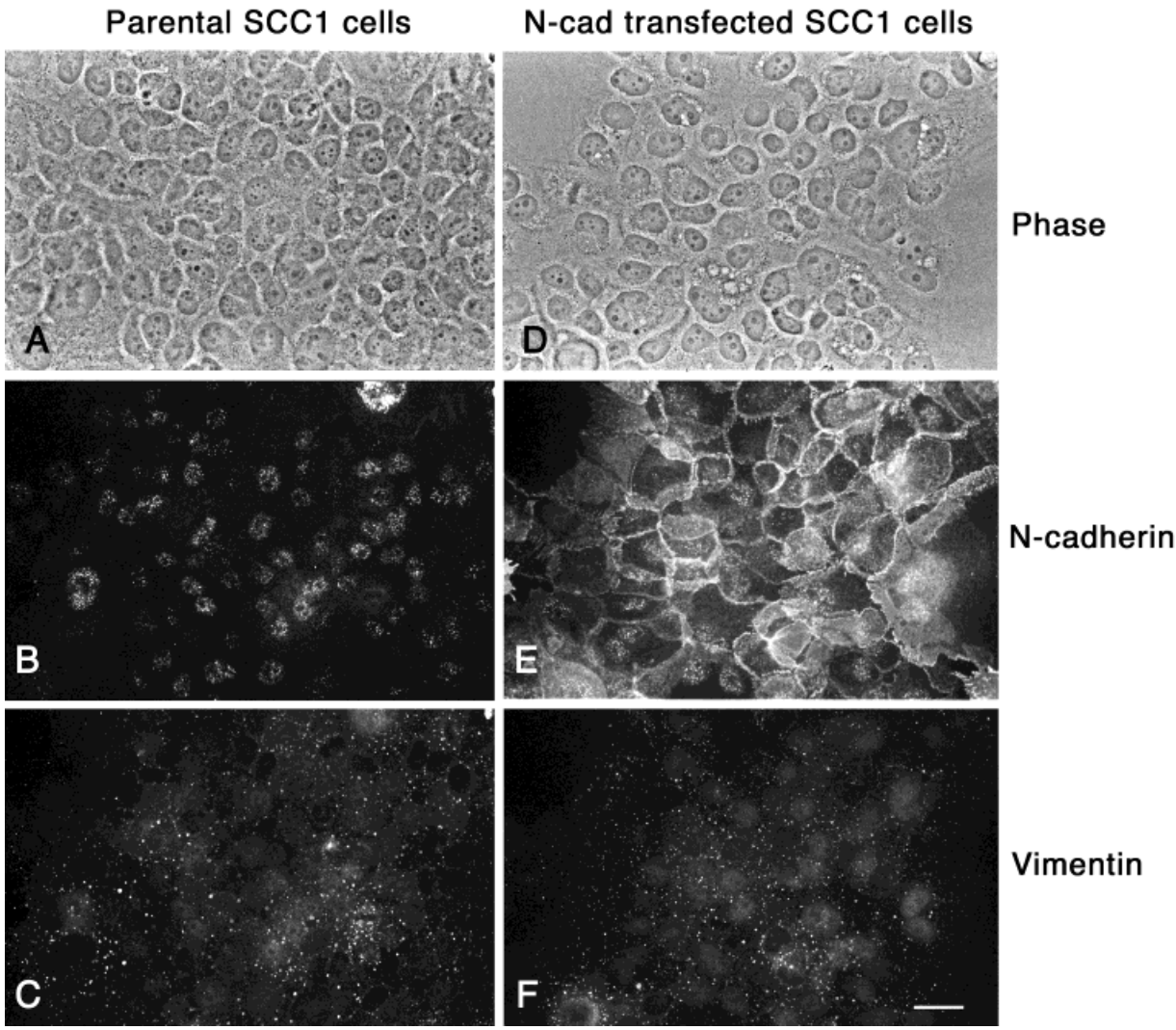

Fig. 5. SCC1 cells transfected with N-cadherin do not express vimentin. Parental SCC1 cells (**Panels A–C**) or stable clones of SCC1 cells expressing human N-cadherin were grown on glass cover-slips and processed for double-label immunofluorescence with the monoclonal IgM antibody LN-6 to vimentin (**Panels C and F**) and the monoclonal IgG antibody 13A9 to N-cadherin (**Panels B and E**). The secondary antibodies were rhodamine-conjugated anti-mouse IgM and FITC-conjugated anti-mouse IgG. Panels A and D show phase microscopy of the cells. Bar equals 10 μm.

be a mechanism by which cells undergo an epithelial-to-mesenchymal transition. To investigate this, we cloned out vimentin-positive and vimentin-negative cells from the SCC11B population of squamous epithelial cells. The vimentin-negative cells tended to express higher levels of E-cadherin. However, we failed to observe a correlation between N-cadherin expression and vimentin expression, even though the vimentin-positive cells had a more mesenchymal morphology than did the vimentin-negative cells.

Studies by Hendrix et al. [1997] showed that transfection of mouse vimentin into human breast cancer cells resulted in an epithelial-to-mesenchymal transition. In our studies, we did not see a dramatic morphologic change when we transfected mouse vimentin into human oral squamous epithelial cells, suggesting that increased expression of vimentin alone is not sufficient to initiate an epithelial-to-mesenchymal transition in these cells. The explanation for the differences between our studies and those in the breast cancer cells may involve an inherent difference between the cell types. Our studies were done on squamous epithelial cells, whereas the breast cancer cells are polarized epithelial cells.

A number of studies have shown that epithelial-to-mesenchymal transitions occur in

response to certain growth factors such as hepatocyte growth factor (scatter factor), fibroblast growth factor, epidermal growth factor, keratinocyte growth factor, and members of the tumor growth factor families [Valles et al., 1990; Geimer and Bade, 1991; Weidner et al., 1991; Boyer et al., 1992; Sonnenberg et al., 1993; Weidner et al., 1993; Werner et al., 1994; Miettinen et al., 1994; reviewed in Birchmeier et al., 1996; Savagner, et al., 1997; Knudsen et al., 1998; DeLuca et al., 1999]. In addition, fibroblast growth factor has been implicated in N-cadherin–dependent neurite extension [Doherty et al., 1991a]. In these experiments by Doherty et al. [1991a], PC12 cells plated on a monolayer of N-cadherin–transfected 3T3 cells extended longer neurites than did PC12 cells plated on control 3T3 cells. Additional studies by this group showed that N-cadherin–dependent neurite extension was due to second messenger activation [Doherty et al., 1991b; Saffell et al., 1992] and involved activation of the fibroblast growth factor receptor [Williams et al., 1994; Saffell et al., 1997]. Currently, we are investigating whether or not N-cadherin interacts with the fibroblast growth factor receptor to initiate the process of epithelial-to-mesenchymal transition in squamous carcinoma cells.

Further studies are needed to elucidate the mechanisms that regulate vimentin expression, cadherin expression, and the switch from an epithelial to a mesenchymal phenotype in oral squamous epithelial cells. Elucidation of physiologic and environmental molecules that regulate this switch may provide clues to effective treatment or prevention of oral squamous cell carcinoma.

ACKNOWLEDGMENTS

The authors thank Dr. Thomas Carey for the SCC1 and SCC11B cell lines, Drs. Katrina Trevor, David Berg, and Nicholas Fasel for reagents, and Dr. Karen Knudsen of The Lankenau Medical Research Institute for critically reading this manuscript. The authors are grateful to Tammy Sadler, Jill Nieset, Kristin Sass, and Jim Wahl for technical help. This work was supported by National Institutes of Health grants GM51188 to M.J. Wheelock and DE12308 to K.R. Johnson.

REFERENCES

Baker SR. 1985. An in vivo model for squamous cell carcinoma of the head and neck. Laryngoscope 95:43–56.

Berg DT, McClure DB, Grinnell BW. 1993. High-level expression of secreted proteins from cells adapted to serum-free suspension culture. Biotechniques 14:972–978.

Birchmeier C, Birchmeier W, Brand-Saberi B. 1996. Epithelial-mesenchymal transitions in cancer progression. Acta Anat 156:217–226.

Boyer B, Dufour S, Thiery JP. 1992. E-cadherin expression during the acidic FGF-induced dispersion of a rat bladder carcinoma cell line. Exp Cell Res 201:347–357.

DeLuca SM, Gerhart J, Cochran E, Simak E, Blitz J, Mattiacci-Paessler M, Knudsen K, George-Weinstein M. 1999. Hepatocyte growth factor/scatter factor promotes a switch from E- to N-cadherin in chick embryo epiblast cells. Exp Cell Res 251:3–15.

Domagala W, Lasota J, Dukowicz A, Markiewski M, Striker G, Weber K, Osborn M. 1990. Vimentin expression appears to be associated with poor prognosis in node-negative ductal NOS breast carcinomas. Am J Pathol 137:1299–1304.

Doherty P, Rowett LH, Moore SE, Mann DA, Walsh FS. 1991a. Neurite outgrowth in response to transfected NCAM and N-cadherin reveals fundamental differences in neuronal responsiveness to CAMs. Neuron 6:247–258.

Doherty P, Ashton SV, Moore SE, Walsh FS. 1991b. Morphoregulatory activities of NCAM and N-cadherin can be accounted for by G-protein dependent activation of L- and N-type neuronal calcium channels. Cell 67:21–33.

Fuchs E, Weber K. 1994. Intermediate filaments: structure, dynamics, function, and disease. Annu Rev Biochem 63:345–382.

Fuchs E. 1995. Keratins and the skin. Annu Rev Cell Dev Biol 11:123–153.

Geimer P, Bade EG. 1991. The epidermal growth factor-induced migration of rat liver epithelial cells is associated with a transient inhibition of DNA synthesis. J Cell Sci 100:349–355.

Hay ED, Zuk A. 1995. Transformations between epithelium and mesenchyme: normal, pathological, and experimentally induced. Am J Kidney Dis 26:678–690.

Heins S, Aebi U. 1994. Making heads and tails of intermediate filament assembly, dynamics and networks. Curr Opin Cell Biol 6:25–33.

Hendrix MJ, Seftor EA, Chu YW, Trevor KT, Seftor RE. 1996. Role of intermediate filaments in migration, invasion and metastasis. Cancer Metastasis Rev 15:507–525.

Hendrix MJ, Seftor EA, Seftor RE, Trevor KT. 1997. Experimental co-expression of vimentin and keratin intermediate filaments in human breast cancer cells results in phenotypic interconversion and increased invasive behavior. Am J Pathol 150:483–495.

Hirt RP, Poulain-Godefroy O, Billotte J, Kraehenbuhl J-P, Fasel N. 1992. Highly inducible synthesis of heterologous proteins in epithelial cells carrying a glucocorticoid-responsive vector. Gene 111:199–206.

Islam S, Carey TE, Wolf GT, Wheelock MJ, Johnson KR. 1996. Expression of N-cadherin by human squamous carcinoma cells induces a scattered fibroblastic phenotype with disrupted cell-cell adhesion. J Cell Biol 135: 1643–1654.

Iyer PV, Leong AS. 1992. Poorly differentiated squamous cell carcinomas of the skin can express vimentin. J Cutan Pathol 19:34–39.

Johnson KR, Lewis JE, Li D, Wahl JK, Soler AP, Knudsen KA, Wheelock MJ. 1993. P- and E-cadherin are in separate complexes in cells expressing both cadherins. Exp Cell Res 207:252–260.

Klymkowsky MW. 1995. Intermediate filaments: new proteins, some answers, more questions. Curr Opin Cell Biol 7:46–54.

Knudsen KA, Wheelock MJ. 1992. Plakoglobin, or an 83-kDa homologue distinct form β-catenin, interacts with E-cadherin and N-cadherin. J Cell Biol 118:671–679.

Knudsen KA, Frankowski C, Johnson KR, Wheelock MJ. 1998. A role for cadherins in cellular signaling and differentiation. J Cell Biochem (Suppl) 30-31:168–176.

Miettinen PJ, Ebner R, Lopez AR, Derynck R. 1994. TGF-β induced transdiffrentiation of mammary epithelial cells to mesenchymal cells: involvement of type I receptors. J Cell Biol 127: 2021–2036.

Peralta Soler A, Knudsen K A, Jaurand M-C, Johnson KR, Wheelock MJ, Klein-Szanto AJ, Salazar H. 1995. The differential expression of N-cadherin and E-cadherin distinguishes pleural mesotheliomas from lung adenocarcinomas. Hum Pathol 26:1363–1369.

Raymond WA, Leong AS. 1989. Vimentin—a new prognostic parameter in breast carcinoma? J Pathol 158:107–114.

Radice GL, Rayburn H, Matsunami H, Knudsen KA, Takeichi M, Hynes RO. 1997. Developmental defects in mouse embryos lacking N-cadherin. Dev Biol 181:64–78.

Sacco PA, McGranahan TM, Wheelock MJ, Johnson KR. 1995. Identification of plakoglobin domains required for association with N-cadherin and α-catenin. J Biol Chem 270:20201–20206.

Saffell JL, Walsh FS, Doherty P. 1992. Direct activation of second messenger pathways mimics cell adhesion molecule-dependent neurite outgrowth. J Cell Biol 118: 663–670.

Saffell JL, Williams EJ, Mason IJ, Walsh FS, Doherty P. 1997. Expression of a dominant negative FGF receptor inhibits axonal and FGF receptor phosphorylation stimulated by CAMs. Neuron 18:231–242.

Savagner P, Yamada KM, Thiery JP. 1997. The zinc-finger protein slug causes desmosome dissociation, an initial and necessary step for growth factor-induced epithelial-mesenchymal transition. J Cell Biol 137:1403–1419.

Schwarz MA, Owaribe K, Kartenbeck J, Franke WW. 1990. Desmosomes and hemidesmosomes: constitutive molecular components. Annu Rev Cell Biol 6:461–491.

Soler AP, Knudsen KA. 1994. N-cadherin involvement in cardiac myocyte interaction and myofibrillogenesis. Dev Biol 162:9–17.

Sonnenberg E, Meyer D, Weidner KM, Birchmeier C. 1993. Scatter factor/hepatocyte growth factor and its receptor, the c-met tyrosine kinase, can mediate a signal exchange between mesenchyme and epithelia during mouse development. J Cell Biol 123:223–235.

Tomson AM, Scholma J, Meijer B, Koning JG, de Jong KM, van der Werf M. 1996. Adhesion properties, intermediate filaments and malignant behavior of head and neck squamous cell carcinoma cells in vitro. Clin Exp Metastasis 14:501–511.

Valles AM, Boyer B, Badet J, Tucker GC, Barritault D, Thiery JP. 1990. Acidic fibroblast growth factor is a modulator of epithelial plasticity in a rat bladder carcinoma cell line. Proc Natl Acad Sci USA 87:1124–1128.

Volk T, Geiger B. 1984. A 135-kDa membrane protein of intercellular adherens junctions. EMBO J 3:2249–2260.

Weidner KM, Arakaki N, Hartmann G, Vandekerckhove J, Weingart S, Rieder H, Fonatsch C, Tsubouchi H, Hishida T, Daikuhara Y, Birchmeier W. 1991. Evidence for the identity of human scatter factor and human hepatocyte growth factor. Proc Natl Acad Sci USA 88:7001–7005.

Weidner KM, Sachs M, Birchmeier W. 1993. The Met receptor tyrosine kinase transduces motility, proliferation and morphogenetic signals of scatter factor/hepatocyte growth factor in epithelial cells. J Cell Biol 121:145–154.

Werner S, Smola H, Liao X, Longaker MT, Kreig T, Hofschneider PH, Williams LT. 1994. The function of KGF in morphogenesis of epithelium and re-epithelialization of wounds. Science 266:819–822.

Wheelock MJ, Knudsen KA. 1991. N-cadherin-associated proteins in chicken muscle. Differentiation 46:35–42.

Wheelock MJ, Buck CA, Bechtol KB, Damsky CH. 1987. Soluble 80kDa fragment of Cell-CAM 120/80 disrupts cell-cell adhesion. J Cell Biochem 34:187–202.

Williams EJ, Furness J, Walsh FS, Doherty P. 1994. Activation of the FGF receptor underlies neurite outgrowth stimulated by L1, NCAM and N-cadherin. Neuron 13: 583–594.