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Cellular Polarity Correlates with Vimentin Distribution, but not to Keratin, in Human Renal Cell Carcinoma Cells *in vitro*

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ABSTRACT. **To investigate the relationships among vimentin, keratin and cellular polarity, reorganized glands composed of renal cell carcinoma cells were investigated *in vitro*. We employed two different three-dimensional collagen gel culture methods, the "floating sandwich method (FSM)" and the "dispersed embedding method (DEM)." The cells composed of reorganized glands formed by FSM culture showed distinct polarity. In contrast, the cellular polarity of the cells formed by DEM culture was less distinct. Keratin was evenly distributed throughout the cytoplasm regardless of the culture method. In contrast, in reorganized glands obtained by FSM culture, vimentin was distinctly polarized at the basal pole while glands obtained by DEM culture showed random distribution of vimentin. These results suggest that there is a close relationship between cell polarity and intracellular localization of vimentin, and that there may be different mechanisms controlling the organization of the two intermediate filament (IF) networks.**

Keratin and vimentin are the main components of intermediate filaments (IFs), that have generally been accepted to be specific markers for epithelial cells and mesenchymal differentiation, respectively. Recently, however, co-expression of the two types of IFs has been reported in many kinds of epithelial cells *in vivo* and *in vitro*, showing that keratin is diffusely distributed throughout the cytoplasm, whereas vimentin is mostly concentrated at the basal pole in gland-forming areas (1–6). Futhermore, in anaplastic thyroid carcinoma, less-differentiated areas of follicular carcinomas (7) and prostatic carcinoma with a high Gleason score (8), vimentin was reported to show paranuclear distribution. Thus, it is likely that cellular polarity has a close relationship with the subcellular localization of vimentin.

Recent advances in culture techniques, in particular, techniques for three-dimensional culture, have made it possible to promote the reorganization of the cellular polarity of cells cultured *in vitro*. Since cells cultured in a monolayer lose their spatial organization and have altered intracellular organization as compared to cells *in vivo*, three-dimensional culture systems seem to be much more useful for studying polarized structures of epithelial cells. In fact, cellular polarity *in vitro* is quite similar to that observed *in vivo*.

In the present experiments we compared the localization of vimentin in human renal cell carcinoma cells co-expressing vimentin and keratin cultured under three

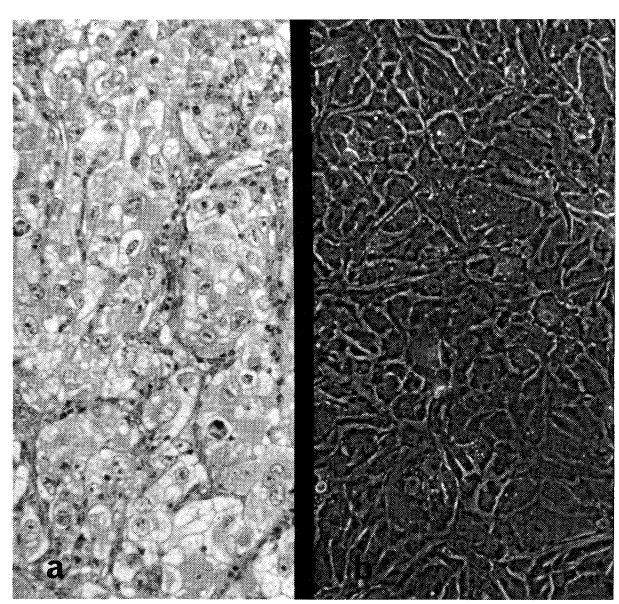

Fig. 1. Microscopical features of SMKT-R 2. a; Histopathological features of the original tumors (H&E, ×200), b; Phase contrast photograph of the confluent monolayer of SMKT-R-3. (×100)

Abbreviations: IF; intermediate filament, FSM; floating sandwitch method, DEM; dispersed embedding method.

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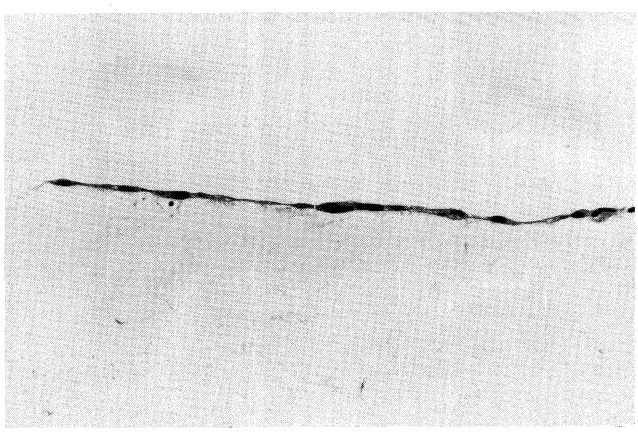

Fig. 2. Monolayer of SMKT-R-3 cells on collagen gel after culture for 3 days. (H&E, ×400)

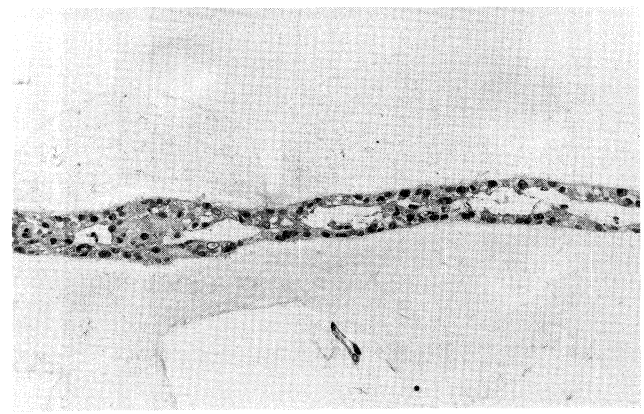

Fig. 4. Photomicrograph of gland-like structures formed by FSM culture. (H&E, ×200)

different sets of conditions using collagen gels (9) to clarify the relationship between cellular polarity and vimentin.

MATERIALS AND METHODS

Cells and cell culture. Human renal cell carcinoma cell lines (SMK-R-3) (10) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum, and subcultured by trypsinization.

Preparation of collagen solution. Type I collagen solution was prepared from rat tail tendons, mixed with concentrate Ham's F 12 solution and allowed to gel as previously described (9).

Three-dimensional cell culture using collagen gel. (i) Monolayer culture of cells on top of collagen gel.
(ii) Culture of cells between double-layered floating collagen gel (floating sandwich method: FSM).

When cells formed a confluent monolayer on the surface of collagen gel they were covered with another layer of collagen gel. After cells were cultured for 24 hours, the collagen gels were allowed to float from the dish into the culture medium.
(iii) Culture of cells in collagen gel (dispersed embedding method: DEM).

Cells were mixed with a collagen-mixture solution, poured into the culture dish and allowed to harden at 37°C in a CO_2 incubator.

Processing for light and electron microscopy. After being

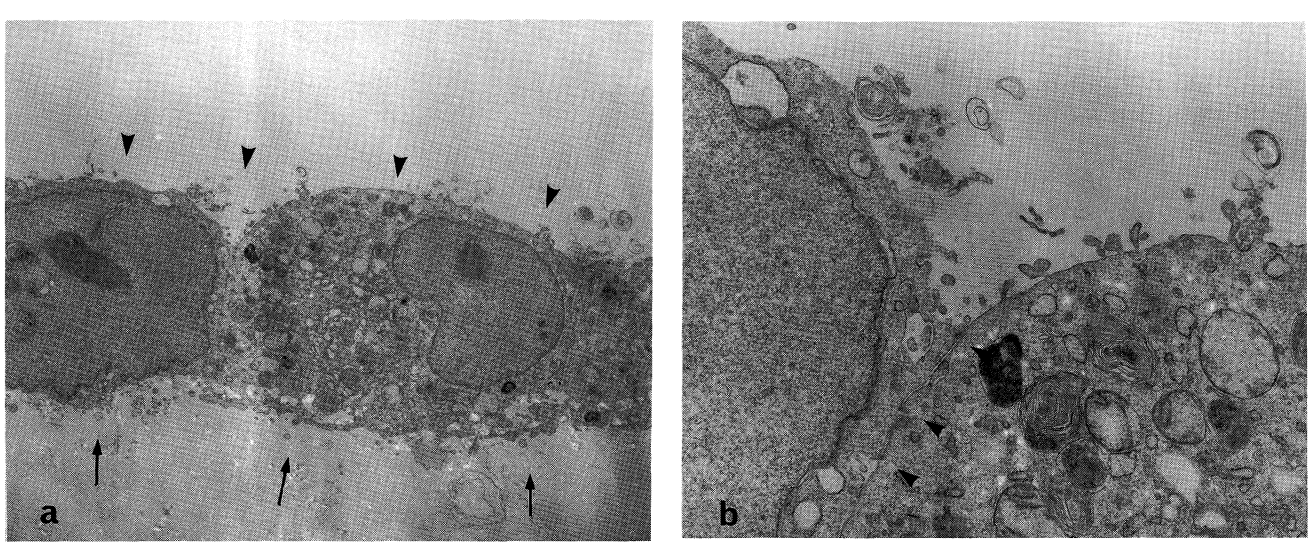

Fig. 3. Electron micrograph of the SMKT-R-3 cells shown in Fig. 2. a; Note the microvilli protruding into the culture medium, indicating the apical-basal polarity of the cells. Between adjacent cells, junctional complex-like structures. ×8,000 (Arrowheads indicate the apical pole and arrows indicate the basal pole.) b; Higher magnification of the junctional complex-like structures shown in Fig. 3a (×20,000) (arrowheads).

cultured for 2 weeks, the cells were fixed in situ in 25% buffered formalin for hematoxylin and eosin staining and for immunohistochemical staining of keratin (DAKO; polyclonal) and vimentin (DAKO; monoclonal). We also used these staining techniques on the primary tumor. For electron microscopic examination, cells were fixed in situ in a mixture of 1% glutaldehyde-4% formaldehyde in 0.1M cacodylate buffer (pH 7.4).

RESULTS

SMKT-R-3, a cell line established from primary renal cell carcioma consisting of granular cells in a papillary arrangement (Fig. 1a), showed good growth in the confluent monolayer on noncoated culture dishes (Fig. 1b).

When cells were cultured on collagen gel, they had a flattened configuration (Fig. 2) but showed cell polarity with microvilli protruding into the medium (Fig. 3a). Between adjacent cells, junctional complex-like structures were formed (Fig. 3b). After sandwiching of these monolayers by adding another layer of collagen gel and floating the gels for 2 weeks, a row of many gland-like structures was observed between the two collagen gel layers (Fig. 4). These gland-like structures often showed

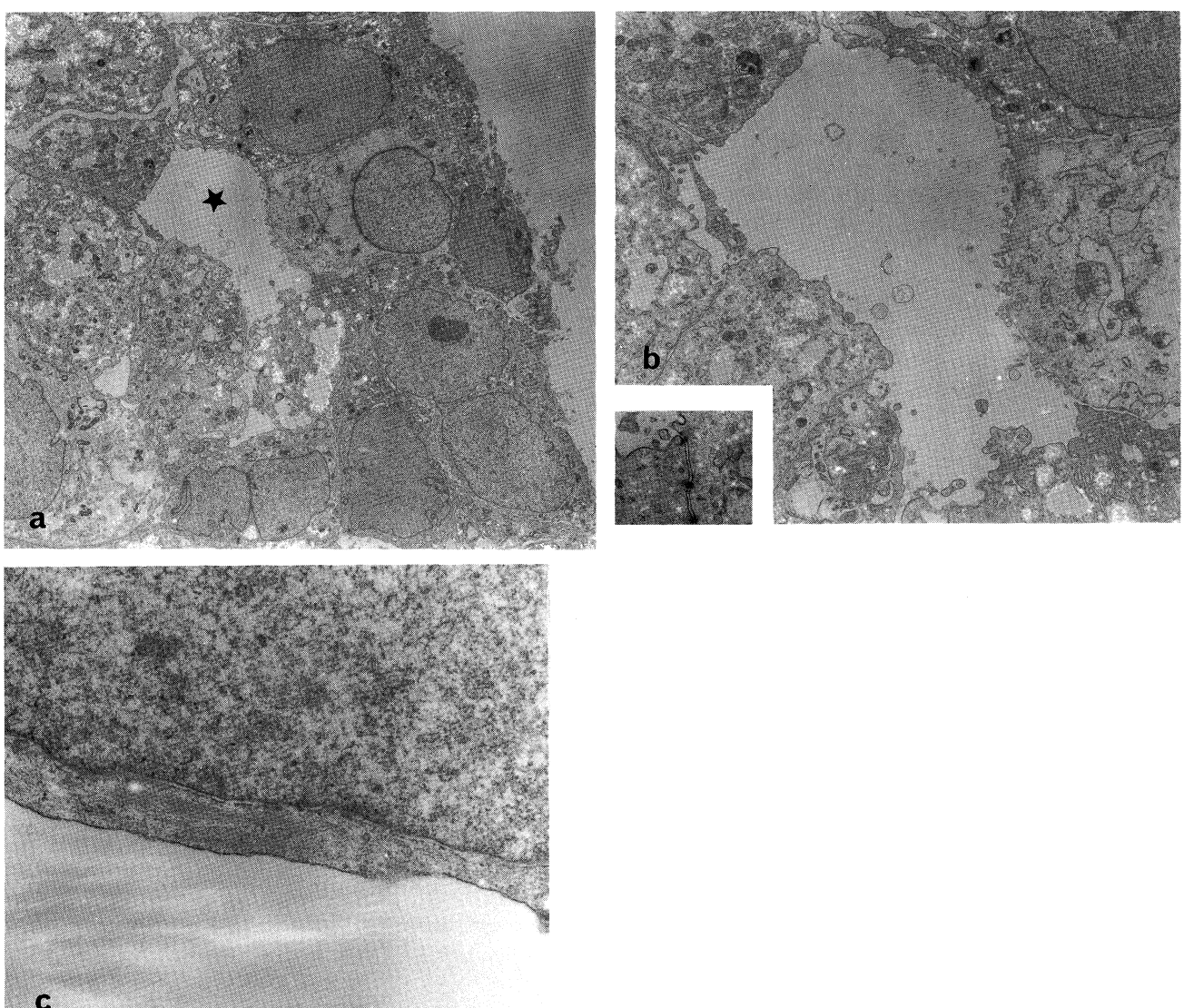

Fig. 5. Electron micrographs of the gland-like structures illustrated in Fig. 4. a; Low magnification of these structures. The stars indicate the lumina. (×2,000) b; Many microvilli protruded into the lumen. In cytoplasm, many glycogen granules are observed. The cells forming the gland-like structures are attached to each other by junctional complexes (×5,000). Inset: higher magnification of desmosomes. (×10,000) c; IFs occasionally run along the plasma membrane at the basal pole. (×25,000)

large lumina and the nuclei were near the basal region of the cells. Ultrastructurally these gland-like structures were composed of cells with distinct polarity (Fig. 5a). The apical pole with many microvilli faced a follicle-like lumen (Fig. 5b) and the basal pole faced the collagen gel. Junctional complexes were observed between adjacent cells (Fig. 5b: inset). Many intermediate filaments running along the plasma membrane were occasionally observed in the cytoplasm near the basal pole (Fig. 5c).

In contrast, when cells were cultured by DME, randomly distributed single or small cellular aggregates were found in the collagen gel (Fig. 6). Only a few of them showed small gland-like structures. On electron microscopical examination, the follicle-like structures consisted of cell aggregates with small lumina and showed less distinct polarity than cells cultured with FSM (Fig. 7a). Intercellular junctions were abortive (Fig. 7b) compared to those obtained by FSM. No IFs were observed at the basal pole. On immunohistochemical examination for keratin and vimentin in the primary tumor, keratin was found to be evenly distributed throughout the cytoplasm (Fig. 8a) but vimentin had a tendency to polarize at the basal pole (Fig. 8b).

As for SMK-R-3 cells cultured in the three-dimensional system, keratin was evenly distributed throughout the cytoplasm regardless of the culture method (Fig. 8c, e, g). In contrast, the intracellular localization of vimentin differed depending on the culture method. Monolayer cells cultured on collagen cells showed the same pattern as keratin (Fig. 8d). Cells composed of glandular structures obtained by FSM culture showed distinct basal distribution (Fig. 8f). In contrast, those cells obtained by DEM culture showed randomly distributed vimentin (Fig. 8h).

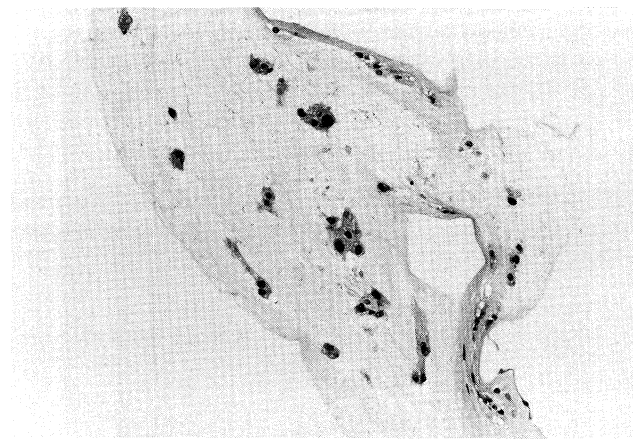

Fig. 6. Gland-like structures formed by DEM culture. a; Photograph of the gland-like structures. (H&E, ×200)

DISCUSSION

In this study using three different collagen gel culture techniques (9) we found that localization of vimentin became distinct with the development of cellular polarity, whereas localization of keratin was not markedly influenced by culture conditions.

There are many papers describing co-expression of keratin and vimentin in a number of normal and neo-

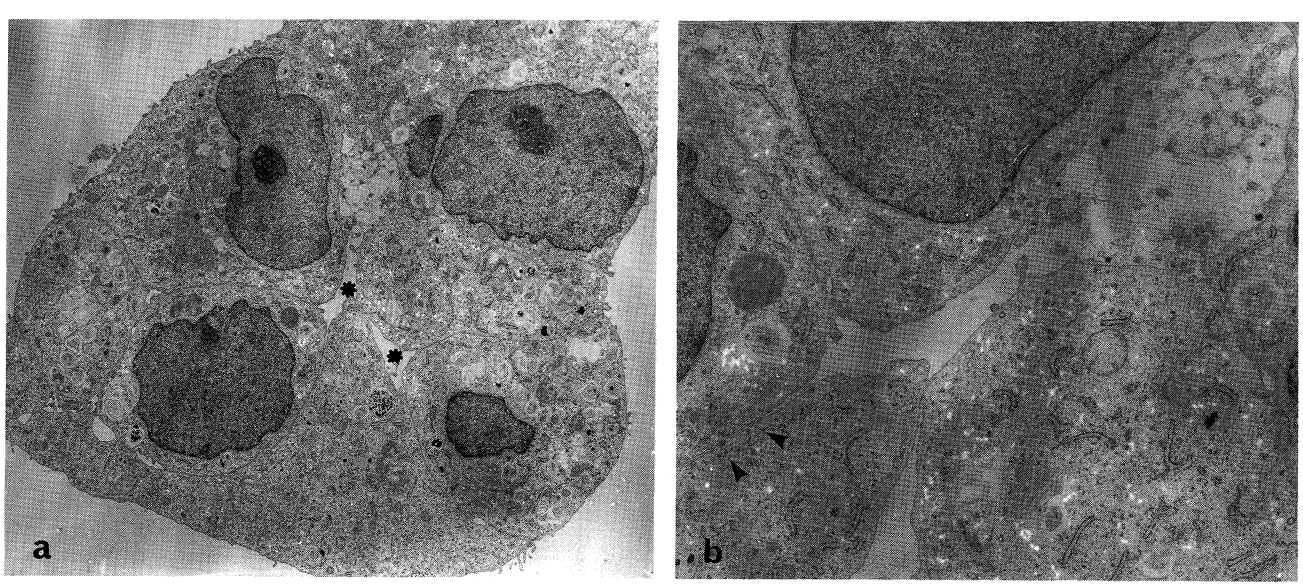

Fig. 7. a; Electron micrograph of the gland-like structures with a small lumen illustrated in Fig. 6 (star). (×2,000) b; Higher magnification view of a small lumen with microvilli. Abortive junctional complexes are observed between adjacent cells (arrows). (×5,000)

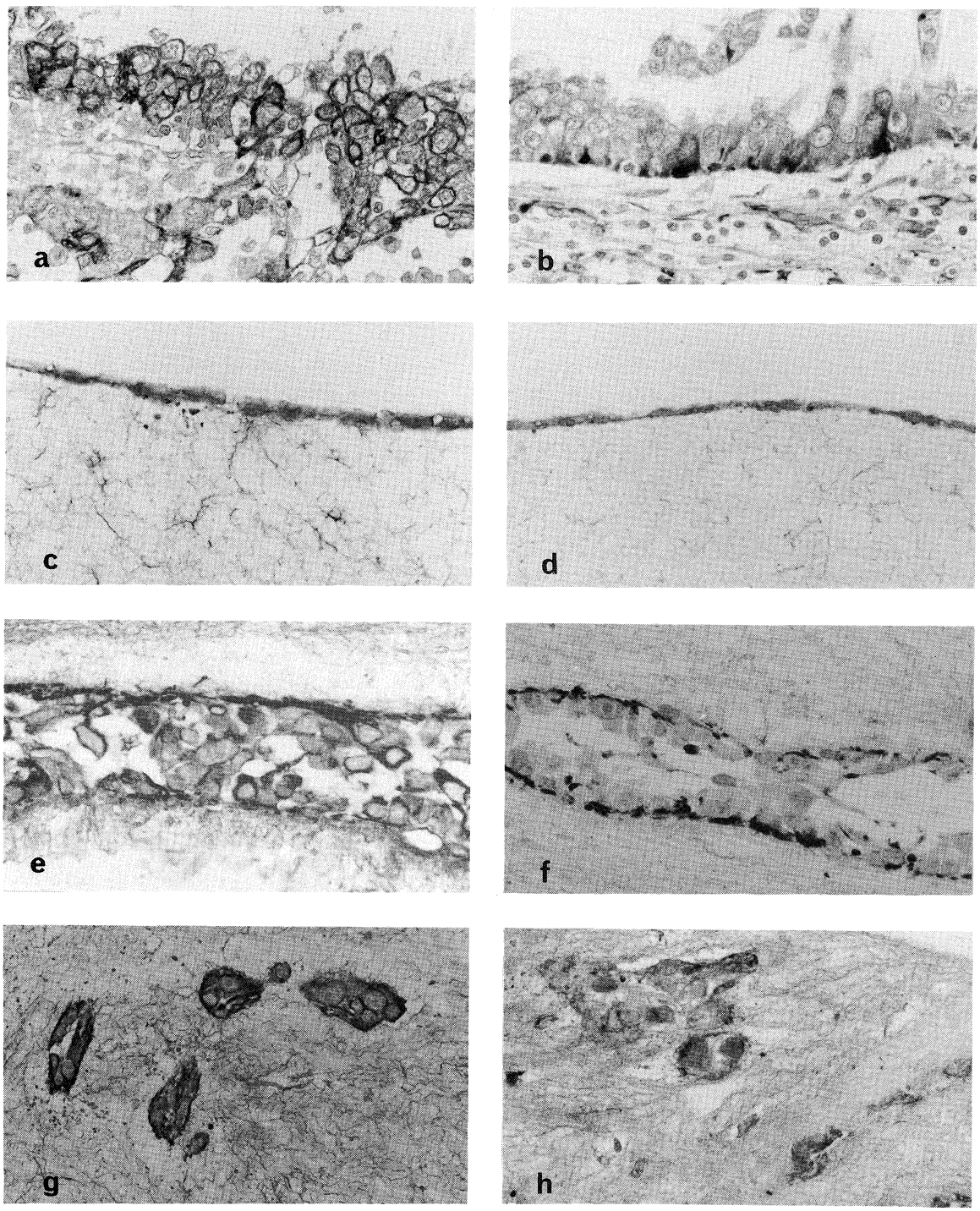

Fig. 8. Immunohistochemical localizations of keratin (a, c, e, g) and vimentin (b, d, f, h). a, b; Original tumor (×400). c, d; Monolayer culture (×200). e, f; FSM culture (×400). g, h; DEM culture (×400). Keratin (a, c, e, g) is always diffusely distributed throughout the cytoplasm, but vimentin in b and f polarized almost at the basal pole. Vimentin in d is diffusely distributed and in h is randomly distributed throughout the cytoplasm.

plastic epithelial tissues (5, 11). In these papers several investigators reported that vimentin was concentrated at the basal pole of the cytoplasm of epithelial cells while keratin did not show such subcellular compartmentalization (1–6, 12, 13, 14). These observations suggest that the two types of IFs, keratin and vimentin,

play different roles in the cells. The reorganized multicellular structures obtained by FSM closely resembled those observed *in vivo*. Furthermore, the localization of vimentin in the culture system is comparable to that observed *in vivo*, consistent with previous observations on the tissues. In the present experiments, we focused on the relationship between the development of cellular polarity and the distribution of vimentin in the cells by using three different culture systems.

Thus, our observations clearly showed that the intracellular localization of vimentin was closely related to the development of cellular polarity.

Extracellular matrices might induce the localization of vimentin in epithelial cells. In the present experiments, however, the epithelial cells were always in contact with collagen gels, regardless of the culture system. Since the effects of extracellular matrices on the localization of vimentin appeared to be negligible, comparison of the three different collagen gel culture systems would seem to be useful for studying reorganized cellular polarity *in vitro*.

The selective aggregation of vimentin, but not keratin, into perinuclear bundles upon treatment with colchicine and colcemid has been observed (15–17). Moreover, when keratin or vimentin was microinjected into PtK2 cells, exogenous keratin was incorporated directly into the networks of keratin bundles in the whole cytoplasm, whereas exogenous vimentin was first incorporated into the perinuclear bundles and then redistributed to the bundles in the whole cytoplasm (18). These observations suggest that the mechanism of the organization of vimentin is different from that of keratin bundles, though vimentin and keratin are the main components of IFs. The close relationship between cellular polarity and the localization of vimentin futher suggests that vimentin and keratin play different roles in the cytoplasm.

We previously reported aggregation of actin microfilaments at the basal pole in glands consisting of normal human epithelial cells from thyroid glands obtained by the FSM (9). However, we could not find such aggregation in the present study using human renal carcinoma cells. The present data are in close agreement with the recent data showing that when cells are transformed the expression of the actin-containing sheath decreases (19). Takasu *et al.* (20) reported that when porcine thyroid cells are cultured on a collagen gel-coated cover glass in the presence of TSH, microfilaments are dominant at the apical pole and intermediate filaments are dominant at the basal pole. Actin filaments, as well as intermediate filaments, might play an important role in determining cellular polarity. Further studies are required to clarify this problem.

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