Research Paper

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Influence of Cholesterol Derivatives on Cytoskeletal Organization of Human Carcinoma Cells

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Abstract
Recent developments of immunotherapeutic approaches have shown that artificial ordering of tumor cell membranes with cholesterol hemisuccinate (CHS) or 25-hydroxycholesterol (25-OH) may significantly enhance the immunogenicity of human renal adenocarcinoma cells.

To gain further insight into the molecular mechanism of these sterols, we investigated cytoskeletal modification, which is related to the cell membrane. After treatment of human renal carcinoma cells with these cholesterol derivatives (at 10^{-6} and 10^{-7} *M*) for 5 days, we observed a desorganization of the submembrane end of the cytoplasmic actin stress fibers by cytofluorescence. The microtubule network was not affected. Thus, in the present study, we found that changes in membrane physicochemical properties impaired the anchorage of actin microfilaments in the plasma membrane of human renal cancer cells. Under the same experimental conditions, such modifications were not observed in normal cells (human fibroblasts) or in human hepatoma cells. We suggest that incubation of cancer cells with these sterols induced a redistribution of the cholesterol-rich membrane microdomains which are linked to the cytoskeleton through submembrane proteins.

Introduction

Biological membranes are dynamic structures which contain a variety of lipids and proteins and exhibit a high degree of molecular orientational order. It is generally believed that both molecular order and motion of the membrane components are essential for the maintenance of protein functions [1–4]. Membrane lipids may exist in discrete domains, differing greatly in their composition and properties [5–7]. Nonesterified cholesterol is an important component of plasma membrane in animal cells and influences both the structural and dynamic organization of the membranes.

In recent years, it became clear that although proteins are highly mobile in the plasma membrane of animal cells, this motility is regulated among others, by attachment of the cytoskeleton to the cell membrane in the living cell [8–11]. The cytoskeletal control of receptor movement on the cell surface, lateral distribution of surface components and spatial order of proteins within func-

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tional domains on the plasma membrane has been characterized extensively in human erythrocytes [12], neurons [13] and polarized epithelial cells [14–17].

Among the different classes of cytoskeletal elements, the actin microfilaments in particular have been shown to form stable contacts with the cytoplasmic face of the plasma membrane [9]. Microfilament-membrane interaction plays an important role in the regulation of cell motility, morphology and organization of cell surface components. The current view stresses that actin microfilaments are anchored to the cytoplasmic face of the membrane by integral receptors and peripheral linking proteins [9].

We were interested in the interaction between membrane structure and cytoskeletal organization. The present study was conducted on cells isolated from human renal carcinoma biopsies, in which we previously demonstrated that treatment by some cholesterol derivatives induced increased immunogenicity [18]. To perform multiple and repeated experiments over a long time interval and to be sure to study a homogeneous cell population, we adapted human renal carcinoma cells to monolayer growth in vitro. The present study shows that two cholesterol derivatives, namely cholesterol hemisuccinate (CHS) and 25-hydroxycholesterol (25-OH) were able to alter the organization of the actin microfilaments within the cells, without affecting microtubule or intermediate filament distribution. These modifications were correlated with changes in the distribution of cholesterol in the outer leaflet of the plasma membrane, as visualized by the fluorescent polyenic antibiotic filipin. Our results suggest the possibility of an interaction between actin filaments and/or their binding proteins and specific lipid domains in the core of the plasma membrane of human renal adenocarcinoma cells.

Material and Methods

Cell culture reagents were from Gibco (Grand Island, N.Y., USA). CHS and 25-OH were purchased from Sigma; their purity was controlled by thin-layer chromatography. Antibodies were obtained from Amersham; rhodamine-phalloidin, from Molecular Probes (Eugene, Oreg., USA).

Cell Culture

Renal cancer cell lines were established from primary kidney tumors from 55- to 75-year old patients. After surgical resection, the biopsies of characterized renal adenocarcinoma were immersed in cold RPMI medium and immediately transported to the laboratory where they were mechanically dissociated under sterile conditions. The isolated cells were washed by low-speed centrifugation through a Hanks' balanced salt solution to eliminate debris and blood cells. Thereafter they were inoculated into 2-cm diameter culture dishes in DMEM containing *D*-valine and 5 g/l *D*-glucose, and supplemented with 10% fetal calf serum and antibiotics (100 U/ml penicillin and 1 mg/ml streptomycin). For the following subcultures, the carcinoma cells were dispersed by trypsinization every 2 weeks and passaged in DMEM medium at a 1:3 split ratio.

Normal kidney cells were isolated and cultured under the same conditions than tumor cells. The cultures of normal or tumoral renal cells, devoid of contaminating fibroblasts, were used until the 3rd passage and between the 3rd and the 10th passage, respectively.

Human fibroblasts were derived from foreskin punch biopsies. These were cut into small pieces and inoculated into culture flasks in DMEM with 1 g/l *D*-glucose, supplemented with 10% fetal calf serum and antibiotics. Primary fibroblast cultures were propagated by the explant outgrowth culture procedure and subcultured every week by mild trypsinization.

Hep3B cells are a human hepatocellular carcinoma cell line (ATCC HB 8064). MDCK is a normal canine kidney cell line, which forms monolayers of polarized epithelial-like cells at confluence (ATCC CCL 34). Hep3B and MDCK cells were routinely grown in DMEM with 5 g/l *D*-glucose supplemented with 10% fetal calf serum and antibiotics.

All cell lines were incubated at 37 °C in a CO_2 incubator set at 5% (v/v) CO_2 in air. The culture medium was renewed every 2–3 days.

Cell Treatment with Cholesterol Derivatives

CHS and 25-OH were dissolved in ethanol at 10^{-2} *M* concentration. Aliquots were stored at −20 °C and diluted in the culture medium to a final concentration of 10^{-6} to 10^{-7} *M*. In control cells, ethanol alone was added to the culture medium. However, at a concentration of 0.2% (v/v) or less, it did not induce any modification in cell growth or morphology, as compared to cells grown in the absence of ethanol.

Unless otherwise specified, the cells were grown in the presence of the cholesterol derivatives for 1 week, and the respective medium was renewed three times during this period.

Transmission Electron Microscopy

After discarding the medium, the cell layers were rapidly washed with PBS at 37 °C and fixed in situ for 15 min in 5% glutaraldehyde and 1 h in 1% osmium tetroxide, both solutions being buffered with 0.1 *M* sodium phosphate (pH 7.0). The fixed cells were then gently scrapped off and collected in small pellets by centrifugation. After dehydratation in series of ethanol, the pellets were embedded in an Araldite-Epon mixture. Ultrahin sections were stained with uranyl acetate and lead citrate, and examined in a Siemens Elmiscope 102.

Scanning Electron Microscopy

After discarding the medium, the cells cultured onto glass coverslips (1.2 cm) were rapidly washed with PBS at 37 °C and fixed in situ for 15 min in 10% glutaraldehyde and 1 h in 1% osmium tetroxide, both solutions being buffered with 0.1 *M* sodium phosphate (pH 7.0). The fixed cells were then dehydrated in series of ethanol, and dried by evaporation in hexamethyldisilasane (Carlo Erba) during 1 min. After gold metallization (SCD040, Baltzers Corp.), the preparations were observed with a Cambridge 360 (Leica) scanning electron microscope.

Cytochemical Staining of Unesterified Cholesterol

Fluorescent cytochemical detection of unesterified cholesterol with filipin was carried out on living cells. The filipin solution was

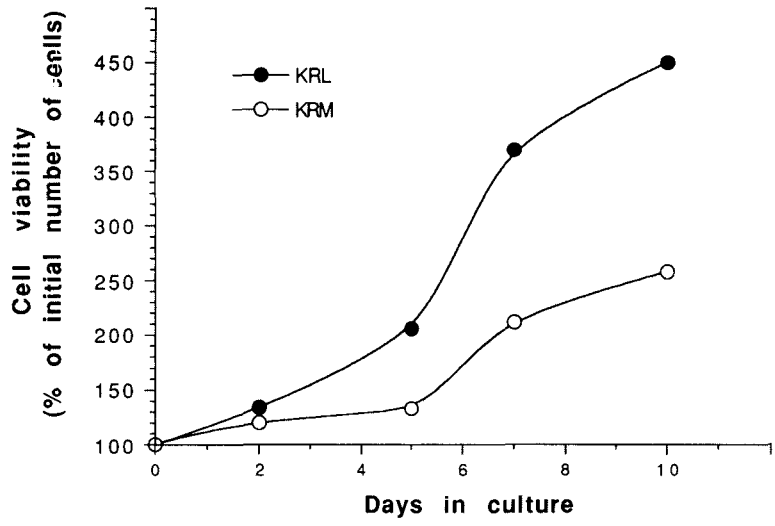


Fig. 1. Growth curves of two renal carcinoma cell lines obtained from cancer patients biopsies. Two human renal carcinoma cell lines, KRL (•) and KRM (⊙), obtained from surgical biopsies were grown in conventional medium for up to 10 days after plating. Cultured cells were detached from the flasks by trypsinization and numbered in a Neubauer cytometer, in the presence of 10% trypan blue to exclude dead cells.

prepared in dimethylsulfoxide according to Behnke et al. [19, 20]. Living cells grown on glass coverslips were rinsed with PBS and incubated for 5 min at room temperature in PBS containing 10^{-5} *M* filipin. The unreacted filipin was eliminated through 3 washes with PBS and the cells were fixed in 1% glutaraldehyde for 10 min. After fixation, the coverslips were rinsed with phosphate buffer 0.1 *M* pH 7.0, mounted on a glass slide with citifluor. The observation was made at an excitation wavelength of 490–495 nm and an emission wavelength of 520 nm.

Fluorescent Staining of the Cytoskeleton

Cells grown for 5 days in the presence of the different concentrations of sterol were detached by trypsinization and plated at low density on 12-mm diameter glass coverslips in their respective medium. After 48 h in culture, the coverslips witch attached cells were washed twice with PBS, prefixed and permeabilized in a 0.1% Triton, 0.5% glutaraldehyde pH 6.9 buffered solution for 3 min, fixed in 1% glutaraldehyde for 10 min and processed for immunochemistry [21]. Actin filaments were visualized either directly with rhodamine-phalloidin which specifically binds to F-actin or indirectly with monoclonal antibodies to actin as primary antibody, followed by FITC-rabbit anti-mouse IgG. Microtubules were detected with a monoclonal anti-β-tubulin. For double-label experiments, the coverslips were processed for tubulin immunofluorescence and then double-stained with rhodamine-phalloidin, according to Schmit and Lambert [21]. Intermediate filaments were visualized using a monoclonal antibody raised against vimentin.

Microscopy and Micrography

The stained preparations were analyzed with a Leitz Orthoplan microscope (Leica) equipped for epifluorescence, with an HBO 100/w2 lamp and a 63× oil fluorescent objective Na 1.30 (Leitz). Micrographs were taken with a Vario Orthomat Camera (Leica) using Tri-X Pan 400 Asa Films or using a Cristal Saphir image processing system (Quantel, France).

DNAse I Assay for Actin

The unpolymerized (G) and filamentous (F) actin pools in extracts of renal carcinoma cells were characterized using the inhibition of DNAse I by G actin, according to the procedure of Blikstad et al. [22].

Results

The renal carcinoma cell lines consisted mainly of epitheloid polygonal cell. Cells often showed vacuoles, particularly those in the middle of colonies. The histology showed that the cultured tumor cells closely ressemble the original renal tumor with regard to the immunocytological staining using commercial anti-cytokeratin antibodies.

The growth rate of cultured human renal carcinoma cells is generally low, as shown in figure 1 representing typical growth curves of two renal carcinoma cell lines obtained from cancer patients biopsies, named KRM and KRL. We determined a doubling time of 7 days for KPM and 5 days for KRL during the logarithmic growth phase.

Effects of CHS and 25-OH Treatment on Cell Growth and Cell Shape

Cells were treated with CHS or with 25-OH at concentration of 10^{-6} and 10^{-7} *M* the next day after plating, and their growth and morphology were studied. The effects of the cholesterol derivatives on the growth of the renal adenocarcinoma cell line KRL is presented in figure 2. It appears that CHS did not markedly alter cell growth, whereas

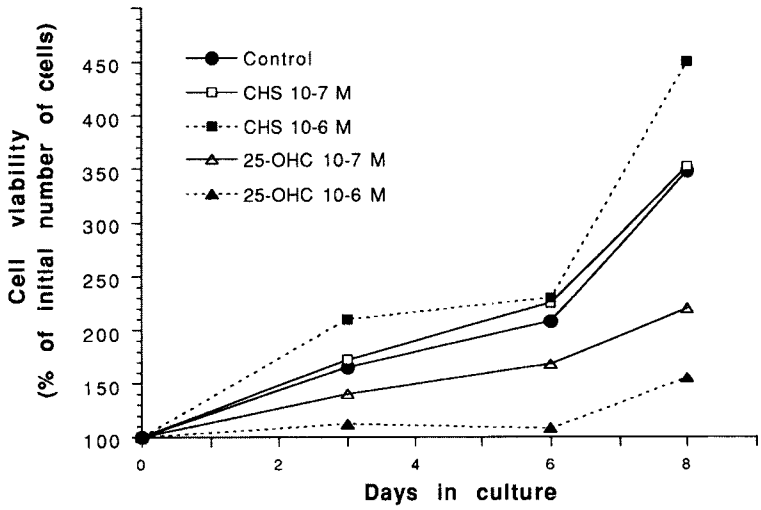


Fig. 2. Effects of CHS and 25-OH treatment on KRL cell growth. The KRL human renal carcinoma cell line was grown in conventional medium (●), or in medium supplemented with CHS at 10^{-7} *M* (□) and 10^{-6} *M* (■), and 25-OH at 10^{-7} *M* (△) and 10^{-6} *M* (▲). After the indicated number of days in culture, viable cells were counted as indicated in figure 1.

25-OH markedly delayed the growth rate, apparently depending on sterol concentration. The effect was clearly visible after 3 days of treatment at both doses tested.

In figure 3, the effects of concentrations of 10^{-6} *M* CHS and 10^{-6} *M* 25-OH on cell morphology are compared in the human renal adenocarcinoma cell line KRL and in human nontumoral renal cells. The renal adenocarcinoma cells in culture consisted of firmly adhering cells with an epithelial-like morphology; the cells were arranged in slowly growing colonies and exhibited a granulous cytoplasm with a clear nucleus (fig. 3A). Whereas this was not noticeably affected by several days of treatment with CHS (fig. 3B), after growing for 7 days in the presence of 25-OH, the cells became elongated and their cell-to-cell contacts were weaker (fig. 3C). However, the effect of the oxysterol was reversible within a few days. Indeed, after elimination of the compound, the cells continued to grow and again constituted small colonies of polygonal cells, typical for the untreated ones.

The morphology of the human nontumoral renal cells was apparently unaffected by CHS (fig. 3E) or 25-OH (fig. 3F).

Ultrastructural Modifications of Renal Adenocarcinoma Cells by CHS and 25-OH

Ultrastructural observations of renal adenocarcinoma cells show that their cytoplasm is rich in organites like mitochondria, lysosomes, rough endoplasmic reticulum, microfilaments and lipid droplets (fig. 4A). The nucleus exhibits a regularly rounded outline and homogeneously dispersed chromatin. The plasma membrane was folded only in few microvilli. After a 1-week treatment with CHS at 10^{-6} *M*, development of endoplasmic reticulum and accumulation of lipid droplets could be observed (fig. 4B). The most noticeable modifications in the 25-OH-treated cells were the outburst of microvilli and granular cytoplasmic expansions in defined cell surface areas (fig. 4C). In the cytoplasm, the endoplasmic reticulum, smooth reticulum and Golgi cisternae also appeared more developed than in control cells.

Changes in Membrane Cholesterol Distribution after CHS and 25-OH Treatment

The effects of CHS and 25-OH on cholesterol distribution on the cell surface was assessed by the filipin staining of unesterified cholesterol. This compound reveals the cholesterol directly accessible on the outer leaflet of the cell membrane. In untreated renal adenocarcinoma cells, filipin staining appeared as regular dots on the cell periphery (fig. 5A). No changes in the staining pattern were visible in cells treated by CHS or 25-OH for 24 h (results not shown). The first modifications appeared in cells treated for 4–5 days (fig. 5B, C). In CHS-treated cells, the fluorescence was concentrated in a continuous bright rim in some areas of the cell surface, besides unstained areas (fig. 5B). Cells treated with 25-OH displayed patches of bright fluorescence (fig. 5C).

In human nontumoral renal cells, the filipin staining was very weak and remained unaffected by the sterol treatments (data not shown).

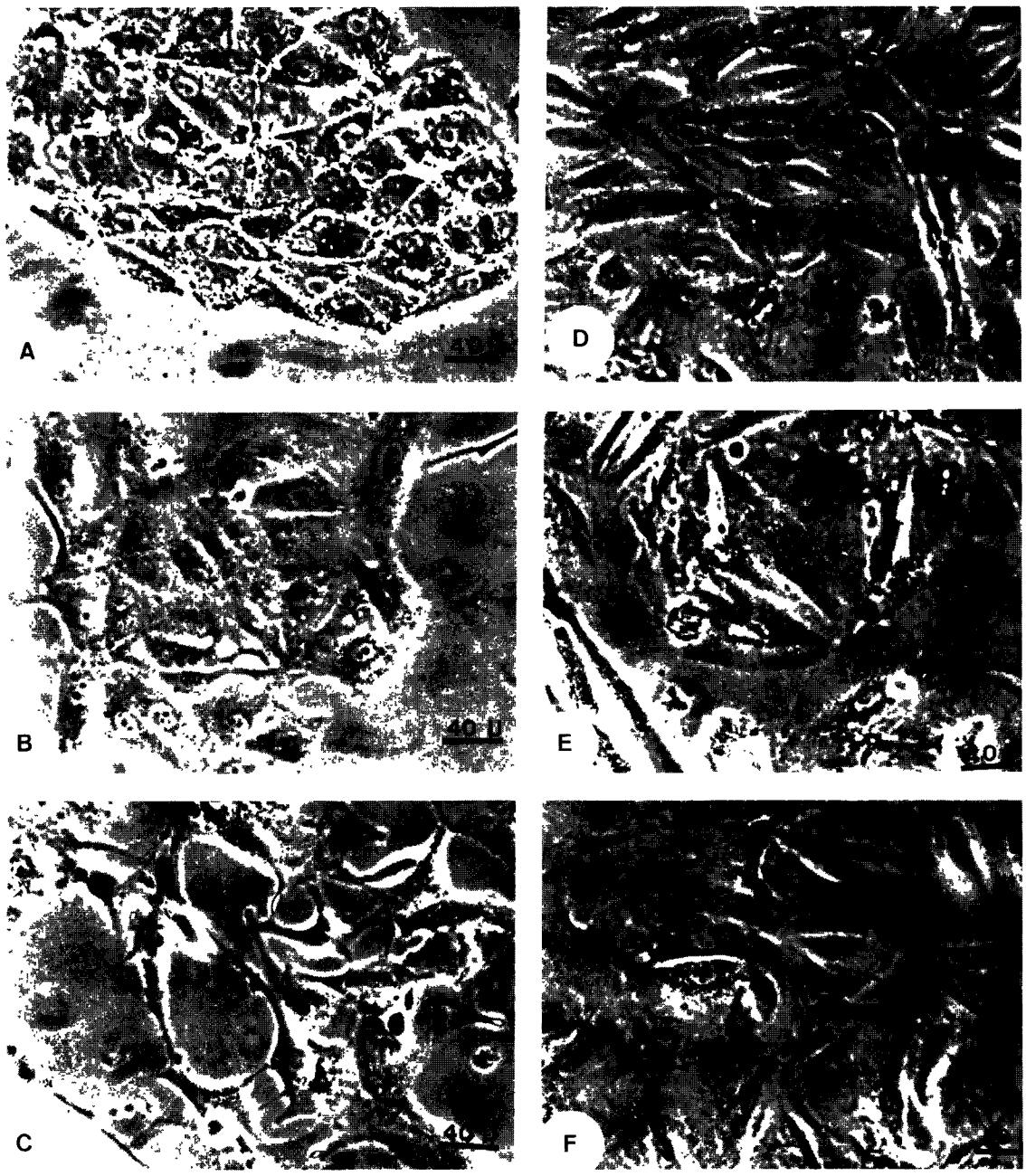


Fig. 3. Effects of CHS and 25-OH treatment on renal carcinoma (KRL) and normal human kidney cell morphology. The KRL human renal carcinoma cell line (**A–C**), and normal human renal cells (**D**, **E**, **F**) were grown on coverslips for 1 week in conventional medium (**A**, **D**) or in medium supplemented with CHS 10^{-6} *M* (**B**, **E**) and 25-OH 10^{-6} *M* (**C**, **F**). Scale bar = 40 μm.

Effect of CHS and 25-OH on the Organization of the Cytoskeleton in Cultured Human Renal Carcinoma Cells

The untreated renal carcinoma cells contained many prominent microfilament bundles of F-actin visualized with fluorescent phalloidin running throughout the cytoplasm and characteristic of animal cells in culture (fig. 6A, 7A). In contrast to actin, the microtubules visualized with the anti-tubulin antibody formed a regular network radiating from the cell center to the whole cytoplasm (fig. 6D). A similar cytoskeletal pattern could be observed in cells treated with increasing doses of CHS or 25-OH for

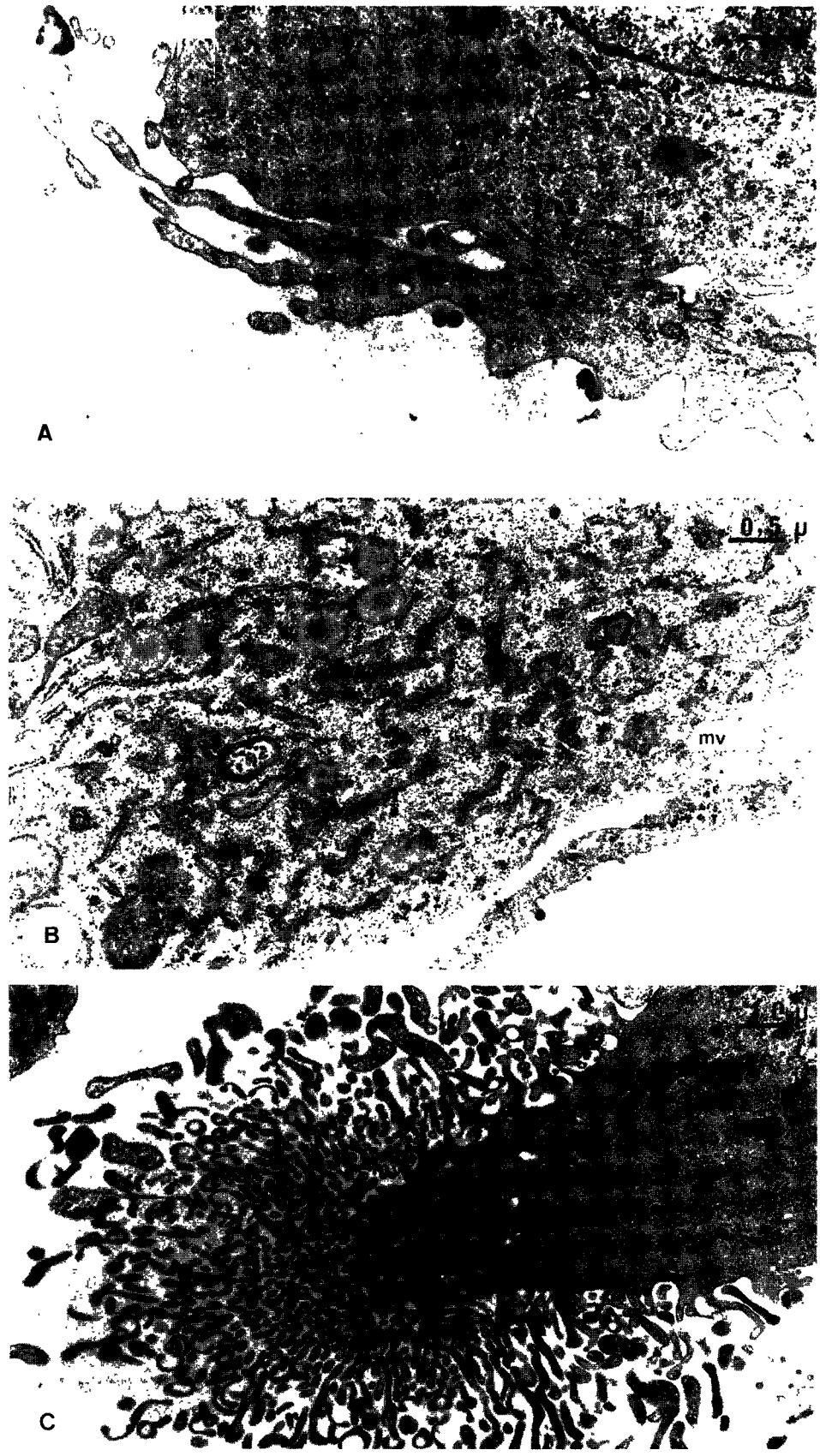
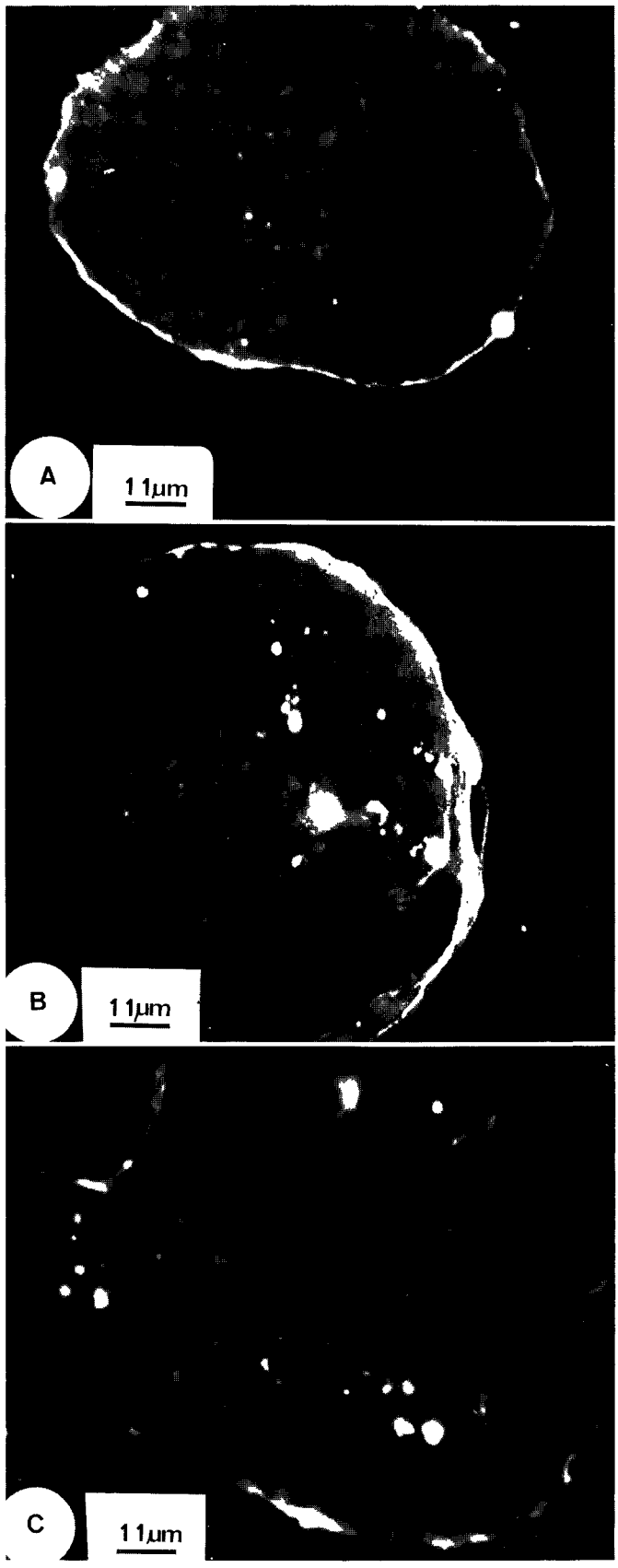


Fig. 4. Ultrastructural modifications of KRL cells by CHS and 25-OH. The KRL human renal carcinoma cell line (**A–C**) was grown for 1 week in conventional medium (**A**), or in medium supplemented with CHS 10^{-6} *M* (**B**), and 25-OH 10^{-6} *M* (**C**). Cells were prepared for transmission electron microscopy as described in Materials and Methods. Scale bar = 0.5 μm (**A**, **B**) or 1 μm (**C**).



24 h (results not shown). However, a striking reorganization of the actin cytoskeleton occurred in the cells treated with CHS or 25-OH for 4–5 days. This effect was dependent on the dose of sterol. The cells treated with CHS (fig. 6B) or 25-OH (fig. 6C) showed an imporant decrease in the number of stress fibers, even in apparently spread cells. The cytoplasmic actin filaments appeared detached from the plasma membrane and confined to some areas of the cytoplasm. Virtually every cell in the treated cultures displayed some degree of actin redistribution. At the highest doses of sterol (10^{-6} *M*), mostly punctuated and needle-shaped actin aggregates organized in reticular structures could be seen (fig. 7B,C). However, the tubulin network revealed by double fluorescence staining in parallel with actin remained unaffected by the cholesterol derivatives (fig. 6E,F). The intermediate filament distribution revealed by an anti-vimentin monoclonal antibody also remained unaffected by the sterol derivatives in all the cells studied (results not shown).

We quantified the polymerization status of actin in renal carcinoma cells after treatment by the cholesterol derivatives using the DNAse I inhibition assay (table 1). Treated cells exhibited no substantial increase in the G-actin pool or decrease in the F-actin pool despite disruption of microfilament bundles. Moreover, there were no significant changes in the total actin content in the renal carcinoma cells after treatment.

Table 1. Polymerization status of actin after a 5-day treatment with CHS or 25-OH

Treatment	Total actin ng/mg protein	G/F, %
Control	1.33 ± 0.15	5.28 ± 2.5
CHS	1.09 ± 0.08	3.97 ± 2.03
25-OH	0.78 ± 0.11	2.98 ± 0.47

Fig. 5. Filipin labelling of membrane cholesterol of treated KRL cells. The KRL human renal carcinoma cell line (**A–C**) was grown for 1 week on coverslips in conventional medium (**A**), or in medium supplemented with CHS 10^{-6} *M* (**B**), and 25-OH 10^{-6} *M* (**C**). Living cells were stained with 10^{-5} *M* filipin for 5 min at room temperature, then washed and fixed in 1% glutaraldehyde for 10 min. The cells were then observed with a Leitz Orthoplan microscope equipped for epifluorescence. Excitation and emission wavelengths were 490–495 and 520 nm, respectively. Scale bar = 20 μm.

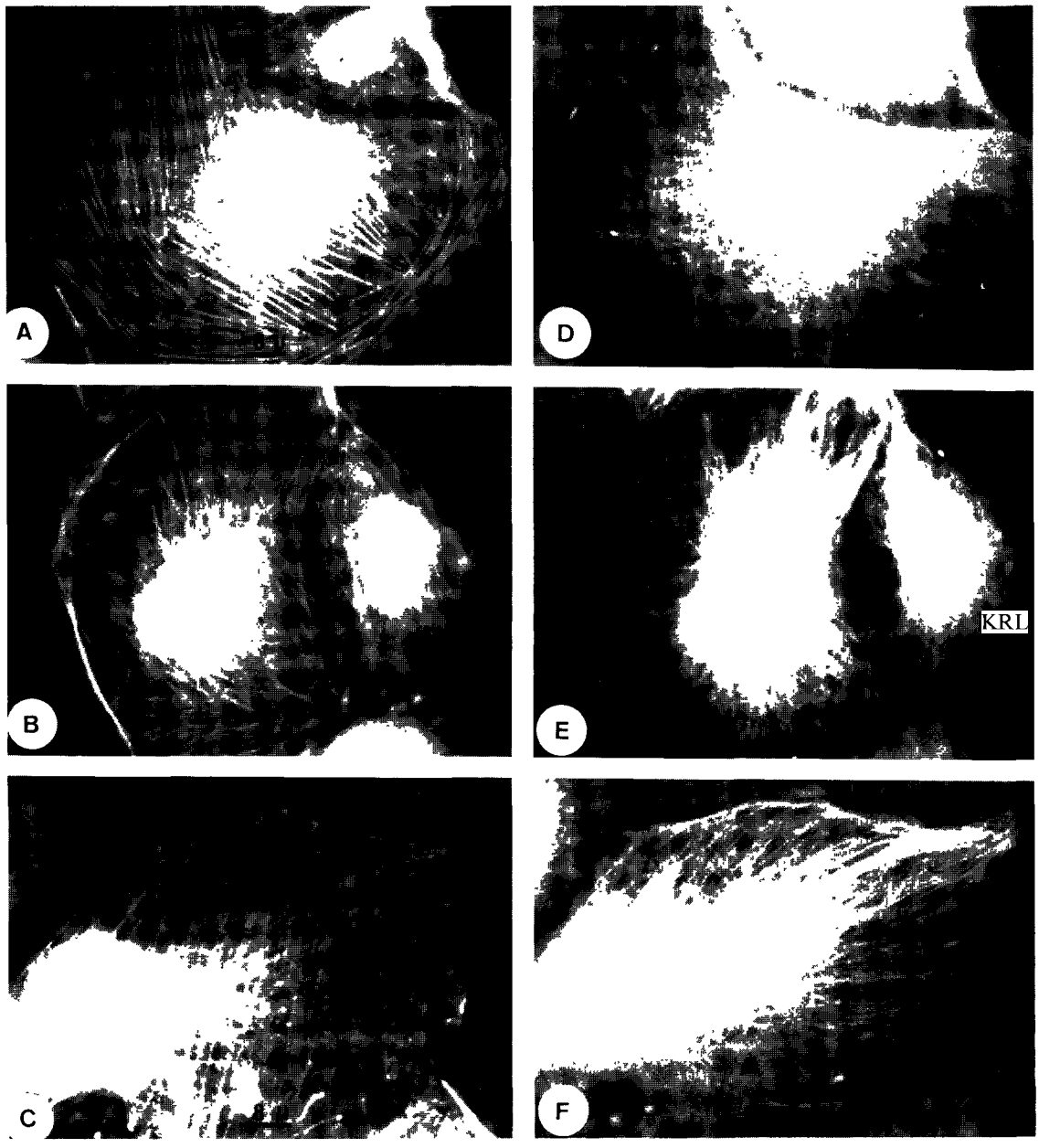
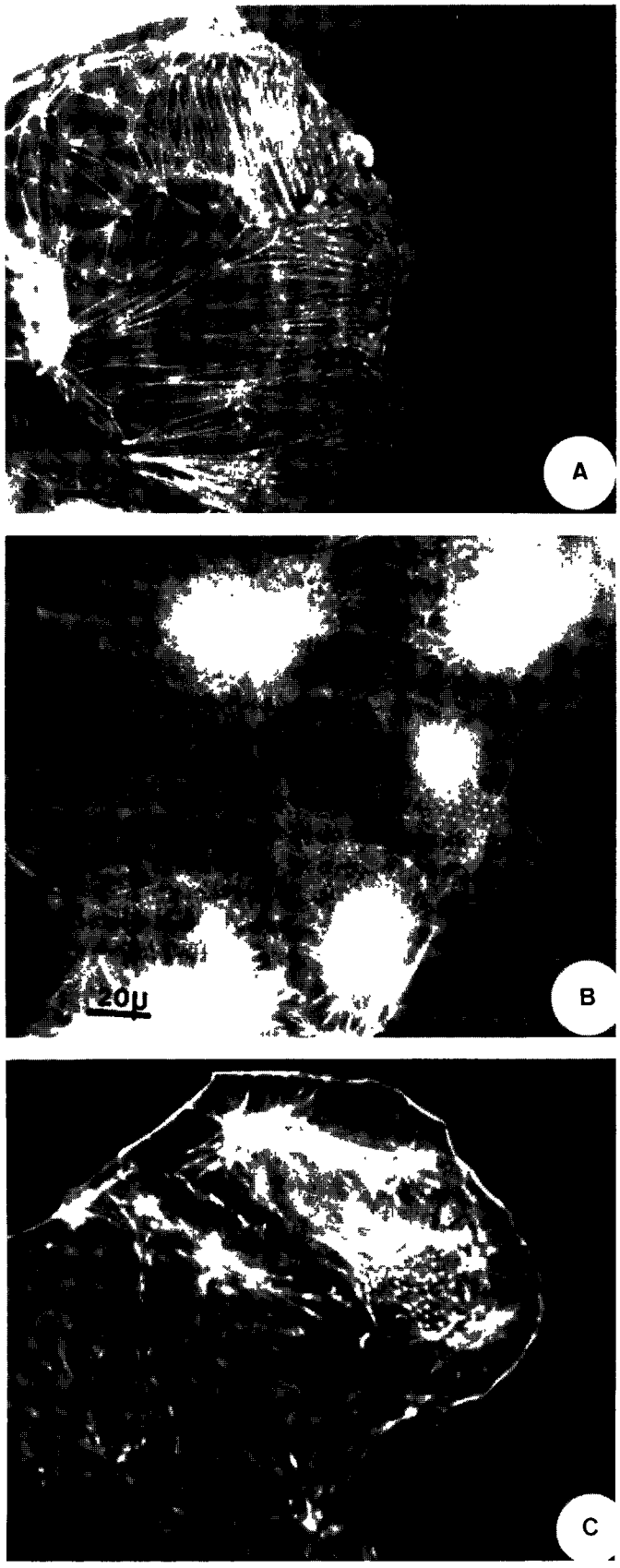


Fig. 6. Effect of CHS and 25-OH on the organization of actin and β-tubulin in cultured human renal carcinoma cells. The KRL human renal carcinoma cell line was grown for 1 week on glass coverslips in conventional medium (**A**, **D**), or in medium supplemented with CHS 10^{-7} *M* (**B**, **E**) and 25-OH 10^{-7} *M* (**C**, **F**). Cells were stained for actin with rhodamine-phalloidin (**A–C**) and for β-tubulin with a specific monoclonal antibody (**D**, **E**, **F**), and examined with a Leitz Orthoplan microscope equipped for epifluorescence, as described in Material and Methods. Scale bar = 8 μm.

Absence of Effect of the Cholesterol Derivatives on Normal Cells and Hepatomas

The previous observation raises the question of the cellular specificity of the effects of sterol on microfilament reorganization. For this purpose, we examined the effect of CHS or 25-OH on the microfilaments in nontumoral cells, namely normal human kidney cells and skin fibroblasts, and on another carcinoma, the Hep3B cell line. In none of these cells was the organization of the actin fibers altered by sterol treatment carried out under the same



conditions as in renal carcinoma cells. As an example, the results obtained in human skin fibroblasts are given in figure 8.

Discussion

In the present study, we report that changes in the structural organization of membrane cholesterol induced by long-term CHS or 25-OH treatment in human renal carcinoma cells was accompanied by a striking disorganization of F-actin microfilaments. These filaments appeared disrupted from the plasma membrane, and the number of distinctive microfilament bundles was decreased. Treatment by cholesterol derivatives did not affect the microtubule or intermediate filament networks, nor did it affect the organization of actin microfilaments in normal renal cells, fibroblasts or hepatomas. Early changes, after minutes or hours, were not observable in renal adenocarcinoma cells, which needed to be treated for at least 4–5 days before any modification could be detected in growth rate, cell morphology, cholesterol staining by filipin and actin distribution. This could be due to the low rate of CHS or 25-OH insertion in the membrane bilayer due to the low growth rate of the cultures. Moreover, it cannot be excluded that during this long treatment time, CHS and 25-OH may act at a transcriptional level [23].

Cholesterol is a major component of the plasma membrane of animal cells and is required for building up new membranes during cell proliferation and maturation. A variety of methods are available to modulate the relative composition of cell membranes imitating physiological cell processes.

Some authors assume that stiffening of the plasmic membrane by enhancing membrane cholesterol content may induce a better immunogenicity of tumor cells. So, Shinitzki and Skornick [24] showed that membrane incorporation of cholesterol derivatives such as the hydrophobic ester CHS decreases the membrane lipid fluidity of tumor cells and promotes the expression of latent tumor-associated antigens. These observations were at-

Fig. 7. Modifications of F-actin bundles in KRL cells after treatment with high doses (10^{-6} *M*) of sterols. The KRL human renal carcinoma cell line was grown for 1 week in conventional medium (**A**), or in medium supplemented with CHS 10^{-6} *M* (**B**), and 25-OH 10^{-6} *M* (**C**). Cells were stained with rhodamine-phalloidin as described for figure 6. Scale bar = 20 μm.

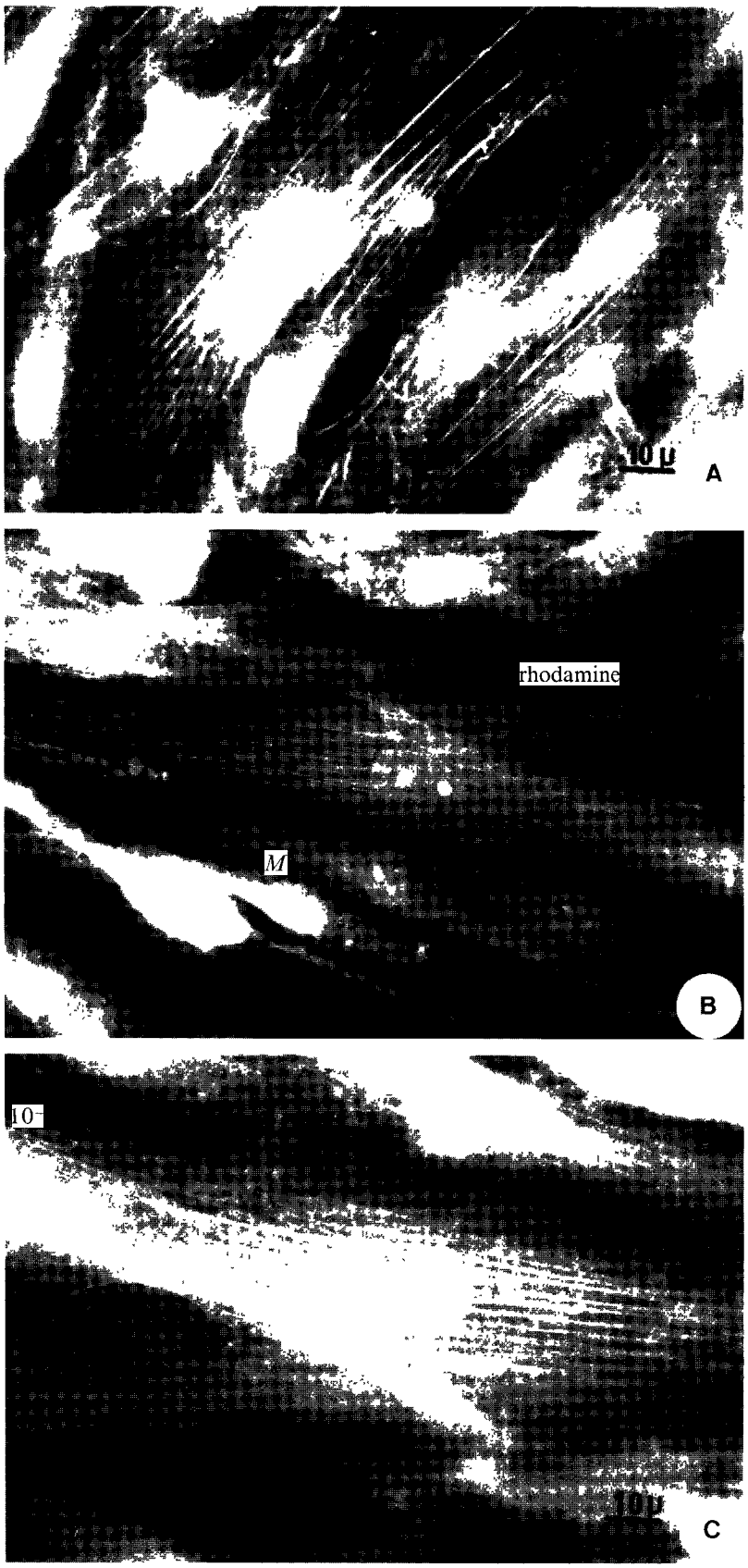


Fig. 8. Effect of CHS and 25-OH on the organization of actin in normal human skin fibroblasts. Normal human skin fibroblasts were grown on coverslips for 1 week in conventional medium (**A**), or in medium supplemented with CHS 10^{-6} *M* (**B**), and 25-OH 10^{-6} *M* (**C**). Cells were stained with rhodamine-phalloidin as described for figure 6. Scale bar = 10 μm.

tributed to a better recognition of tumor antigens due to the decreased fluidity of cancer cell membranes in vitro, which can be used as a source of vaccine against residual tumor.

Oxysterols, another cholesterol derivatives class, have many biological effects, including immunomodulation, angiotoxicity and atherogenesis. Oxysterols have also been shown to have profound effects on cell morphology and membrane-associated functions [25].

In living systems, oxygenated sterols are produced as normal compounds of the metabolic pathway of cholesterol. They decrease cholesterol synthesis and inhibit cholesterol esterification [26].

Also, substantial evidence indicates that one important property of oxygenated sterol compounds may be their physical insertion into biomembranes [27].

With a hydroxyl group at both ends of the molecule and a perpendicular orientation in the phospholipid bilayer, oxygenated sterols synergistically immobilize acyl chains and enhance protein helical structure in human erythrocyte membranes [28].

Using both CHS and 25-OH known to induce more potent stiffening, we previously showed that manipulation of the physicochemical state of the membrane of human renal carcinoma cells could increase their antigenicity in the donor patients. In contrast to previous findings, measurements of membrane fluidity did not clearly establish the causal relationship between increased immunogenicity and decreased fluidity due to stiffening of the tumor cell membranes [18].

In the present study, we evidenced by filipin staining of the living cells that several days of treatment with CHS or 25-OH induced the formation of areas of high concentrations of cholesterol or cholesterol derivatives, although the distribution of the areas revealed by filipin markedly differed between the two compounds. Alterations in cholesterol levels and metabolism are frequently observed in tumor cells, with an increased intracellular cholesterol content, as compared to their patches near the upper and lower surface of tumor cells, whereas normal cells stained diffusely with filipin [29].

It can be argued that a nonrandom distribution of components may exist in the fluid matrix of membranes in the form of lipid domains or specific cholesterol-rich regions with particular altered physicochemical properties. Thus, it could be assumed that reorganization of some lipids in the plasma membranes by the cholesterol derivatives can affect membrane proteins, in particular those involved in the contacts of actin microfilaments at the inner face of the plasma membrane.

A direct demonstration of such a spatial relationship between cholesterol-rich surface components, called microdomains, and the cytoskeleton has been obtained by double immunolabelling experiments in 3T3 cells.

Observation of cultured cells by double fluorescence staining indicated that regions of the plasma membrane which are in close contact with actin fibers have characteristics differing from other regions of the membrane. It was also found that several integral membrane proteins are excluded from these regions of membrane-fiber apposition [11]. A few studies suggested that in A 431 cells, a human epidermoid carcinoma, the interactions between the cytoskeleton and the epidermal growth factor receptors (EGF-R) were involved in the mechanism of action of EGF [30]. Recently it was found that the actin bundles delineating cell margins indeed take part in capping of the EGF-R of A 431 cells. The submembrane actin binding proteins are assumed to be involved in the linkage of microfilaments of EGF-R. Spectin is one of the proteins that plays an important role in anchoring actin filaments to membrane proteins, as has been demonstrated in human erythrocytes. This protein is one of those which may be considered as a linker in receptor-microfilament interactions. After treatment of the renal cancer cells with the sterols, we observed a decrease in the fixation of EGF linked with rhodamine to the EGF-R in the treated cells (unshown data).

Conclusion

Alteration of the actin cytoskeleton of cultured cells is associated with changes in cell shape induced by various agents. These effects include disassembly of stress fibers and/or reorganization into aggregates of short filaments [31]. The mechanisms underlying these changes are not known, but similar changes have recently been described by cross-linking of actin filaments to the cytoplasmic domain of integrins via talin [32].

Qualitatively similar changes in actin filament distribution have been described in cells treated by a variety of agents [33]. It should be hypothesized that the distinctive morphology of cells may be established and maintained in culture by a mechanism involving plasma membrane attachment and orientation of actin filaments, mediated by extracellular matrix receptors such as integrins [34].

It can be suggested that the incubation of the cancer cells with CHS and 25-OH induced a redistribution of the cholesterol-rich membrane microdomains which are

linked to the cytoskeleton through submembrane proteins such as vinculin, spectrin, talin.

One hypothesis prevails at the present time. It is that the insertion of 25-OH in the membrane bilayer perturbs its dynamic state, which in turn alters its physiological properties. This alteration seems to decrease cell surface EGF-R after treatment with the sterols.

In conclusion, the nature of the association of membrane domains with the actin cytoskeleton is currently under investigation. It will be of considerable interest in the future to determine how these interactions may be altered when the membrane domains are changed in different physiological or pathological states.

Acknowledgements

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