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The Ultrastructure of MCF-10A Acini

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MCF-10A human mammary epithelial cells cultured inside reconstituted basement membrane form acini that resemble the acinar structures of mammary lobules. This three-dimensional culture system has been used for identifying and characterizing the signal transduction pathways controlling cell proliferation and death, and for studying their disregulation in malignant progression. We have compared the ultrastructure of MCF-10A acini, MCF-10A cells grown in monolayer, and the acinar structures of human breast lobules. The tissue architecture of MCF-10A acini was formed by hemidesmosomes connected to a basement membrane and by abundant desmosomes between acinar cells. Intermediate filaments that joined into large and abundant filament bundles connected hemidesmosomes and desmosomes to sites at the nuclear surface. Fewer and thinner bundles of filaments were observed in monolayer MCF-10A cells and even fewer in breast tissue. Tight junctions were observed between cells in breast tissue but missing in MCF-10A acini. The cytoplasm of MCF-10A acinar cells had a polar organization similar to that observed in breast tissue, with centrosomes and the Golgi apparatus on the apical side of the nucleus. MCF-10A acinar nuclei had an irregular, frequently invaginated surface and had a single nucleolus. The distribution of heterochromatin was similar to that in the epithelial cells of breast tissue. The nuclei of monolayer MCF-10A cells had multiple nucleoli, a more regular profile, and less heterochromatin. Electron microscopy has the resolution required to survey features of MCF-10A cell and acinus architecture that may change with manipulations designed to induce malignant phenotypes. J. Cell. Physiol. 208: 141–148, 2006.

Malignant transformation takes place within a three-dimensional tissue and requires a profound dysregulation of normal tissue structure and homeostasis, altering the balance between survival and cell death. While much has been learned by studying malignant changes in two-dimensional monolayer cultures, this method does not recapitulate the microenvironment of a tissue or the dynamic and reciprocal crosstalk between the extracellular matrix and nuclear gene expression (Weaver et al., 1996).

Normal MCF-10A mammary epithelial cells, and similar HMT-3522 cells, when cultured in three dimensional reconstituted basement membrane (rBM) culture, reproduce important features of normal breast tissue in an increasingly well characterized temporal and spatial program (Petersen et al., 1992; Weaver et al., 1995, 1996, 2002; Debnath et al., 2002, 2003a). An initial stage of proliferation produces loosely connected groups of cells and is followed by cell cycle arrest. Acini form from these groups of cells by basal deposition of a basement membrane with collagen IV and laminin V. Apical-basolateral polarization of acinar cells is achieved with attachment of $\alpha6\beta4$ integrin to the basement membrane. Cells attached to the basement membrane survive, while unattached cells undergo apoptosis, creating a luminal space. Cell nuclei reorganize and display a differentiated nuclear architecture more characteristic of mammary epithelial cells in tissue than those cultured in monolayer (Lelievre et al., 1998). Acini assembled in three-dimensional culture resemble the corresponding structures of normal breast tissue, which are clustered in lobules that connect to intralobular ductules that in turn connect to interlobular ducts. These similarities to in vivo breast biology have made three-dimensional culture systems important for elucidating the mechanisms of cancer-related genes in a tissue context.

In one experimental approach, cancer-related genes can be ectopically expressed in normal mammary epithelial cells. Then three-dimensional culture in rBM can be used to determine the consequences on tissue formation, cessation of proliferation, development of basal-apical cell polarity, basement membrane formation, apoptosis of luminal cells, and other parameters (Debnath et al., 2003a). For example, the forced expression of Akt in the PI3 kinase pathway of MCF-10A cells causes them to form large, misshapen structures in three-dimensional rBM culture (Debnath et al., 2003b). These more tumor-like aggregates arise from an mTOR-dependent mechanism producing abnormal proliferation and increased cell size. In another example, the MCF-10A system has been used to explore the regulation of apoptosis and autophagy to clear luminal spaces during three-dimensional acinus formation (Debnath et al., 2002). Lumen-filling, characteristic of early

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malignancy, required the expression of oncogenes enhancing proliferation and those inhibiting apoptosis, or the enforced homodimerization of ErbB2 (HER2/Neu), which upregulates both types of oncogenes and also cooperates with TGF-beta to increase MCF10A cell migration and invasiveness (Seton-Rogers et al., 2004).

In a reciprocal experimental design, mammary tumor cells cultured in rBM form disorganized and continually proliferating masses. For example, the application of certain combinations of (1) drugs targeting the PI3 kinase or MAP kinase pathways, (2) antibodies targeting β1 integrin or the EGF receptor, and (3) appropriate genetic manipulations causes these cultures revert to more normal patterns of morphology, proliferation, and apoptosis (Weaver et al., 1997; Wang et al., 1998, 2002; Chen et al., 2000).

One of the first studies of the MCF-10A cell line was an ultrastructural characterization of the cells grown in monolayers (Tait et al., 1990). A comprehensive ultrastructural study of MCF-10A acini has not appeared, though electron microscopy has played a role in establishing features of acinar biology, including the demonstration of apoptosis and autophagy in cells not in contact with the basement membrane (Debnath et al., 2002; Mills et al., 2004). Because of the growing importance of the MCF-10A system and the great potential of electron microscopy to characterize malignant changes in breast cell and tissue structure at high resolution, we present a detailed analysis of MCF-10A acinar ultrastructure. We demonstrate that electron microscopy can directly image important biological features of MCF-10A tissue structures, features that can only be indirectly inferred by immunohistochemistry for the light microscope.

MATERIALS AND METHODS

MCF-10A cells were cultured on plastic tissue culture dishes (Nunc) in Reduced Growth Factor Matrigel without phenol red (BD Biosciences, San Jose, CA) as described by Debnath et al. (2003a).

For confocal microscopy, cells were cultured in Reduced Growth Factor Matrigel on 4-well chamber slides (Nalge Nunc, Rochester, NY), before fixation and staining as described (Debnath et al., 2003a) and then imaged with a Leica SP1 confocal microscope. DNA was stained with DRAQ5 after the second antibody wash.

For electron microscopy of MCF-10A acini, tissue structures were harvested by suspending the cultures in ice cold DMEM/F12 without serum or additives and centrifuging at 600*g* for 5 min, followed by three washes in cold DMEM/F12 to remove most of the Matrigel. Alternatively, in some experiments, embedded acini were fixed without removing Matrigel and then processed. Acini were fixed in 2.5% glutaraldehyde in 0.1M sodium cacodylate, pH 7.4 at 4°C for 1–2 h, then washed in 0.1M sodium cacodylate. Monolayer cultures of MCF-10A cells on Thermanox coverslips or on tissue culture dishes were either fixed without washing or washed in DMEM-F12 medium without additives at 37°C before fixation in 2.5% glutaraldehyde in 0.1M sodium cacodylate, pH 7.4 for 1 h at 4°C.

Samples were postfixed in 1% osmium in 0.1M sodium cacodylate at 4 degrees for 30 min, washed again, and dehydrated in graded ethanols. For samples compatible with EDTA regressive staining, 0.1M Sorensen's phosphate buffer, pH 7.3 replaced the sodium cacodylate and osmium postfixation was eliminated. When MCF-10A cells were grown in monolayer on tissue culture dishes, ethanol replaced propylene oxide as the transitional solvent. In some MCF-10A monolayer experiments osmium postfixation was replaced with the ferrocynanide reduced osmium–thiocarbohydrazide–ferrocyanide reduced osmium method (Willingham and Rutherford, 1984) which gave better preservation of mitochondrial ultrastructure.

For embedment in Epon, the transitional solvent was propylene oxide. Thin sections were stained with 1.4% uranyl acetate in 40% ethanol and then with lead citrate. Sections were imaged with a Philips CM10. Negatives were scanned and processed digitally. Size measurements were made from the digitized images.

Normal human breast tissue samples from a reduction mammoplasty were immediately dissected in Hanks balanced salt solution with Toluidine blue staining to aid in the location of terminal ductal lobular units. Tissue was fixed in 3% glutaraldehyde in 0.1M sodium cacodylate, pH 7.4 at 4°C for 3 h and processed as described above.

RESULTS AND DISCUSSION

MCF-10A cells grown in rBM form acinar structures with a diameter of 71 ± 30 μM. Observed 20 days after seeding in Matrigel (Fig. 1), these acini are stable tissue structures with one layer of live cells surrounding a lumen created by the death of internal cells. The evidence that acini are stable structures is that they maintain a constant size from day 12 to day 20, have few

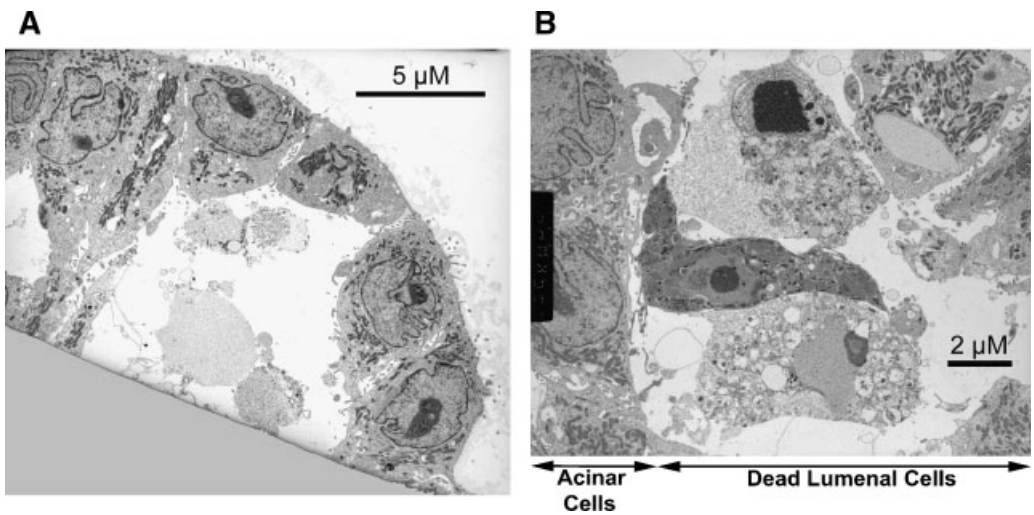

Fig. 1. The architecture of MCF-10A acini. MCF-10A cells were grown in rBM culture for 20 days before fixation and processing for electron microscopy. **A**: MCF-10A formed acini consisting of one layer of live cells. **B**: The lumens of these acini contained dead cell remnants. Many of these dead cells had many cytoplasmic vacuoles, consistent with autophagy.

cells with KI-67 staining, have no mitotic figures, and show no evidence of cell death in the one surviving layer of cells ((Debnath et al., 2002; Debnath et al., 2003a) and our unpublished results).

Lumens

Cell death is essential for clearing MCF-10A lumenal spaces (Debnath et al., 2002). Cell remnants were frequently observed in the lumens of MCF-10A acini. As seen in Figure 1B, some of these cells had highly condensed and electron dense inclusions in remnant nuclei, properties that are characteristic of apoptotic cells. As previously observed (Debnath et al., 2002), the cytoplasm of many remnant lumenal cells was highly vesiculated, a characteristic of autophagy. It is possible that these cells induced autophagy as a survival mechanism before succumbing to apoptosis (Lum et al., 2005). Alternatively, autophagy is a form of programmed cell death that, like apoptosis, can involve caspase activation (Yu et al., 2004). In fact, a role for tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) both in inducing autophagy and in clearing the lumenal spaces of MCF-10A acini has been demonstrated (Mills et al., 2004).

These mechanisms may be important in maintaining clear lumens, insuring that cells detaching from the basement membrane into the lumen die and do not proliferate. The suppression of lumenal cell death may be an important early step in the progression to breast cancer (Debnath et al., 2002). This mechanism for clear lumen maintenance may be somewhat different from the mechanisms initially creating lumens in developing breast. Nests of epithelial cells appear under the future nipple during early embryogenesis, and the postnatal stages of development may consist of the elongation and branching of existing ducts (discussed in Pitelka et al., 1973).

Elements of tissue architecture

To support mammary tissue architecture, the cytoskeletons of individual cells connected to specialized junctions at the cell membrane. At desmosomal junctions the cytoskeletons of adjacent cells were linked together, while hemidesmosomes connected the cytoskeletons to molecules of the extracellular matrix. The disruption of these connections that occurs during breast tumor progression causes the disorganization of tissue structure and allows cells to become

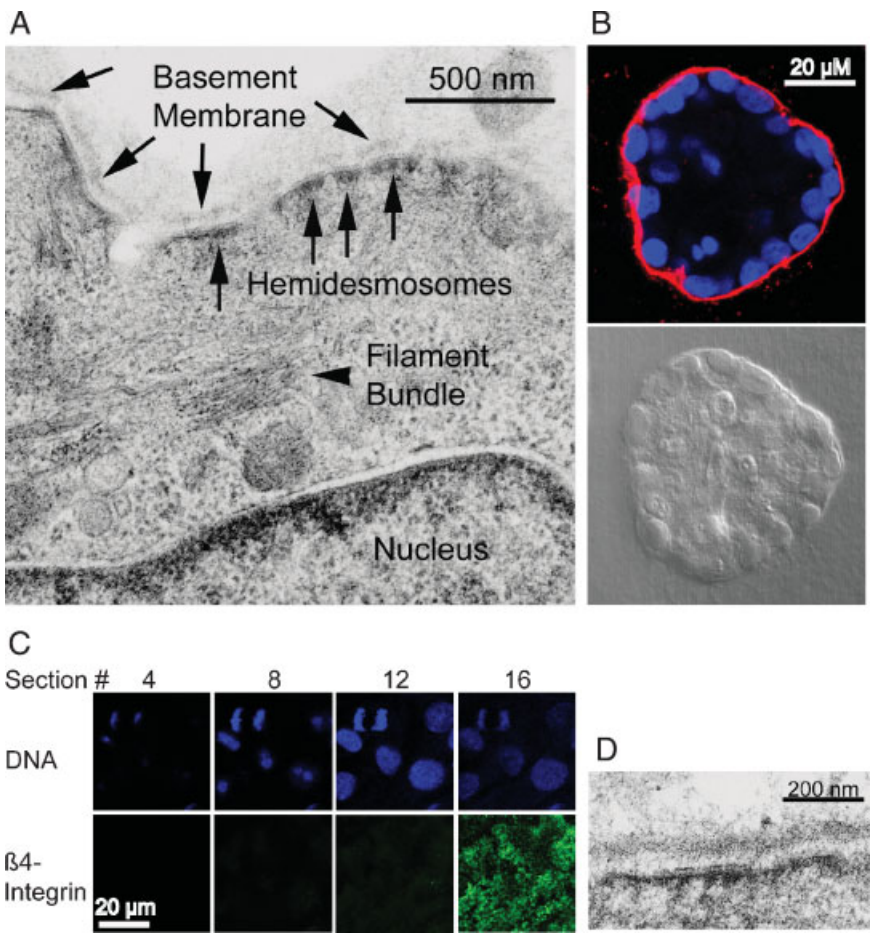

Fig. 2. Junctions between cells and the basement membrane. **A**: Acini formed a basement membrane (upper arrows) apposed to the basal surface of cells. Hemidesmosomes (lower arrows) connected cells to this basement membrane. **B**: These connections contain β4 integrin (in the form of α6β4) as shown in the red channel of the single confocal section of the upper part. Nuclei shown in the blue channel were stained with Draq5. The bottom part shows the differential interference contrast image of the same section. **C**: The distribution of β4 integrin (green) in MCF-10A cells grown in monolayers on coverslips. Section 16 corresponds to the bottom surface of cells where they are attached to the coverslips. Nuclei stained with DRAQ5 are shown in blue. **D**: The epithelial cells of a normal breast biopsy had hemidesmosomes apposed to the basement membrane with an appearance and distribution similar to that of the MCF-10A acini of part A.

motile and invasive (Ren et al., 1990; Bergstraesser et al., 1995).

Cell-extracellular matrix junctions

Matrigel rBM is enriched in extracellular matrix molecules but these matrix molecules lack the normal architecture organizing a basement membrane. MCF-10A cells grown in this disorganized extracellular matrix generate their own basement membrane, containing both laminin-5 and collagen-IV (Muthuswamy et al., 2001; Debnath et al., 2002). This basement membrane, seen in Figure 2A, had a dark band of the lamina densa (upper arrows) running parallel to the cell membrane. Spanning the membrane were dense structures that are hemidesmosomes (lower arrows). $\alpha 6\beta 4$ integrin is concentrated in these structures and connects to laminin in the basement membrane (Debnath et al., 2002; Weaver et al., 2002). The $\beta 4$ integrin constrained to these sites at the basal surface of cells was detected by antibody staining and confocal microscopy (Fig. 2B). Primary mammary epithelial cells (Bergstraesser et al., 1995) and MCF-10A cells (Stahl et al., 1997) grown in monolayer have been reported to have hemidesmosomes. For monolayer cultures of MCF-10A cells, most of our sections for electron microscopy did not bisect the growth surface so it was difficult to estimate the density of hemidesmosomes. Confocal microscopy of MCF-10A cells grown in monolayer showed that $\beta 4$ integrin was concentrated in hemidesmosome-like structures at the growth surface (Fig. 2C). Human breast lobules observed in normal biopsies had hemidesmosomes very similar in appearance and distribution to those of the MCF-10A acini (Fig. 2D). Ribosomes were sometimes observed in clusters close to hemidesmosomes (Fig. 4B). It has been reported that mechanical tension induces ribosome localization to sites of integrin clustering (Chicurel et al., 1998).

Cell–cell junctions

In normal breast tissue (Fig. 3A) tight junctions were frequently seen at cell–cell junctions near the apical surface (Pitelka et al., 1973) but they were not observed between adjacent MCF-10A cells in the acini formed in rBM culture (Fig. 3B). This is an important difference between in vivo breast tissue and in vitro assembled tissue. Tight junctions may contribute to the development and maintenance of cellular polarity by restricting lipids and membrane proteins to either an apical or basolateral distribution. Tight junctions also participate in signal transduction pathways (Matter and Balda, 2003), and play an important role as a permeability barrier to milk molecules during lactogenesis (Itoh and Bissell, 2003). Since the structure of tight junctions in mammary tissue is regulated by lactogenic hormones and progesterone, it may be that the absence of these structures in MCF-10A acini reflects the altered hormone composition of the culture medium-Matrigel environment. Consistent with this view, tight junctions are observed by both electron microscopy and dye exclusion assay in acini formed during rBM culture of primary mammary epithelial cells from pregnant mice (Barcellos-Hoff et al., 1989). These cells are primed for lactation and the resulting acini produce and secrete milk proteins. The MCF-10A three-dimensional culture system could be a useful system for testing hormone effects on tight junction formation. This may be important for understanding breast tissue tumorigenesis, since loss of tight junctions and their molecules is associated with poor prognosis in breast cancer patients (Martin et al., 2004).

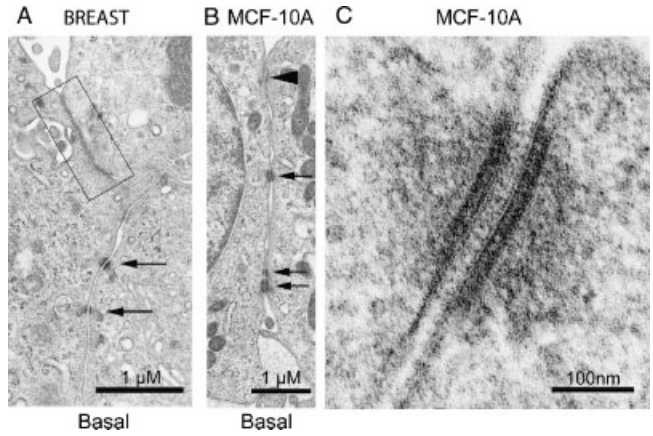

Fig. 3. Cell–cell junctions. **A**: Mammary epithelial cells of a normal breast tissue biopsy had both tight junctions (bounded by the box) close to the lumen and desmosomes (arrows). **B**: MCF-10A acini had many desmosomes (arrows) but lacked tight junctions. Occasionally, an adherens junction (arrowhead) could be seen at the cell border near the lumen. Compared to desmosomes, these junctions were connected to fewer cytoplasmic filaments and had less electron density in the cleft between cells. **C**: Seen at higher magnification, the desmosomes of MCF-10A acini were connected to cytoplasmic filaments and the cleft was characterized by short filaments between cells.

The cells of MCF-10A acini were held together by frequent desmosomes (Fig. 3B, arrows, and 3C). These were recognized as dense structures at and parallel to the plasma membrane that were connected to a dense network of cytoplasmic filaments with short filamentous structures crossing the cleft (Figs. 3C and 4C). Occasionally the junction nearest the lumen had a different appearance with fewer cytoplasmic filaments and with much less density in the cleft (Fig. 3B, arrowhead). These may be adherens junctions. MCF10A cells grown in monolayer also had frequent desmosomes (data not shown) and these may be responsible for the epithelial clustering of these cells.

The cytoskeleton

Although thin filaments and microtubules were occasionally observed, by far the dominant cytoskeletal structures were large bundles of filaments (Figs. 2A, 4, 5A,B). These filaments were most concentrated in three cellular regions: first, connecting to a network of individual filaments at the hemidesmosomes (Fig. 4A, and 4B), second, connecting to desmosomes at cell–cell junctions (Fig. 4C), and finally, at the surface of the nucleus where they were sometimes seen connecting to the nuclear lamina (Fig. 4E). Based on this cellular geometry, the epithelial origin of the cells, and the diameter of individual filaments (8–10 nm), we suggest that these are bundles of intermediate filaments made from cytokeratins. Such bundles have been called tonofibrils (Gaffney, 1982). The filament bundles were much less abundant in monolayer cultures of MCF-10 A cells than in acini assembled in three-dimensional rBM culture (data not shown).

Human breast tissue had even fewer cytoplasmic filament bundles than MCF-10A monolayer cells. These tonofibrils and their constituent intermediate filaments may be important for the structural integrity of epithelial tissues (Fuchs and Cleveland, 1998). MCF-10A acini are constructed from a single type of cell and

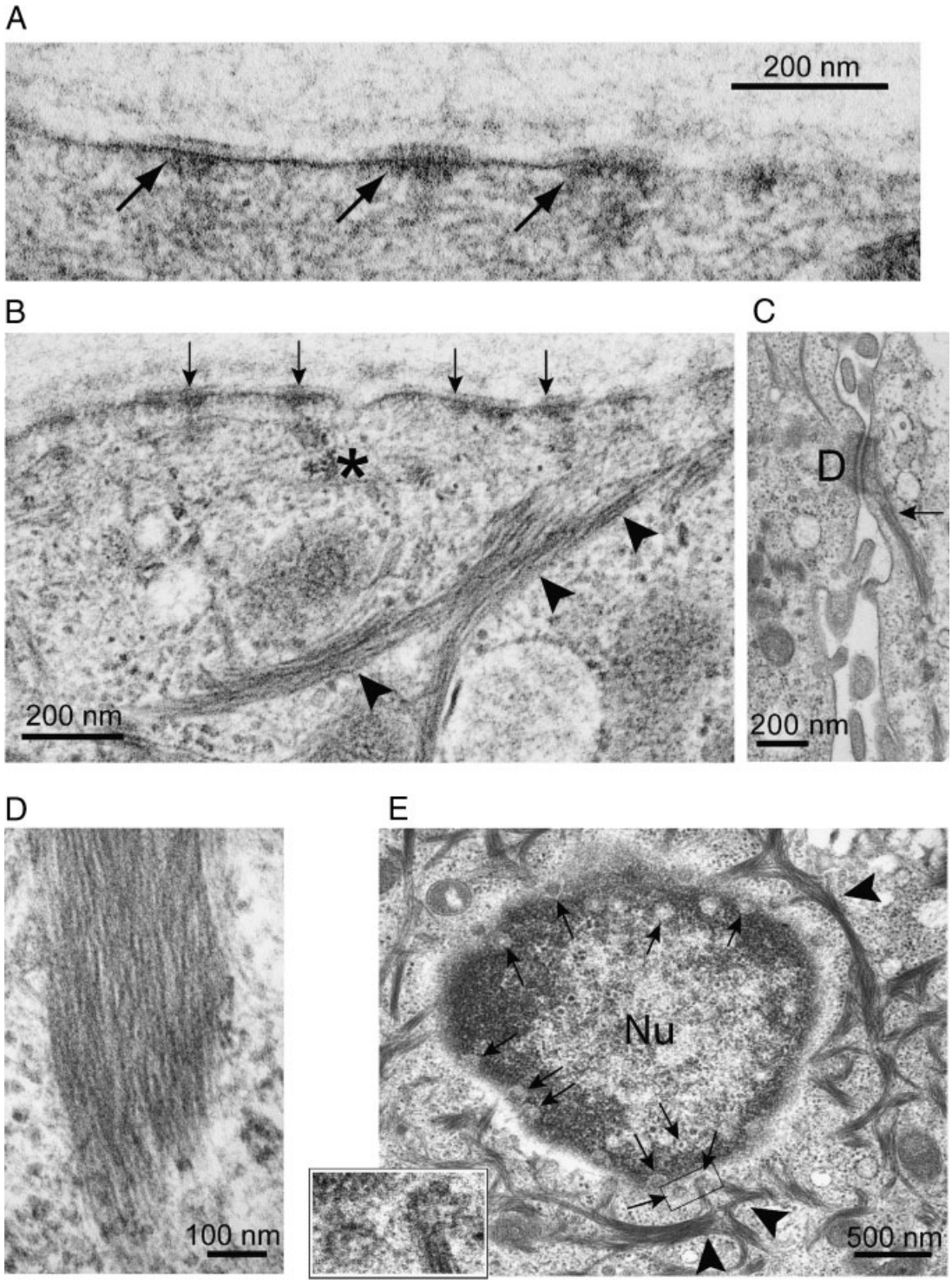

Fig. 4. The cytoskeleton of MCF-10A acinar cells. **A**: Hemidesmosomes (arrows) were connected to a network of 8–10 nm cytoplasmic filaments. **B**: These hemidesmosome-connected filaments were bundled together into tonofibrils (arrowheads). Ribosomes and clusters of ribosomes (*) were seen in the cytoplasm close to hemidesmosomes. **C**: The cytoplasmic filaments that were connected to desmosomes were also bundled together (arrows). **D**: Seen at higher magnification, bundles consisted of many individual filaments of uniform diameter. **E**: Filament bundles (arrowheads) were also frequently seen at the surface of the nucleus (Nu). In this section near the top surface of the nucleus, the nuclear lamina has been cut tangentially and sites where filament bundles connect at the lamina were seen. Arrows mark nuclear pores embedded in the nuclear lamina. The inset shows a filament bundle (right) terminating at the nuclear lamina about 100 nm from a nuclear pore (left).

those cells must generate all the architectural support for the tissue. This may require a greater bundling of intermediate filaments. In actual breast tissue, however, much of this support can be provided by a more substantial and structured stroma and by myoepithelial cells that wrap around the lobule, contract to expel milk, and share cytoskeletal features of smooth muscle cells (Linzell, 1955; Warburton et al., 1981). Myoepithelial cells may develop from epithelial cells (Pitelka et al., 1973) and can restore normal polarity to mammary epithelial cells grown in a collagen-I gel, most probably by producing laminin-1 (Gudjonsson et al., 2002).

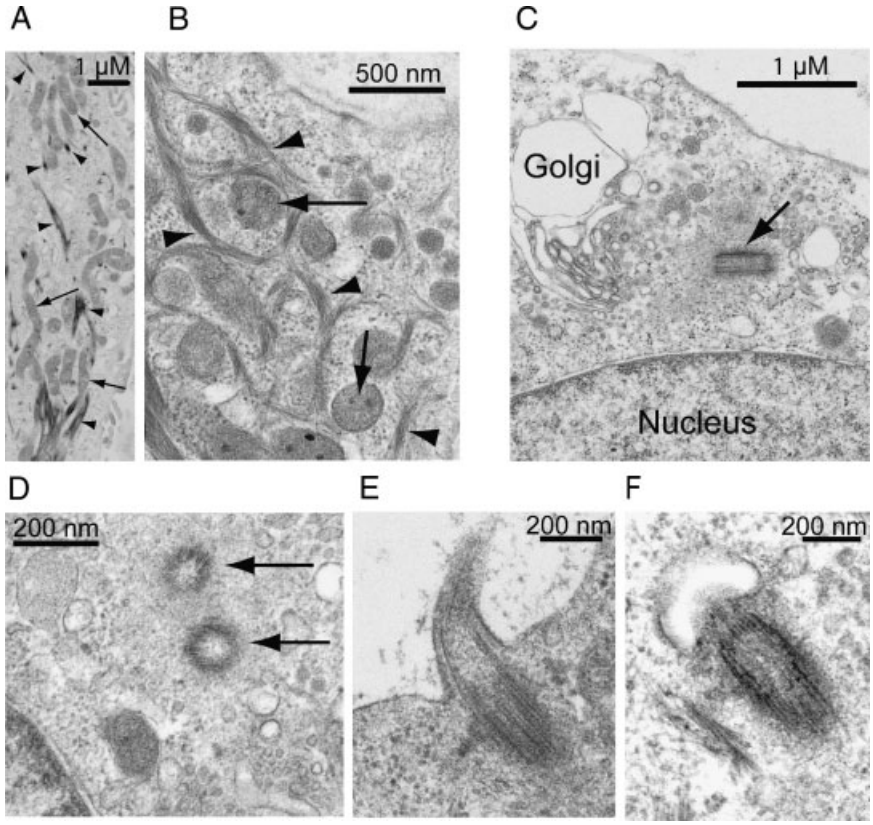

Fig. 5. The cytoplasmic organization of MCF-10A acinar cells. **A**: The cytoplasm of MCF-10A acini had abundant and unusually elongated mitochondria. A few are marked by arrows. Regions enriched in mitochondria were frequently also sites where cytoplasmic filament bundles (arrowheads) were concentrated. **B**: Mitochondria, seen in this part in cross section (arrows) were in cytoplasmic regions enriched in filament bundles (arrowheads) and were often surrounded by bundles. **C**: The cytoplasm had a polar organization, with the nucleus nearer the basal surface and both the centrosomes (arrow) and the Golgi apparatus oriented apically toward the lumen. **D**: A different plane of section showing the centriole pair of a centrosome (arrows) in a region of the cytoplasm rich in Golgi apparatus and located to the apical side of the nucleus. **E**: Occasional primary cilia were on the apical surface of cells. **F**: The cap structure seen in this section is the tunnel though the cytoplasm from which the primary cilium emerges from the cell.

Cytoplasmic organization

The cytoplasm of acinar cells was characterized by abundant and unusually elongated mitochondria (Figs. 1 and 5A). Regions of the cytoplasm that were rich in mitochondria also had abundant filament bundles (Fig. 5A,B). When seen in cross section (Fig. 3B), these mitochondria (arrows) were often enmeshed in networks of bundled filaments (arrowheads). Fewer mitochondria were observed in monolayer MCF-10A cells or in the epithelial cells of breast tissue. The cytoplasm had sparse endoplasmic reticulum. This was expected since these acini are not secreting milk molecules. Primary mammary epithelial cells collected from pregnant mice and grown on rBM form acini that secrete milk proteins and milk fat globules (Li et al., 1987; Barcellos-Hoff et al., 1989; Aggeler et al., 1991). Cells in these in vitro lactating structures have highly abundant rough endoplasmic reticulum, recapitulating the hypertrophy of rough endoplasmic reticulum that supports milk production in the lactating mammary gland (Hollmann, 1969).

The cytoplasm of acinar cells had a polar organization. Nuclei were closer to the basal surface (Figs. 1 and 6A). Centrosomes were close to the nucleus but only toward the apical side toward the lumen (Fig. 5C,D). Most of the Golgi apparatus that was observed was also positioned to the apical side of the nucleus and was frequently near centrosomes. Occasional short primary cilia were observed at the apical surface (Fig. 5E,F). As seen in Figure 5F, the bases of these cilia were easily identified by the tunnel structure adjacent to the cilium. In normal breast tissue, occasional short cilia project into the lumenal spaces of ducts, though they have been considered to be of myoepithelial origin (Stirling and Chandler, 1976a,b, 1977). Highly abnormal cilia have been seen in breast carcinomas (Reilova-Velez and Seiler, 1984).

Nuclei

The shape of MCF-10A acinar nuclei was less oval and regular than observed in nuclei of the same cells grown in monolayer (compare Fig. 6A and B with C). A range of shapes was observed. Some acinar nuclei had shallow indentations in the nuclear surface as in Figure 6A while other nuclei had deep invaginations as shown in Figure 6B. Multiple examples of these nuclear shapes can be seen in the low magnification view of Figure 1A. Occasional nuclear invaginations were observed in MCF-10A monolayer cells, though at a lower frequency than for acinar cells and in a plane of section tangential to the surface of the nucleus, where a groove seen in the one plane of section may appear to be an invagination or a channel. In acinar MCF-10A nuclei, the invaginations appeared to be deeper into the nucleus and to be more abundant. Nuclei in human breast tissue had fewer invaginations than MCF-10A cells in acini. A typical nuclear profile from epithelial cells in breast lobules is

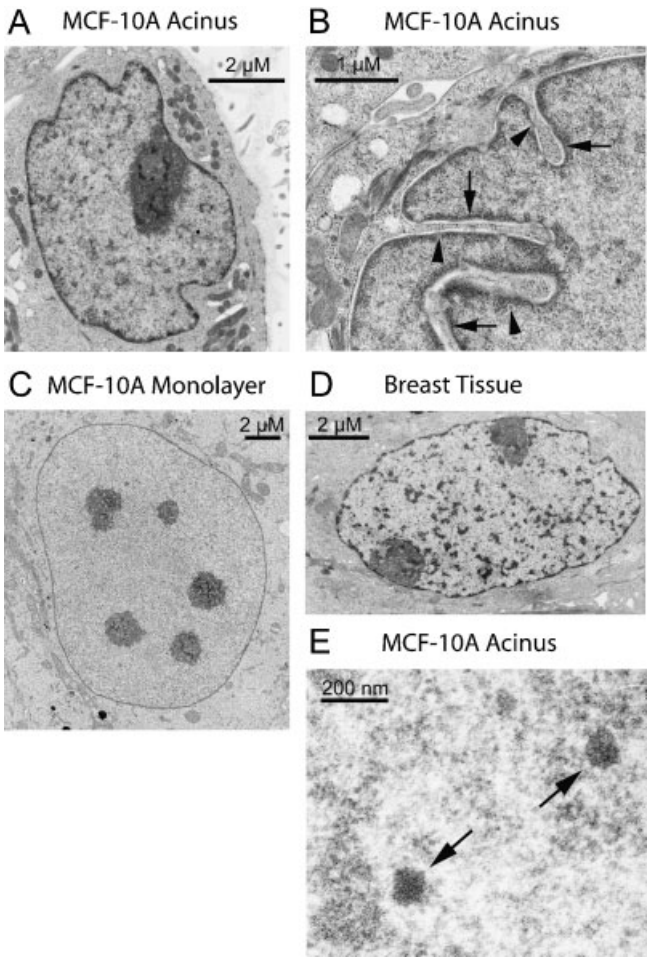

Fig. 6. The nuclei of MCF-10A cells and breast mammary epithelium. **A**: The nuclei of MCF-10A cells in acini had a single nucleolus. The nuclear profile was irregular with many indents and invaginations. The more densely staining heterochromatin was distributed in patches at the nuclear periphery as well as in small masses through the nucleoplasm. **B**: Many cells had multiple deep invaginations (arrows) in the surface of the nucleus. Nuclear pores were seen in these invaginations (arrowheads). Arrows mark the heterochromatin at the inside surface of the nuclear lamina. **C**: The nuclei of MCF-10A cells cultured in monolayer had multiple nucleoli. They had less heterochromatin, seen by the relative absence of electron dense masses at the periphery and throughout the nucleoplasm. Nuclear profiles were more regular with fewer invaginations observed. **D**: The epithelial cells in a normal breast tissue biopsy had nuclei with one or two nucleoli. The amount and distribution of heterochromatin was similar to that of MCF-10A acinar cells (part A) but fewer indentations and invaginations in the nuclear profile were observed. **E**: MCF-10A acini had abundant nuclear bodies (arrows) that resembled Cajal bodies.

seen in Figure 6D. Cytoplasmic invaginations into the nucleus have been observed in mouse mammary epithelial cells grown on rBM (Barcellos-Hoff et al., 1989; Aggeler et al., 1991). Channels have also been observed in the nuclei of many cell types cultured in monolayer (Fricker et al., 1997), though these channels were smaller than we have observed in MCF-10A acinar nuclei. Larger channels of cytoplasm have been observed in HeLa nuclei where they may participate in calcium signaling (Lui et al., 2003).

The nuclei in MCF-10A acini after 20 days in three-dimensional culture always had a single nucleolus. The nuclei of the same MCF-10A cells cultured in the same medium in monolayers (Fig. 6C) had an mean number of 3.5 ± 1.1 nucleoli per nucleus ($n = 56$). Nuclei in the acinar epithelial cells of human breast lobules had 1 or 2 nucleoli (Fig. 6D). The regulation of nucleolar number is not well understood. Normal diploid human cells have nucleolar organizing regions on the five acrocentric chromosomes and can therefore form a maximum of 10 nucleoli per nucleus. The clustering of these regions, in practice, results in a much smaller number of nucleoli, with the number being characteristic of cell type. MCF-10A cells have a stable, near-diploid karyotype (Soule et al., 1990; Yoon et al., 2002) with modest genetic modifications typical of culture-adapted breast epithelial cells (Yaswen and Stampfer, 2002). In MCF-10A acini, the nucleolar organizing regions of all 10 nucleolus-forming chromosomes must be located in close proximity, imposing greater constraints on the spatial organization of chromosome territories (Gilbert et al., 2005) than is found in MCF-10A cells grown in monolayers.

MCF-10A cells forming acini in rBM culture had more heterochromatin than the same cells grown in monolayer (compare Fig. 6A and C). This heterochromatin was distributed at the nuclear lamina and in small masses in the nucleoplasm. These features were similar to the heterochromatin in the nuclei of breast tissue (Fig. 6D).

Many nuclear bodies were seen in MCF-10A acinar nuclei. These included structures of about 100 nm (Fig. 6E) that resembled Cajal bodies (Monneron and Bernhard, 1969), though an immunohistochemical analysis will be required to confirm this. There were many more of these structures in acini nuclei than in MCF-10A cells grown in monolayer.

SUMMARY

The formation of breast tissue structures by normal mammary epithelial cells cultured in rBM has become an important system for identifying and manipulating signal transduction pathways controlling cell proliferation and death, pathways important in the progression of normal mammary epithelial cells to malignancy. Malignancy in the mammary gland is accompanied by changes in tissue organization and cell structure. While gross changes in the morphology of tissues can be imaged directly by light microscopy, many important features of cell and tissue architecture have sizes below the resolution limit of light optics. These features can sometimes be imaged indirectly by immunohistochemistry, but this requires that the structure that is changing be identified in advance. Only electron microscopy has the resolution to survey features of cell and tissue architecture on scales smaller than $\sim$0.2 μM and to identify changes that cannot be predicted in advance.

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