Altered Microfilament Structure in Cells Transformed with a Temperature-sensitive Transformation Mutant of Murine Sarcoma Virus

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SUMMARY

The structure and distribution of microfilaments were examined by electron microscopy in uninfected normal rat kidney (NRK) cells, murine sarcoma virus (MSV)-transformed NRK cells, and NRK cells infected with a cold-sensitive transformation mutant of MSV. Normal NRK (MSV-1b) cells, grown at both permissive (39°C) and nonpermissive (33°C) temperatures. The uninfected cells contained numerous microfilaments which were especially prominent at sites of intercellular adherens junctions. In contrast, the MSV-transformed cells contained few microfilaments and did not form adherens junctions. At 33°C, the NRK (MSV-1b) cells appeared normal but formed an altered form of adherens junction with disorganized microfilaments. At 39°C, these cells resembled NRK cells transformed by wild-type MSV but still formed a few of the altered type of adherens junctions. Disorganized adherens junction microfilaments were also found in cells newly infected with wild-type MSV. These results suggest that the perturbed assembly of microfilaments at adherens junctions may be an intermediate stage in the loss of adherens junctions during viral transformation.

INTRODUCTION

Microfilaments, 5 to 8 nm in diameter, have been observed in the cytoplasm of a wide variety of cells. In cultured mammalian cells they are seen both as a fine network beneath the plasma membrane and as bundles or sheaths of parallel fibers. Recently, results from several laboratories have shown that microfilaments contain significant amounts of actin and myosin and are a component of microfilament bundles. The presence of both actin and myosin in microfilament bundles supports the widely held concept that contractility is involved in the functions of these structures. Contractile filaments have been implicated in many diverse cellular processes, a number of which are relevant to changes which occur during malignant transformation, including: (a) maintenance of cell shape; (b) mobilization of surface lectin receptors and antigenic determinants; (c) regulation of cellular movements; (d) promotion of cellular adhesion; and (e) contact inhibition of growth.

Microfilaments of normal and oncogenically transformed cells have been compared. When examined by electron microscopy or by staining with fluorescent antibodies, normal cells are seen to contain numerous filament bundles that are especially prominent at sites of cell-to-cell adherens (intermediate) junctions. In contrast, cells transformed by simian virus 40 virus and adenovirus contain significantly fewer microfilament bundles and rarely form the microfilament-rich adherens junctions.

Studies in this laboratory have previously focused on membrane-associated changes related to viral transformation. As part of these studies we have utilized NRK cells infected with a cold-sensitive transformation mutant of murine sarcoma virus in order to relate more clearly observed changes with expression of the transformed phenotype. Studies included examination of the structure and distribution of microfilaments in NRK (MSV-1b) cells grown at permissive and nonpermissive temperatures as well as in the parental MSV-transformed NRK cells and in newly infected NRK cells. The results of these studies demonstrate the relationship between decreased numbers of microfilaments and expression of the transformed phenotype and point to a perturbation in microfilament assembly at adherens junctions during oncogenic transformation.

MATERIALS AND METHODS

Cell Lines. The normal rat kidney (NRK), NRK (MoLV), NRK (MSV-MoLV), and NRK (MSV-1b) cell lines used in this study have been previously described. The NRK (MSV-1hp) line was isolated as a helper independent isolate of MSV from cultured normal rat kidney cells transformed by a helper independent isolate of MSV. All cells were grown on 60-mm plastic dishes in Eagle’s minimum essential medium supplemented with 10% fetal bovine serum.
fetal calf serum (Grand Island Biological Co., Grand Island, N. Y.). NRK (MSV-1b) cells were maintained at the permissive temperature, 39°. When nonpermissively grown cells were required, they were used during their 2nd passage at 33°.

Electron Microscopy. Cells grown in plastic culture dishes were fixed and embedded in situ by the procedure of Brinkley et al. (2). Thin sections were cut either parallel or perpendicular to the growth substrate, placed upon uncoated copper grids, and stained with saturated aqueous uranyl acetate followed by Reynold’s lead citrate (17) and examined in an RCA EMU3 electron microscope operated at 100 kV.

Virus Infection. Infection of NRK cells with MSV-MoLV was performed as previously described (18). Virus stocks were obtained from cell-free supernatant fluids of exponentially growing NRK (MSV-MoLV) cells.

RESULTS

NRK and NRK (MSV-MoLV) Cells. Ultrastructural examination of the NRK cell line and of the wild-type transformed and virus-producing cell line, NRK (MSV-MoLV), was performed. Micrographs of uninfected NRK cells revealed an abundance of cytoplasmic microfilaments, especially near the bottom of the cells where they occur as bundles and large sheets. In dense cultures, these filaments were particularly numerous where adherens junctions had formed between adjacent cells (Fig. 1). A closer view of a representative adherens junction between 2 NRK cells can be seen in Fig. 2. At the junctional sites, prominent bundles of microfilaments extend into the cytoplasm from condensations of a dense fibrillar material located just beneath the surface of the plasma membranes. The distance separating the apposing cell membranes at adherens junctions varies between 10 and 50 nm (Fig. 2, arrowheads). In contrast to normal cells, the wild-type MSV-transformed NRK cells show a dramatic decrease in the number of microfilaments and do not form adherens junctions between cells. For the most part, adjacent NRK (MSV-MoLV) cells (Fig. 3) appear to be held together loosely by intertwining microvilli, and areas of substrate attachment are very small. Occasional small bundles of microfilaments are seen in the cytoplasm, sometimes extending into the microvilli.

NRK (MSV-1b) Cells. As previously reported (19), NRK (MSV-1b) cells grown at the nonpermissive temperature (33°) appeared to be essentially normal, i.e., flat, poorly refractile, and relatively contact inhibited, although they produce significant levels of MSV-specific proteins (20). However, when examined by electron microscopy, the NRK (MSV-1b) cells grown at 33° typically display abnormal microfilament development associated with adherens junctions (Figs. 4, 5). The bundles of parallel microfilaments usually present at junctional sites have been replaced by masses of a fine filamentous or granular material, and the apposing plasma membranes are closer together than those in normal adherens junctions. Microfilament bundles not associated with adherens junctions appear normal in NRK (MSV-1b) cells grown at 33°, but they are less abundant. In a few of the cells grown at 33°, small normal adherens junctions (Fig. 6) and partially normal adherens junctions (Fig. 7) were seen. The partially normal junctions have small bundles of microfilaments extending into the cytoplasm from apposing points on the 2 plasma membranes as well as the amorphous, granular material.

When grown at the permissive temperature, 39°, NRK (MSV-1b) cells acquired the transformed phenotype. Comparable to cells transformed by wild-type MSV, they contained very few cytoplasmic microfilaments. The occasional adherens junctions formed between NRK (MSV-1b) cells at 39° were of the altered type (Fig. 8), similar to those formed at 33° (Fig. 4). Neither partially normal nor normal adherens junctions have been observed in permissively grown NRK (MSV-1b) cells.

Transformation of NRK Cells with MSV-MoLV. In order to examine whether altered adherens junctions were associated with the process of transformation, we infected cultures of NRK cells with a sufficient amount of MSV-MoLV derived from the culture fluids of NRK (MSV-MoLV) cells to yield well-defined foci. Samples were fixed for electron microscopy at appropriate time points following infection. By 16 hr, a disorientation of cells in localized areas of the plates was observed and representative areas were selected for sectioning. Microfilaments were abundant and adherens junctions appeared to be normal (Fig. 9). Foci were clearly visible 36 hr after infection. Ultrastructural examination of cells in several of the foci revealed a few budding and extracellular virus particles. Microfilaments were slightly less abundant than in uninfected cells, but adherens junctions remained normal (Fig. 10). In samples fixed 69 to 72 hr after infection, the foci were prominent and the cells contained many budding virus particles. Some bundles and sheets of normal microfilaments were present, but most of the adherens junctions had microfilament alterations similar to those in NRK (MSV-1b) cells (Fig. 11). Seven days after infection, adherens junctions were absent and the cells appeared to be fully transformed. Although the precise time after infection of the complete disappearance of normal adherens junctions varied slightly between experiments, the presence of altered adherens junctions was a constant feature during acquisition of the transformed phenotype by MSV-MoLV-infected NRK cells.

NRK (MoLV) and NRK (MSV-1hp) Cells. In order to determine whether virus replication per se was associated with alterations in microfilament structure, we examined 2 cell lines that produce virus but are not transformed. The NRK (MoLV) cell line is infected with and produces MoLV. The NRK (MSV-1hp) cell line was isolated as a flat revertant from helper-independent, MSV-transformed NRK cells. Although the NRK (MSV-1hp) cells appear flat and nonrefractile, they produce virus capable of infecting and transforming NRK cells (K. Somers, manuscript in preparation). The results show that the morphology and microfilament content of the NRK (MoLV) (Fig. 12) and NRK (MSV-1hp) (Fig. 13) cells resemble those of uninfected NRK cells. In addition, it can be seen that adherens junctions of normal type are produced.
DISCUSSION

Although the precise role of adherens junctions in normal cellular structure and function is incompletely understood, they are thought to be involved primarily in intercellular adhesion (12, 21). It has been suggested that the presence of stable adhesions between normal cells may function to limit cellular growth to a monolayer (13), while the absence of these structures in oncogenically transformed cells results in piling up of the cells. In this regard, McNutt et al. (11) have suggested that the large numbers of microfilaments associated with adherens junctions of normal cells may promote contact inhibition of movement and growth by immobilizing the cell at regions of intercellular contact. We have examined the microfilaments associated with adherens junctions in cells infected with a cold-sensitive transformation mutant of murine sarcoma virus. At the nonpermissive temperature, 33°, these cells resemble normal NRK cells by light microscopy, i.e., appear flat and poorly refractile, but they produce significant amounts of MSV-p30 antigen (20) and show slightly elevated hexose transport (9). Thus, there appears to be at least partial expression of the transformed phenotype even at the nonpermissive temperature. Examination of these cells at 33° reveals a large number of adherens junctions in which the microfilaments appear disorganized. It is reasonable to suggest that the altered adherens junctions are due to partial expression of the MSV-transforming genome and represent an intermediate stage in the loss of adherens junctions and microfilaments that normally accompanies transformation. At the permissive temperature, 39°, there is a noticeable decrease in the number of adherens junctions although the altered type is still clearly in evidence. It is worthwhile mentioning that at 39° the NRK (MSV-lb) cells are more transformed than at 33°, but, as judged by a number of parameters (9, 22), the cells do not appear to express the transformed phenotype as fully as wild-type MSV-transformed NRK cells. Hence we feel that at 39° the NRK (MSV-lb) cells represent a further stage in the loss of adherens junctions which accompanies viral transformation. In support of this thesis we cite the results obtained with newly MSV-MoLV-infected NRK cells in which the altered type of adherens junction is just a transient one, which suggests that they are capable of producing normally assembled microfilaments but are inhibited from doing so.

Whatever the basis of the altered adherens junctions in normal contact inhibition of growth. On the basis of the evidence of others (3, 11) and of these studies with a cold-sensitive mutant of MSV, it is reasonable to suggest that loss of adherens junctions plays a central role in the expression of oncogenic transformation.

REFERENCES

B. C. Altenburg et al.


Fig. 1. Thin section of NRK cells cut parallel and very close to the growth substrate. Extensive sheets and bundles of microfilaments (mf) are present as well as an adherens junction (arrows). × 15,200.

Fig. 2. Adherens junction between uninfected NRK cells. Apposing plasma membranes are separated by a 35-nm space (arrowheads). Bundles of 5- to 8-nm microfilaments (mf) extending from just inside the plasma membranes are prominent. × 49,500.

Fig. 3. Thin section cut perpendicular to the growth substrate showing a group of NRK (MSV-MoLV) cells. The cells appear to be loosely associated by intertwining microvilli (mv). × 18,000.

Fig. 4. Abnormal adherens junction between NRK (MSV-1b) cells grown at 33°. An amorphous granular material (asterisks) has replaced the microfilaments seen in Fig. 2. Plasma membranes are separated by 9 nm at arrowheads. × 56,800.

Fig. 5. Perpendicular section of NRK (MSV-1b) cells grown at 33°. Abnormal adherens junctions are marked by arrows. × 6,800.

Fig. 6. Small normal adherens junction between NRK (MSV-1b) cells grown at 33°. × 14,500.

Fig. 7. Partially normal adherens junction between NRK (MSV-1b) cells grown at 33°. Both assembled microfilaments (mf) and amorphous material (asterisk) can be seen. × 14,500.

Fig. 8. Abnormal adherens junction between NRK (MSV-1b) cells grown at 39°. × 14,500.

Fig. 9. NRK cells 16 hr postinfection with MSV-MoLV. Adherens junctions (arrows) are sectioned obliquely. Microfilaments are abundant. × 21,200.

Fig. 10. Normal adherens junction between NRK cells 36 hr postinfection with MSV-MoLV. × 26,300.

Fig. 11. Abnormal adherens junction between NRK cells 72 hr postinfection with MSV-MoLV. Junction microfilaments (asterisks) appear disorganized. Some normal microfilaments (mf) are also present near the adherens junction. × 47,000.

Fig. 12. Adherens junctions between NRK (MoLV) cells. × 15,500.

Fig. 13. Adherens junctions between NRK (MSV-1hp) cells. × 20,000.
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