A SCANNING ELECTRON MICROSCOPIC STUDY OF SV40 INFECTED CELLS

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SUMMARY: The surface of the SV40-infected African green monkey kidney (AGMK) cells was studied morphologically by scanning electron microscopy. In 24 hr post infection (p.i.), the cell surface was covered with slightly elongated microvilli. The microvilli increased in number. In 96 hr p.i., most of the cells showed SV40-specific cytopathic effects (CPE). Nuclear swellings and the elongation of microvilli were eminent. Microvilli were observed projecting with high densities especially on the nuclear portions of the cell surfaces. Features suggesting cytoplasmic vacuolization were also observed in some cells. Spherical particles viewed in some of the cells at the late stage of infection were considered SV40 virions. Their origin was also discussed.

INTRODUCTION

The morphological aspects of the replication of SV40 in permissive cells have been well documented (Granbouran et al., 1963; Mayor et al., 1966; Oshiro et al., 1967; Hummeler, Tomassini and Sokol, 1970). Transmission electron microscope studies of AGMK cells infected with SV40 revealed that the site of development of the virus was the nucleus, the cytoplasmic vacuolization was actually the consequence of its multiplication in the nucleus, and the progeny virus particles were released into extracellular fluid. A more detailed morphological study of the multiplication of this virus and the cellular lesions it induces would be useful for better understanding of the virus-cell interaction following SV40 infection.

The development of techniques for examining cultured cells and soft tissues by scanning electron microscopy (SEM) has greatly enhanced exploration of the topography of the cell surface (Porter, Fonte and Weiss, 1974). SEM studies of cells infected with vesicular stomatitis virus (VSV), murine oncornavirus or herpes simplex virus have recently been reported (Holmes, 1975; de Harven et al., 1973; Panem and Kirsten, 1975; Aranyi, Fenters and Tolkacz, 1970). By SEM with high resolution power and the improved coating technique, the author observed subtle constitutions of the cell surface and such small particles as 40-50 nm in diameter. This communication describes SEM of the surface of the SV40-infected AGMK cells.

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MATERIALS AND METHODS

Virus: SV40 ConII strain was used throughout this study. This strain was isolated in this laboratory from a commercial polio vaccine lot of Salk type (Miyamura and Kitahara, 1975). Primary AGMK cell cultures were infected with the virus and supernatant fluid harvested at maximum CPE was centrifuged at 3,000 rpm. The supernate was stored at -20 C as a virus stock.

Cell culture: Monolayers of AGMK 9186, a line of AGMK cells established in this laboratory, were grown to confluency on glass coverslips (12×32 mm) in Leighton tubes. They were infected with SV40 at a multiplicity of infection of about 100 TCID50 per cell. AGMK 9186 cells were used at 90-100 passage levels. Infected and mock-infected cells were cultured in equivoluminal mixtures of Eagle’s minimal essential medium and YLE (Earle’s salt solution with 0.5% lactalbumin hydrolysate and 0.1% yeast extract) supplemented with 0.15% sodium bicarbonate. After 24 and 96 hr, infected cells were harvested, fixed and prepared for SEM.

Preparation of cells for SEM: Cells were washed twice with phosphate-buffered saline (PBS), pH 7.2, and fixed in 2% glutaldehyde in PBS with 4.5% of glucose for 20 min at 0 C. After rinsed twice with PBS, the cells were dehydrated by serial passages through a gradient of 25, 50, 70, 90, 95 and 100% ethanol. Coverslips were dipped in each gradient for 5 min, followed by further three 20-min treatments with 100% ethanol. Dehydrated cells were then dipped in amyl acetate for 10 min at room temperature. The cells were then dried at the critical point of CO2 in a critical-point-drying apparatus (JEOL, Akishima, Japan), and coated with a thin film of Au-Pd (about 150 A) in a vacuum evaporator with a rotary coater attachment. Samples were observed with a JSM-35 scanning electron microscope at 20 KV. Pairs of stereoscopic photographs were taken with specimen tilt of 6° difference. Three dimensional images were obtained by observing the photographs with a stereoscopic viewer. For higher magnifications, a JEM-100C/ASID electron microscope was used.

RESULTS

Uninfected AGMK Cells

The surface morphology of the uninfected AGMK cells is shown in Fig. 1-a,b,c. In some cells, discrete swellings of the nuclei with a plenty of microvilli were observed (Fig. 1-a). Other cells were flat with the microvilli scattered sparsely. Round blebs were also seen. Ruffled membranes and numerous filipods were observed at the edges of most cells. Most of microvilli were standing erect, fairly

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Fig. 1. Scanning electron micrographs of the surfaces of uninfected AGMK cells.

a) Some cells are flat and have microvilli sparsely scattered. Others have a plenty of microvilli and blebs. ×1,400
b) A portion of a cell (arrow in Fig. 1-a). This cell is rich in microvilli. ×14,000
c) A higher magnification of (b). Microvilli are relatively short and uniform in diameter. ×42,000

straight, and had rather uniform diameters (90–130 nm). The diameters might have increased slightly by coating with Au-Pd (Fig. 1-b,c).

**SV40-Infected Cells**

In 24 hr after infection, the AGMK cells were harvested and processed for SEM. At this time no morphological change was observed by a light microscope. As shown in Fig. 2, most cells had a large number of microvilli distributed homogeneously on all over the cell surfaces. The microvilli per se were similar in diameter but slightly elongated compared with those of the uninfected AGMK cells.

In 96 hr after infection, most of the cells showed SV40 CPE. Pairs of Fig. 3-a,b,c show three dimensional images of the cells showing CPE. The nuclear swelling was a characteristic feature (Fig. 3-a,b). The microvilli of the cells of this stage differed distinctly from those of noninfected cells. They increased in number and gathered mainly over the nuclear portion. They were longer than those of uninfected cells, and were sometimes of tortuous forms including nodular swellings and elbow-like bends. Occasionally, clusters of two or more microvilli
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4a

4b
Fig. 6. Scanning electron micrograph of an AGMK cell infected with SV40. On the cell surface between the elongated microvilli, spherical particles are viewed. (×40,000) These particles were re-examined by a JEM 100C/ASID electron microscope and typical one is shown in the inset. ×100,000

Fig. 2. Scanning electron micrographs of AGMK cells 24 hr after SV40 infection.
   a) Microvilli are scattered homogeneously on the whole surface. ×4,200
   b) A higher magnification of (a). Microvilli are slightly elongated. ×42,000

Fig. 3. These represent stereoscopic views of AGMK cell which is just beginning to show SV40 CPE.
   a) Nuclear swelling with a plenty of microvilli is seen. Contacts with the adjacent cells have been disrupted during shrinkage induced by dehydration. ×660
   b) A higher magnification of the cell shown in (a). Most of the microvilli are standing erect from the cell surface. ×2,000
   c) A portion of the same cell as (a) and (b). The elongation of the microvilli is prominent. ×6,600

Fig. 4. Scanning electron micrographs of the later stage of SV40 CPE.
   a) A cell just detaching from the glass (96 hr after infection). ×3,000
   b) The cell surface is covered with extraordinarily elongated microvilli. ×10,000

Fig. 5. Scanning electron micrographs of the AGMK cells showing the cytoplasmic vacuolization.
   a) The unevenness of the cell surface is observed. The cell surface is relatively free of microvilli. ×2,800
   b) In other cells, rugged cell surfaces are covered with elongated microvilli. ×4,200

were observed, which appeared to emerge from a single locus on the cell surface. Some microvilli of large diameters (300 nm) were also observed (Fig. 3-c).

At the final stage of CPE, when cells were going to detach from the glass, extraordinarily elongated microvilli were observed on the surface (Fig. 4-a,b). Abnormal projections, regarded as fused microvilli, were also seen.
In some cells, the surface was rugged and the nucleus was difficult to locate (Fig. 5). The unevenness of the cell surface might be due to the cytoplasmic vacuolization observed with a light microscope at this stage of infection (Sweet and Hilleman, 1960; Mayor et al., 1962). Dehydration of the specimen during the processing for SEM might have induced the unevenness.

At a higher magnification (×40,000), small particles, uniform in shape and size (60–65 nm), were observed on the cell surface (Fig. 6). By correction for the metal coating thickness, the real diameters of these particles were calculated to be 45–50 nm. No spherical particles were found in noninfected or mock-infected cells. These particles were smaller in diameter than the microvilli, and might, therefore, not be confused with the microvilli viewed end-on. The possibility that these particles might be SV40 particles released from infected cells will be discussed later.

**DISCUSSION**

The surfaces of uninfected AGMK cells was not always morphologically uniform (Fig. 1-a,b,c). Whether such variation bears any relation to the different phases of the cell cycle has not been determined. It has been suggested that microvilli are transient structures normally associated with the particular physiological state of the cell (Follett and O’Neill, 1969; Follett and Goldman, 1970). This was confirmed by several experiments using synchronized cell cultures in both suspensions and monolayers (Porter, Prescott and Frye, 1973; Hale, Winkelhake and Weber, 1975; Knutton, Sumner and Pasternak, 1975). In the present study, confluent stational AGMK cells were used and most of the cells were considered to be arrested at G1 phase, but further studies on this point by using synchronized cell populations are under way.

The most striking difference between normal AGMK cells and SV40-infected cells with CPE was the length of microvilli. Although the length of microvilli of uninfected cells is up to 400 nm, SV-infected cells showing CPE had long tortuous microvilli (~1500 nm).

Although discussions on the role of microvilli on the surface of cells are still speculative, suggestions that they are concerned in the selective attachment or aggregates of cells were accumulating (Pethica, 1961; Lockwood and Allison, 1966). It has been reported that SV40 infection increases cell agglutinability by wheat germ agglutinin (Sheppard, Levine and Burger, 1971). It seems probable when one views three dimensional structures of SV40-infected AGMK cells with long and densely grown microvilli. By providing a greater surface area or more specific receptor sites, microvilli seem to facilitate association of the cells with agglutinins. Noonan, Levine and Berger (1973) reported that the agglutinability of cells by concanavalin A was also cellcycle dependent.

It was reported that SV40-transformed medullary carcinoma cells showed a high incidence of microvilli (Grimley et al., 1969). On the other hand, SV40-transformed Balb/3T3 cells were shown to be free of microvilli, while 'normal'
Balb/3T3 cells were covered with thick microvilli (Porter, Todaro and Fonte, 1973). Whether this discrepancy was due to the cells used or reflected the different cell stages remains to be determined by more detailed experiments using synchronized cell culture.

At this moment, there is no definite evidence showing that the spherical particles observed on the surface of the cells showing CPE were SV40 virions. But it may be probable that SV40, as small as 40-50 nm in diameter, released from infected cells by cell lysis or degeneration were observed on the surface of the cells. Such budding viruses as VSV and oncornaviruses have been observed to be budding from infected cells (Holmes, 1975; de Harven et al., 1973; Panem and Kirsten, 1975). In the case of VSV, virions were budding from the sides of microvilli as well as from the cell surface between microvilli (Holmes, 1975). As shown in Fig. 6, SV40-like particles were never associated directly with microvilli. If the observed particles were really SV40 virions, the following possibilities may be conceivable as their origin: (i) mature SV40 particles were observed to ooze out through the cell membrane, (ii) mature SV40 were released from degenerated cells into extracellular fluid and re-adsorbed onto the cell surface, (iii) input parental viruses, which were adsorbed on the cells but could not be uptaken, were observed. We can not determine which was the case, but since transmission electron microscopic studies showed that a large number of virus particles were tightly packed beneath the cytoplasmic membrane at this stage of infection, it may not be unreasonable to consider that these particles on the cell surface were those having oozed out little by little.

The cell surface unevenness observed in some of the cells showing CPE (Fig. 5) might be due to extensive cytoplasmic vacuolization. Thin sections of the cells harvested at the same time showed extensive cytoplasmic vacuolization but not such an unevenness of the cell surface, when they were observed by transmission electron microscopy (Miyamura et al., unpublished data). Rapid dehydration processes by the critical point method for SEM might play a role in inducing the unevenness. While most of the cells at this stage of infection showed cytoplasmic vacuolization by light microscopy, the unevenness of the cell surface was observed by SEM only in some of the cells. The reason for this discrepancy is now under investigation.

The morphological changes of microvilli of SV40-infected cells were revealed by scanning electron microscopy. The findings by transmission electron microscopy of SV40-infected cells are accumulating, but these findings have not explained the situation of microvilli. At the present time, it is difficult to discuss the mechanism or meaning of the morphological changes of microvilli. The change might be a result of an event involved in replication of SV40. Twenty-four hours p.i., when microvilli were observed to have slightly increased in length and number, is the time for mature virus to be released into cytoplasm. When the cell surface revealed the unevenness, masses of SV40 particles were packed beneath the cytoplasmic membrane (Miyamura et al., unpublished data). The discrete relationship between the intracellular events and the surface changes
reported here are not clear yet. A freeze-fracture method which is now in progress may be useful to analyze the intracellular structures as well as the surface morphology. Further investigations are required to take clearer pictures of the sequential events of SV40 replication leading to the morphological changes of microvilli.

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REFERENCES


