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Scanning Electron Microscopy of Differentiated Liver Cells Transformed In Vitro by Chemical Carcinogens

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A scanning electron microscopy study was carried out on differentiated liver cells transformed in vitro by three chemical carcinogens into cells that give rise to carcinomas. The results indicate that the transformed cells grow as a rule in tightly adherent monolayers but differ in topography. There is a tendency toward heterogeneity in cell shape compared to the normal and on the whole toward a larger number of surface microvilli in the malignant cell population. However, both in sparse and confluent cultures the topographic differences are often not striking enough to unequivocally distinguish single neoplastic cells from the normal.

Key words: SEM, chemical carcinogenesis in vitro, epithelial liver cell cultures

In earlier studies using scanning electron microscopy (SEM) we investigated the topography of differentiated and chemically induced hepatoma cells in primary and long-term cultures as compared to suitable controls of normal liver cells [1–3].

In the present report we have extended these investigations to study the surface architecture of liver cells transformed in vitro by three different chemical carcinogens into cells that give rise to carcinomas, and we have compared their topography to that of their untransformed parental cells.

MATERIALS AND METHODS

Cell Lines

We used the epithelial cell line TRL_{12} [4] originally derived from the liver of a Fischer rat and three established transformed liver lines [4, 5]. These lines had been developed by treating TRL_{12} cells with one of the following carcinogens: nitrosomethyl urea, 2-aminoanthroquinone, and aflatoxin B_1. The transformed cells are malignant and

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upon injection into the rat give rise to carcinomas [4, 5; E. K. Weisburger, personal communication]. Both normal and transformed TRL cells were kindly donated by Drs. E. K. Weisburger and G. M. Williams.

Preparation and Fixation of SEM

All cells were grown in Williams medium D (Grant Island Biologicals Co.) fortified with 10% fetal calf serum. Normal and transformed cells were seeded at seeding levels of 6×10^3 to 6×10^5 per square centimeter onto coverslips as described previously [6] and prepared for SEM 72 h after seeding, as reported [6].

A Jeol JSM-35 scanning electron microscope with a regular tungsten filament operating at 30 kV accelerating voltage was used. The resolution of the microscope is 70 Å.

RESULTS

Each cell type was viewed in three separate preparations. Dividing cells were observed only in the low-density cell populations. No mitosis was observed microscopically in the high-density confluent cultures. Autoradiography carried out as previously reported [7] on parallel preparations supported the microscopic observations.

The normal TRL liver cells grew in culture as sheets of flat, tightly adhering cuboidal cells in a mode characteristic of liver epithelial cells [4, 5, 7, 8]. Their surface architecture appeared either smooth as previously reported [3] or with a moderate number of microvilli (Fig. 1) sparsely distributed on the surface. Distinct junctional ridges were seen between the cells and fine microvilli extended from one cell to another and overlapped as reported previously for some other normal liver cells in culture [3, 9]. The three transformed cell lines grew in a manner similar to the normal as flat sheets of monolayered cells, and similarly fine microvilli were observed at the junctional ridges. In contrast to the regularity seen among the untransformed parental cells, the shapes of the transformed cells displayed more heterogeneity.

The surface architecture of the transformed cells varied from one cell line to another and also within a single preparation in the density and distribution of microvilli. Within each transformed culture we observed cells that possessed a moderate or large number of surface microvilli (Figs. 2, 3) and others that were relatively smooth (Fig. 4). The transformed epithelial cells in mitosis (not shown) were rich in microvilli similar to transformed mitotic fibroblasts [6] and similarly were indistinguishable from the mitotic normal cells.

DISCUSSION

The importance of recognizing neoplastic cells as they appear within a normal cell population is unequivocal. The detection and isolation of low-frequency early transformants in vitro is of great importance for studies on the progression of the neoplastic state of the cells.

In recent years it has become increasingly clear that many biologic properties that have served well to distinguish neoplastic fibroblasts in vitro from their normal counterparts are inconsistent as markers for neoplastic epithelial cells. For example, a loss of density inhibition and piling up of cells was a striking feature in some transformed epithelial liver cells [8, 10, 11] but was not prominent in others [5, 10, 12], depending on culture conditions [10].

Fig. 1. Surface structure of a normal TRL cell in a confluent non-dividing culture. A moderate number of microvilli are dispersed on the surface. SEM × 16,000.

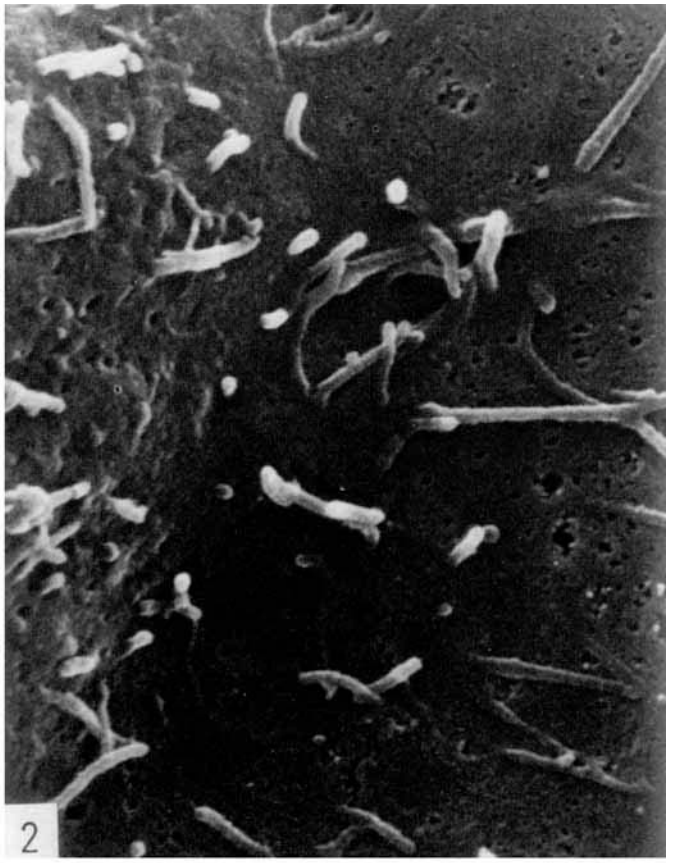

Fig. 2. Surface features of a confluent culture of a TRL cell transformed in vitro by 2-aminoanthroquinone. Note the moderate number of microvilli on the surface. SEM × 18,000.

Fig. 3. Surface features of a confluent culture of a TRL cell transformed in vitro by nitrosomethyl urea. Note the large number of microvilli, clustered in uneven fashion on the cell surface and markedly different from the normal. SEM × 1,600.

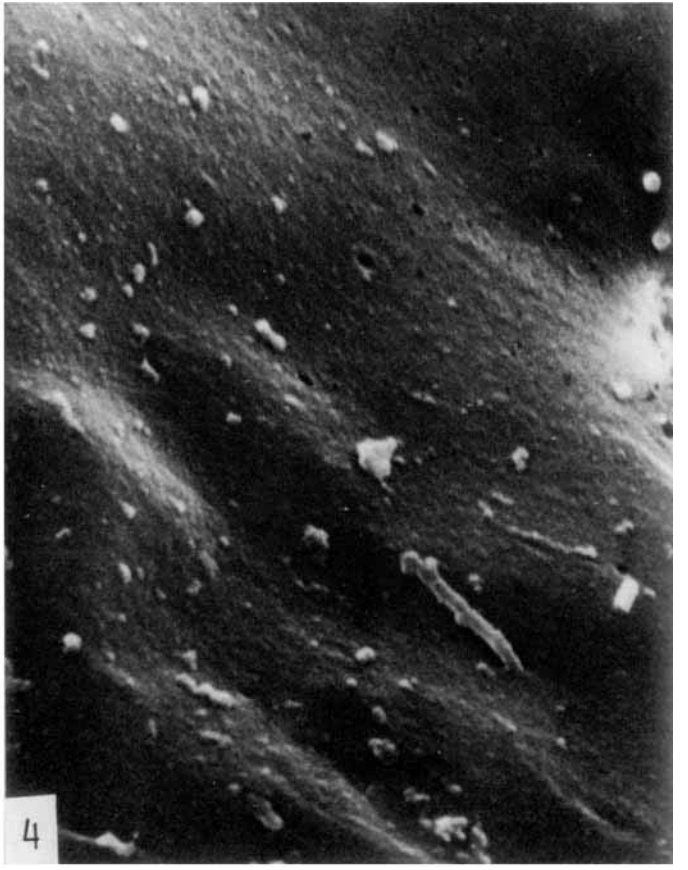

Fig. 4. Surface features of a confluent culture of a TRL cell transformed in vitro by aflatoxin B_1. Note the sparse number of surface features. SEM × 18,000.

A wide range of fibroblasts transformed by various oncogenic agents have been reported to display an altered mode of growth and an increase in surface excrescences compared to the normal counterparts (for some examples see refr. 6, 13–16). These cellular features varied in their deviation from normal, depending on the source of cells and the oncogenic agent. In addition, cells of established hepatoma cell lines [1, 3] and spontaneously transformed liver cells [17] showed a marked increase in surface features compared to normal liver.

In the present report we have studied by SEM the topography of epithelial liver cell lines transformed in vitro by three different chemical carcinogens into malignant differentiated cells (give rise to carcinomas) and compared them to the parental normal cells. We have found that while the growth pattern of the transformed epithelial cells is similar to that of the normal, the cell topography differs. There is a tendency toward heterogeneity in cell shape in the transformed cultures and on the whole toward more surface microvilli in the transformed cell population. However, the topographic changes are inconsistent. With the exception of cells such as the one illustrated in Fig. 3, which had no counterpart in the normal population, the surface changes in the transformed cells (Figs. 2, 4) were not striking enough to unequivocally distinguish *single* neoplastic cells from their normal counterparts.

Studies on Chinese hamster cells have discussed a cell-cycle-dependent variation on cellular surface features [18, 19]. In the present cultures of differentiated epithelial cells these variations in cell surface were observed in confluent noncycling cultures as well as in the sparsely seeded cycling cells.

Liver cells transformed in vitro by chemical carcinogens into malignant differentiated cells appear to undergo fewer surface changes compared to spontaneously transformed liver cells [17], whose fate in the animal was not documented [17]. It remains to be seen whether epithelial cells derived from other sources and transformed in vitro by a variety of oncogenic agents into cells that give rise to carcinomas will show properties similar to those of the transformed liver cells reported here.

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