Ultrastructural Comparison of Two Human Malignant Melanoma Cell Lines

Gerd G. Maul and M. M. Romsdahl

Department of Pathology, Temple University Health Sciences Center, Philadelphia, Pennsylvania 19140 [G. G. M.], and Department of Surgery, M. D. Anderson Hospital and Tumor Institute, University of Texas, Houston, Texas 77025 [M. M. R.]

SUMMARY

The ultrastructure of 2 established human melanoma cell lines (LeCa and MeGo) and their clones was investigated. All strains of line LeCa had premelanosomes with the typical arrangement of "helical filaments" and with cross-striated melanosomes; only the amount of melanosomes produced varied in the different strains.

The strains in line MeGo varied widely in the appearance of their premelanosomes. The helical arrangement of the matrix filaments was missing, and cross-striated melanosomes were not seen. Morphologically, premelanosomes tend to resemble lysosomes in varying degrees in the different strains. Because all strains of both lines are dihydroxyphenylalanine positive and show acid phosphatase activity in melanosomes, two possible explanations are considered: (a) hydrolytic enzymes function in the melanosome to terminate melanogenesis; or (b) the enzymes indicate hydrolysis of melanosomes as they accumulate in the melanoma cells.

INTRODUCTION

Several established human malignant melanoma cell lines exhibited differences in morphology, amount of melanin produced, enzyme activity, growth rate, and cytogenetics (24, 25). Because the ultrastructure of the melanosome varies considerably with different melanomas (2, 3) in vivo, it was decided to investigate different melanoma cell lines at the ultrastructural level. Novikoff et al. (20) have shown that the premelanosomes of mouse melanoma contain acid phosphatase activity, and they therefore considered this organelle a modified lysosome. The ultrastructure of 2 cell lines will be presented since they differed considerably from each other with respect to melanosome morphology, and since they also seem to indicate that most "melanosomes" are lysosomes in melanomas (16).

MATERIALS AND METHODS

Two human malignant melanoma cell lines (LeCa, MeGo) utilized in this study have been described previously (24, 25). Cloned strains of these cell lines were used in this investigation. Line LeCa, obtained from a metastatic malignant melanoma originating on the back of a 56-year-old male patient, had been in continuous culture for 2 years at the time of this study. Line MeGo was derived from metastatic disease in the groin, secondary to a malignant melanoma of the foot. It has been cultured continuously for approximately 1.5 years. Both lines were maintained in modified McCoy's 5A medium supplemented with 20% fetal calf serum. Cloning was performed on both malignant melanoma lines which resulted in the establishment and propagation of strains that were individually unique in regard to morphology, growth, pigment production, and tyrosinase activity. The cells for study were prepared for electron microscopy 48 hr after subculturing into flacon plastic tissue culture flasks. Specimens were fixed for 1 hr at room temperature in 3% glutaraldehyde (phosphate buffered, pH 7.4) (26) and postfixed for 1 hr with 1% OsO4 (phosphate buffered, pH 7.4). Before rapid dehydration in ethanol, the cells were covered with 2% uranyl acetate in water for 1 hr (13). They were then flat-embedded (1) in Epon (14). This technique allowed selection of single cells with the light microscope. Sections were cut on an LKB Ultratome III and stained with uranyl acetate and lead citrate. Observations were made on a Hitachi HU-11-C electron microscope operated at 75 kV. Samples for the histochemical demonstration of enzyme activity were fixed in glutaraldehyde for 15 min. For the demonstration of tyrosinase activity, monolayers of fixed melanoma cells were covered with freshly prepared 5 mM DOPA (Mann Research Laboratories Inc., New York, N. Y.) in 0.1 M phosphate buffer, pH 6.8, for 4 hr at 36°C (18, 20, 22). For the demonstration of acid phosphatase, cells were fixed in cacodylate-buffered glutaraldehyde (3%). The method of Gomori (7) was adjusted for monolayers of cells by incubation for 10 min only. The substrate was omitted in the controls. After incubation, the cells were osmicated and processed as described above.

RESULTS

Low-magnification survey micrographs of the Golgi region and high-magnification micrographs of typical premelano-
Ultrastructural Comparison of 2 Melanoma Cell Lines

Ultrastructural Comparison of 2 Melanoma Cell Lines

...omes in that region in 2 cell lines (LeCa and MeGo) were studied. Also, different strains of each cell line were investigated. No ultrastructural difference was found between the cloned strains of malignant melanoma cell line LeCa that could not be attributed to culture conditions or plane of section. There was, however, a difference in the number of melanosomes present. This report will deal primarily with the heavily pigmented strain 19-4.

Line LeCa, strain 19-4, corresponds to the lines reported in long-term tissue culture cells (32). The individual cells are markedly dendritic and contain all stages of melanosome formation. Some cells also contain autophagosomes, which are filled with melanosomes. Premelanosomes of all representative stages of development are concentrated around the Golgi-centriole region of the cell. Fig. 1 shows a relatively high ratio of premelanosomes with advanced melanization. Some premelanosomes were also found in more “remote” areas of the cell. The Golgi apparatus is extensively developed. Single cisternae consist of approximately 4 to 6 cisternae. Often one side of the cisternae is fenestrated (Fig. 1, parallel arrows). This was observed in all preparations studied (17). Mitochondria were long, narrow, and frequently branched. There were only a few melanosomes present in the Golgi area and very little RER.

“Empty” areas appearing in Fig. 1 (upper left) might be due to the leaching out of glycogen in our preparation method. Glycogen was easily discernible in all strains when stained with periodic acid-Schiff (24). The ultrastructure of premelanosomes in line LeCa showed the classical arrangement of helical fibers. Most stages of premelanosomes are present in Fig. 2; only the very early stages as described previously (15) are not present in this micrograph.

Structures that seem to be helical fibers in Melanosome 1 (m1) can be interpreted as folded sheets. The observation of a light line (m2), which runs in the center of the melanized matrix, demonstrates an area of less melanization. This may be the unmelanized center of the helix inasmuch as it is assumed that several helices fuse into a sheet. This assumption is further supported by the observation of many equally spaced light dots in cross-sectioned melanized strands (not shown in this micrograph).

In comparison of line LeCa with line MeGo, one major difference is that the strains of line MeGo produce less pigment as judged by the pale color of the cell pellet, which led to their designation as amelanotic strains (Amel-4, Amel-4P). However, single cells observed by light microscopy were seen to produce melanosomes, as evidenced by a denser region in the area of the Golgi apparatus. This may indicate the strain Amel-4 from line MeGo was not a pure clone but consisted of a population of genetically different cells or that only a few cells express the potential for melanosome formation.

In Fig. 3, a cell is shown from strain Amel-4, which when viewed by light microscopy contained a dense Golgi region. The arrangement of the melanosomes is similar to that of line LeCa except that they are more irregular and appear to be of uniform density. The relationship of premelanosomes to the tubular SER is the same as described from strain 19-4 of line LeCa (15). Mitochondria that surround the Golgi area are thin and long. The cytoplasm is dense, and many polysomes are present at the cell periphery, although few are present in the area of the Golgi apparatus. SER is more extensively developed than in strain 19-4.

Strain 19-4 demonstrates no dense material in tubular SER connected with premelanosomes (15). In strain Amel-4, however, it is obvious that the tubular SER (diameter is approximately 200 to 400 Å) contains the same electron-dense material as the melanosomes (Fig. 4). There is no highly ordered underlying matrix, but the electron-dense material appears granular or clumped. Only in a few melanosomes was there any suggestion of an ordered substructure (Fig. 4, arrow).

The survey micrograph of another strain of line MeGo, strain Amel-4P (Fig. 5), exhibits the familiar arrangement of “premelanosomes” in the area of the Golgi apparatus. It cannot be stated with much certainty that the dark granules are melanosomes since some or even the majority of them may be lysosomes. The Golgi apparatus is extensive as in the other clones, but the tubular SER is most extensive in this strain (Fig. 5, right lower corner), and there is high variability between single cells. The mitochondria are thin and long in the Golgi area but differ in shape within areas of the cell that contain large amounts of lipid droplets.

At higher magnification (Fig. 6), the laminated body is structurally comparable with a lysosome. The dark bodies (db) contain clumped (db1) or rod-like (db2) electron-dense material reminiscent of melanosomes. In several instances, typical ultrastructural features of a lysosome and an early premelanosome within 1 membrane-bound structure were seen (Fig. 7). Melanosomes were also observed together with externally ingested particles like mycoplasma. These findings indicate that a membrane-delimited body with the structural inclusion of a melanosome may be a lysosome. The demonstration of tyrosinase activity and acid phosphatase activity was therefore used in the hope of distinguishing between the 2 organelles, i.e., melanosome and lysosome.

Tyrosinase activity was found in an anastomosing network of tubular membranes associated with the Golgi apparatus, coated vesicles, and melanosomes of strain 19-4 (Fig. 9). The detailed account of the localization of tyrosinase activity in normal melanocytes will be published elsewhere (G. G. Maul, On the Possible Function of Coated Vesicles in Melanogenesis of the Melanocytes in Regenerating Fowl Feathers, J. Cell Biol., in press) (see also Refs. 20, 22, and 28 for demonstration of tyrosinase activity at the electron microscopic level). Acid phosphatase activity was found in the same organelles as well as in several cisternae of some of the dictyosomes. Fig. 8 shows melanosomes of line LeCa, strain 19-4, with reaction product between the melanized matrix and in Golgi-associated tubular membranes (compare with Fig. 2). However, not all melanosomes contain the reaction product of acid phosphatase activity (arrow). It was present in mature forms more often, but statistical evidence for this observation is not yet available.

The tyrosinase activity as judged by the DOPA reaction was found in both strains of line MeGo. It was present in the Golgi-associated structures as in line LeCa but was difficult to ascertain in the melanosomes because of their...
inherent density and the lack of ordered substructures. Reaction product of the acid phosphatase activity was present in most melanosomes of both line MeGo strains.

The apparent average size of mitochondria is seen in Fig. 1. However, mitochondria were very long and thin as based on the reconstruction of serial sections. For study of the length of mitochondria, cells with well-developed, long, thin dendrites were selected. These were aligned so that section could be made parallel to the knife edge. With this technique, it was possible to estimate the length of mitochondria since they were approximately in the same plane. The mitochondrion shown in Fig. 11 is 9.3 μ long. Reconstruction from 3 serial sections show it to be at least 11 μ long. Fig. 11 shows typical mitochondria found in all cell lines. They were the only type observed in the strain 19-4. In Amel P-4, however, an unusual type were also present. These mitochondria are shown in Fig. 10. They were never observed near the Golgi area but were most frequently noted on the opposite pole of the nucleus and in an area of lipid droplet accumulation. These areas were also rich in RER, the latter coming in close contact with lipid droplets. A dark area on the lipid droplet (arrow) indicates the attachment. In this area, markedly dilated RER, which was densely packed with a fibrous material, was observed.

DISCUSSION

Many investigations have dealt with melanogenesis and the origin of the melanosomes. The generally favored concept is that vesicles are pinched off from the Golgi apparatus and are in turn enlarged by a process of fusion (6). In earlier investigations, Novikoff (19) described the relationship between the various parts of the GERL. This investigation, as well as the one by Holtzman et al. (8), suggests a mechanism for a transport system of precursor of enzymes to the membrane-bound organelles by direct transport through tubular membranes. A different scheme has been proposed by Jamieson and Palade (10, 11). They show that small, smooth-surfaced vesicles derived from RER or the Golgi apparatus fuse with condensing vacuoles, which in turn form the zymogen granules in pancreatic cells.

No convincing mechanism has been described from an ultrastructural viewpoint, which could explain the transfer of precursors from the Golgi complex into the premelanosomes if such a route is assumed to exist. However, the first possible transfer mechanism was suggested by Novikoff et al. (20), who found both acid phosphatase and tyrosinase activities in the premelanosomes and also in tubular membranous structures that belong to the GERL complex. It has been suggested, on morphological grounds, that enzyme systems may be channeled from the Golgi complex through tubular membranous structures into premelanosomes (15). The demonstration of membranous connections of early premelanosomes, tubular elements of the SER, and possibly the dictyosomes were the basis for this hypothesis.

Novikoff et al. (20) considered the melanosome a modified lysosome. The structural evidence presented in this study also suggests that there is a close relationship between the melanosomes and lysosomes in melanoma cells in vitro. Novikoff et al. (20) and Seiji and Kikuchi (27) reported the presence of acid phosphatase activity in premelanosomes. These results are confirmed for human melanoma in vitro, although this seems to be the case only in more mature premelanosomes (preliminary results). If this can be verified statistically, a membranous connection of SER to the premelanosomes might be assumed to exist at a stage of melanogenesis of individual melanosomes later than we had previously anticipated (15), that is, after melanogenesis has ended in an individual premelanosome. If there is no membranous connection of GERL to the acid phosphatase containing more mature premelanosomes, as our results indicate, acid phosphatase may be channeled into the early premelanosome in an inactive form or by a mechanism different from that proposed (8, 19).

In line MeGo, we see an arrangement of dark bodies similar to that in line LeCa, but the characteristic substructures of premelanosomes are missing. We are justified in considering them melanosomes only by our knowledge that line MeGo is a DOPA-positive melanoma cell line, which we determined by the distribution and size of the dark bodies, by their electron density, and by their connection to SER. Many of these characteristics also apply to lysosomes. The dark granules of strain Amel P-4 appear morphologically even more characteristic of lysosomes. Also, most of them contain acid phosphatase activity.

In a recent study, Okun et al. (21) reported peroxidase-mediated synthesis of melanin from tyrosine or DOPA in melanocytes. They claim that mammalian peroxidase may have roles in initiating melanin synthesis in vivo. As the different strains of line LeCa and MeGo have not yet been investigated for peroxidase activity, we cannot exclude the possibility that peroxidase has reacted with DOPA; but, in view of the report of Okun et al. (21) both enzymes would indicate melanin formation. The terminology of a membrane-bound organelle, which has the structural arrangement of a melanin granule, may become more confused if it is named according to the enzymes that it contains. It may be called a peroxisome in the presence of peroxidase (21), a premelanosome in the presence of tyrosinase, and a modified lysosome in the presence of acid phosphatase (16).

As melanosomes and lysosomes cannot be distinguished by the presence or absence of acid phosphatase, morphological criteria seemed to be an additional approach to differentiation between these 2 organelles. It became clear during the course of this investigation that it is difficult to discriminate between the structures of concern in certain strains. Since all strains were DOPA positive and contained acid phosphatase, we can only speculate. We may interpret the electron-dense granules in the micrographs of the line MeGo (Fig. 3) as premelanosomes that lack the characteristic helical matrix onto which tyrosinase could be oxidized and polymerized. This may account for the presence of electron-opaque material in very thin membranous tubules. A backflow may exist because no polymerization occurs onto a preexisting matrix structure. Single deletions of genetic information in melanosome formation may be responsible for the production of incomplete melanosomes. In superficial, spreading melanoma there is a definite formation of the helical matrix...
Ultrastructural Comparison of 2 Melanoma Cell Lines

but no apparent melanization (3). This is also an indication of the interruption of the normal sequence of events in melanosome formation in melanoma. The appearance of a typical premelanosome and a laminated body, typical for lysosomes within one limiting membrane, might be explained by the fusion of the two. This may lead eventually to the formation of autophagosomes if it is a regulated process to eliminate premelanosomes. It does not seem to be a mechanism for the introduction of acid hydrolases into individual premelanosomes, as it was observed only on rare occasions.

The presence of acid phosphatase in membrane-delineated bodies constitutes an intracellular digestive system (4, 5). It is suggestive of protein degradation or the potential thereof. The functioning of melanosomes in the coloration of vertebrae (23) makes it unlikely that an organelle is built, partly degraded, and then used. The possibility must be considered that, in melanoma cells, melanosomes are degraded as they accumulate and cannot be transferred to keratinocytes. Melanocytes may have the option of transferring melanosomes or digesting them in case of overproduction or reduced uptake by keratinocytes. This means that acid phosphatase containing melanosomes must be regarded as lysosomes and not "modified lysosomes" (20), a term that seems to imply that a lysosome has been modified during evolution or ontogenesis of the melanosome. A breakdown of melanin granules within melanocytes had previously been suggested (9, 12). The appearance of autophagic vacuoles in melanoma cells filled with melanosomes seems to be another mechanism with the same result. They may also contain acid hydrolases (20). An investigation of the presence or absence of acid phosphatase in premelanosomes of normal melanocytes could result in suggestive evidence for these special circumstances in melanoma cells. Fitzpatrick et al. (6) did not find acid phosphatase activity in isolated melanosomes from melanocytes in vitro and from retinal pigment epithelium of 10-day-old chick embryos. This was confirmed by electron microscopic histochemistry (27). These findings support the hypothesis that, in melanoma cells, melanosomes containing acid phosphates are degraded. The lack of acid phosphatase in the retinal pigment epithelium of the chick was interpreted as being due to the presence of immature pigment granules, but fully melanized and DOPA-negative melanosomes were found in retinal pigment epithelium of 6-day-old chick embryos (G. G. Maul, unpublished observation). If acid phosphatase were present in fully developed melanosomes of normal melanocytes, an alternative explanation seems likely. Acid hydrolases may be introduced into the membrane-bound melanosomes to stop enzyme activity necessary for melanosome formation. This may, then, be part of a size-limiting control mechanism for melanosomes about which we know absolutely nothing.

On the ultrastructural level, there were only two noteworthy differences between the cell lines besides the differences in premelanosome morphology, that is, the great abundance of free polysomes in line MeGo and the appearance of mitochondria that are more or less round. They deviate strongly from the normal appearance, which is very long and thin. These round mitochondria were found near lipid accumulation. No significance can be inferred from this observation. Similar mitochondria are described in brown adipose tissue (29–31). Differences between in vitro cell lines may not necessarily reflect equal differences under in vivo conditions. The correlation of in vivo structures of the same tumor should be attempted to evaluate the relevance of studies on cultured malignant melanoma cell lines. Such studies are in progress.

REFERENCES


Ultrastructural Comparison of Two Human Malignant Melanoma Cell Lines

Gerd G. Maul and M. M. Romsdahl


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/30/11/2782

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.