Ultrastructure of malignant melanomas of the choroid

Michael J. Hogan and Lynette Feeney

Eleven malignant melanomas of the uveal tract have been studied by light and electron microscopy. Two types of cells were found in the epithelioid tumors: one, a darker type, contained large amounts of free RNP particles in single granules or in clusters, many smooth membranes and vesicles, and numerous mitochondria; the lighter type had similar cytoplasmic components, but in a lesser amount, especially in the number of free RNP particles. The epithelioid cells were closely packed, in contrast with the findings of light microscopy, and the cell walls were smooth and straight. In mixed tumors it was more difficult to distinguish spindle and epithelioid cells, except by prior identification with stained 1 μ sections. The three spindle cell B tumors showed a very uniform pattern in low-power field with the electron microscope, but in higher magnifications exhibited considerable variety in cytoplasmic composition, with numerous large mitochondria, many smooth-surfaced vesicles, and an amorphous component in the cytoplasm. In contrast with the epithelioid cell there is more rough-surfaced endoplasmic reticulum, and an absence of free RNP particles in the cytoplasm. All tumors contained a variable number of macrophages, characterized by large masses of cytoplasmic pigment. It was not possible to determine whether these were true reticulo-endothelial cells, or tumor cells which had phagocytized pigment. The origin of the melanin granules was also not revealed by this study. Possible modes of origin of these tumors were considered. None of the cells resembled Schwann cells of normal human beings, nor did they exactly resemble normal melanocytes. In addition to pigmentation, many similarities to the normal melanocyte could be detected in the spindle B type cells.

Callender4 classified malignant melanomas of the uveal tract according to their cytologic and morphologic features. This classification was later6,7 found to be useful in determining the prognosis in affected patients. The following types were described: spindle A, spindle B, mixed, epithelioid, fascicular, and necrotic. The epithelioid and necrotic tumors are the most malignant and the spindle types the least so.

Francois and associates11,12 described the electron microscopic features of two melanomas of the choroid. Both were of the spindle B variety; one was pigmented, the other was not. Both tumors had essentially the same ultrastructure. The pigmented tumor had pigment granules which were not homogeneous. Each granule was composed of finer particles which were embedded in a less dense material. Some of these granules were surrounded by a delicate, smooth membrane. An additional
feature was the presence in the cytoplasm of larger rounded (about 0.5 μ) or oval spaces surrounded by a delicate membrane and containing melanin particles in a less opaque ground substance. The findings suggested that the rapid development of the malignant cells caused abnormal formation of pigment granules, and that the vacuolar spaces in the cytoplasm were incompletely formed pigment granules.

Wellings and Siegel studied 10 biopsy specimens of melanomas from human skin and described melanin granule formation in the Golgi apparatus. The smallest melanin granules were found in the Golgi zone, arising by deposition of a dense material within the small vesicles of the Golgi apparatus. The authors later described the morphology of the same tumor cells. In general, the cells corresponded to previous descriptions of electron microscopic studies of other normal and neoplastic cells. The melanin granules had an average diameter of 300 μ, were circular or oval, uniform in density, and occasionally showed a substructure of finer granules or rods. Macrophages were found in the tumors and showed conspicuous differences from the melanocytes, having smaller nuclei and nucleoli, fewer mitochondria, less ergastoplasm, less Golgi material, and in the presence of very large ovoid or circular pigmented masses. The internal granular and rodlike structure of the phagocytized granules was more prominent and more easily seen than in those of the tumor cells. The rod-shaped internal units of phagocytized melanin granules sometimes showed a longitudinal beaded appearance or periodicity which was not apparent in the melanin granules of the tumor cell.

Seiji, Fitzpatrick, and Birbeck studied melanoma pigment granules of the mouse and pigment epithelium of the chick and correlated the electron microscopic appearance with enzymatic content. They concluded that the melanin granules and mitochondria are distinct cytoplasmic constituents of melanin-forming cells. They proposed the term "melanosome" for the characteristic enzymatically active particle which is the site of melanin formation. The melanosome is a cell organelle with an internal structure which ranges in size from 0.1 to 0.4 μ. The melanin granule is 0.4 to 20 μ in diameter and is composed of the melanoprotein polymer.

Melanosomal will be used to describe the small striated or granular melanin particles seen in the tumors.

During the past 2 years we have collected 11 malignant melanomas of the uveal tract for study. All specimens were obtained from adult human eyes. Comparisons will be made of the cytology as seen with the light microscope in paraffin-embedded sections of 10 to 12 μ, in methacrylate-embedded sections of 1 μ, and in thin sections with the electron microscope.

Materials
Seven of the melanomas involved the choroid, 2 the choroid and ciliary body, 1 the ciliary body alone, and 1 the iris. Of the 7 choroidal melanomas 6 were of the mixed cell type, and one was of the spindle cell B variety. One of the 2 tumors involving both the choroid and ciliary body was of the mixed type, and the other was of the spindle cell B variety. The ciliary body tumor was an epithelioid tumor, and that of the iris was a spindle cell B type. One tumor of the ciliary body was thought at first to be a spindle cell A type, but on electron microscopy all nuclei were found to contain nucleoli. Restudy of thinner sections showed the tumor to be a spindle cell B type.

Methods
At the time of operation all eyes were opened quickly and in a direction determined by the position of the tumor. A portion of the tumor was removed for formalin fixation and embedding in paraffin, and additional pieces were placed in cold (4° C.) veronal buffered (pH 7.4) osmic acid (OsO₄). After 3 to 5 minutes' fixation they were divided into 1 to 2 mm. portions and transferred to fresh fixative for 2 to 3 hours. They were then dehydrated and embedded in methacrylate, and sectioned with a Porter-Blum microtome or an LKB ultratome. The sections were placed on carbon-coated copper grids, and stained with lead hydroxide. All were viewed with the RCA EMU-3 electron microscope. The paraffin-embedded portion of the tumor was sectioned and stained with hematoxylin and eosin. Other
sections were bleached with potassium permanganate (KmMnO₄) before staining. Comparisons were made between the hematoxylin and eosin paraffin sections, bleached paraffin sections, 1 μ thick methacrylate sections stained with the Goodpasture-MacCallum stain, and the 1/40 μ electron microscope sections.

Results

The paraffin sections were used to determine the type of melanoma and the degree of pigmentation. Six of the choroidal melanomas were of the mixed type and one was of the spindle B variety. Five tumors were moderately pigmented (2 to 3 plus) and 2 were lightly pigmented (1 plus). One of the tumors which involved the choroid and ciliary body was a very lightly pigmented spindle cell B tumor; the other a moderately pigmented (3 plus) mixed cell tumor. The one ciliary body tumor was a lightly pigmented (1 plus) epithelioid tumor. The iris tumor was a lightly pigmented (1 plus) spindle cell B tumor.

The identification of cell types was less easy in the 1 μ thick stained methacrylate sections. Spindle cells in the spindle cell B tumors could be identified because of the uniformity of the cells and their heavy branching processes (Fig. 1). In the mixed tumors it was less easy to differentiate the spindle cell B type from the epithelioid because the lack of cohesion of epithelioid cells was less evident in osmium-fixed tissues, and the spindle cells showed fewer and smaller branching processes (Fig. 2).

The epithelioid cells in the 1 μ methacrylate sections could be recognized by their size, large nuclei, nucleoli, and abundant cytoplasm (Fig. 3).

Macrophages were present in all tumors, but were especially numerous in the heavily pigmented tumors. Their large masses of phagocytized cytoplasmic pigment and other material often obscured the nucleus. Bleached paraffin sections showed these cells to have most features of the adjacent tumor cells. Many sections showed that the lumina of blood vessels contained macrophages.

Fig. 1. (EM 140 B) Spindle cell B melanoma of the iris. One micron thick methacrylate section. A group of spindle B type cells from the central portion of the tumor. (Goodpasture-MacCallum stain. ×630.)

Fig. 2. (EM 77 T,) Mixed cell melanoma of the choroid and ciliary body. One micron thick methacrylate section. Epithelioid cells near the right margin and spindle cells centrally. Large macrophage to the right of center. (Goodpasture-MacCallum stain. ×630.)

Fig. 3. (EM 134 T,) Epithelioid cell melanoma of ciliary body. One micron thick methacrylate section. (Goodpasture-MacCallum stain. ×630.)
Figs. 4 and 5. For legends see page 551.
Fig. 6. For legend see page 551.
Figs. 7 and 8. For legends see page 551.
Figs. 9 and 10. For legends see page 551.
**Epithelioid types of cell.** Unlike the epithelioid cells seen in paraffin sections, those seen in the electron microscope were close together (150 Å intercellular space). The cell boundaries were straight. The nuclei were very large in proportion to the surrounding cytoplasm. The chromatin was evenly dispersed except for a denser layer along the inner nuclear membrane (Fig. 4). The nucleoli were large and dense and were composed of a tangled mass of bands or ropes which had a uniform width of 0.1 to 0.15 μ.

On the basis of the appearance of the cytoplasm, both dark and light cells were seen in various fields (Fig. 5). No intermediate forms were observed. The difference was produced by the quantity of cytoplasmic components. The dark cells were loaded with ribonucleoprotein (RNP) particles which occurred singly or in clusters and rarely were attached to membranes (Fig. 6). Smooth membranes and vesicles were abundant even in areas distant from the Golgi apparatus. A free amorphous component occupied much of the cytoplasm, and numerous mitochondria were seen (Fig. 7). The light cells exhibited the same cytoplasmic components but to a lesser degree, particularly in the number of free RNP particles.

This tumor contained intercellular fibrils, which were not seen in the paraffin sections. Unlike the epithelioid cells seen in paraffin sections, those seen in the electron microscope were close together (150 Å intercellular space). The cell boundaries were straight. The nuclei were very large in proportion to the surrounding cytoplasm. The chromatin was evenly dispersed except for a denser layer along the inner nuclear membrane (Fig. 4). The nucleoli were large and dense and were composed of a tangled mass of bands or ropes which had a uniform width of 0.1 to 0.15 μ.
with 600 to 640 Å periodicity typical of both collagen and reticulin, and bundles of fine (100 to 180 Å) unbanded fibrils. Elastic fibers also were seen in some areas.

The melanin content and morphology in the epithelioid tumors varied. Nearly every cell contained melanosomes. Lighter pigmented cells showed a wider spectrum of pigment forms: melanosomes, densely striated granules, and larger solid, homogeneous melanin granules.

**Spindle B cells.** The cells in these tumors showed the same close alignment and straight borders as the epithelioid cells, except at the very periphery of the tumor or where poor fixation was evident. The nuclei were more oval and many showed extensive convolutions of the nuclear membrane. In proportion to the amount of cytoplasm, they were smaller than those of the epithelioid cells. There was no chromatin layer at the inner nuclear membrane, as was seen in the epithelioid cells.

The cytoplasm of these cells did not show the quantitative differences which were seen in the epithelioid cells, i.e., in a low-power field all cells looked alike. Aside from this, however, there was generally more variety in cytoplasmic composition among the spindle B than among the epithelioid cells (Fig. 8). Like the epithelioid, the spindle B cells had numerous mitochondria and many smooth-surfaced vesicles. There also was a similar amorphous component in the cytoplasm. In contrast with the epithelioid cell, there was more rough-surfaced endoplasmic reticulum, i.e., the RNP particles were attached to the cytoplasmic membranes. At the same time there was not the striking abundance of free RNP particles in the cytoplasm. The rough endoplasmic reticulum was in the form of vesicles and very short double membranes; extensive parallel arrays were not seen (see mixed tumors).

Most cells showed large sacules of rough endoplasmic reticulum the contents of which appeared to have been dissolved by fixation procedures or sublimed by the electron beam. There were frequent bizarre forms of endoplasmic reticulum, usually in the form of spirals. The Golgi vesicles of one tumor (139) contained a dense amorphous material like that seen in secretory cells.

The melanin content was quite variable. One tumor (139) contained no pigment except for an occasional branching melanocyte with mature granules. Another (140) showed only an occasional cell with finely striated melanosomes. Most of the cells of another (814) contained abundant large (about 0.5 μ) pigment granules of the finely striated type. All those of still another (838) contained numerous solid or densely striated melanin granules.

Collagen rarely was seen in these tumors except around the blood sinuses.

**Mixed tumors.** The 7 mixed tumors were composed of epithelioid and spindle B cells in varying proportions. It was frequently difficult, however, to identify a cell as epithelioid or spindle B because more cytoplasmic variation was observed in these tumors.

Cells with the following characteristics of epithelioid cells as observed in 1 μ sections by light microscopy were selected for study: large nuclei, large fibrous nucleoli, extensive cytoplasm, and lack of alignment in bundles. Electron micrographs of these cells showed the features of dark epithelioid cells, with vesicles of all descriptions as the predominant feature. The vesicles were of all sizes, surrounded by thick and thin membranes, and appeared empty or contained amorphous material, particles, and tiny vesicles. They might or might not be studded with RNP particles, and some definitely contained melanin. Large sacules of endoplasmic reticulum which were apparently empty were a common feature, similar to those of spindle cells. Nearly every cell contained large lipid droplets. Mitochondrial sizes varied, but a given cell had uniform-sized mitochondria.

Cells which most nearly fitted the morphologic concept of spindle cells seen by light microscopy also showed much more cytoplasmic variation than those of the
pure spindle B tumors. These cells could be placed in 2, and possibly 3, classes: (1) those with the rough endoplasmic reticulum beautifully arranged in long parallel lamellae, and (2) those with a few short lamellae of endoplasmic reticulum and many free RNP granules occurring singly and in rosettes (Fig. 9). A possible third category contained fingerlike projections of endoplasmic reticulum around the mitochondria. The various cells suggested a progression of dedifferentiation, possibly toward the epithelioid type. In addition, all cells contained lipid droplets and large empty saccules of endoplasmic reticulum.

The melanin content varied from tumor to tumor and from cell to cell. Two tumors described as moderately to heavily pigmented in histologic sections (65 and 77) showed many cells with but few melanin granules, others with numerous melanosomes, too small to be resolved by the light microscope, and still others which contained many large solid granules. In one tumor (133), only a rare cell contained pigment and it was of the mature or solid type.

All the mixed tumors contained some connective tissues, but in varying amounts. Typical collagen, reticulin, and fine fibrils were often seen. One tumor (65) also contained elastic fibers. One of these tumors (133) had groups of cells bound into fasicles 20 to 30 μ wide by strips of connective tissue 1 to 2 μ wide. The cells had interdigitating borders, unlike the 3 spindle B tumors.

**Macrophages.** In all the tumors, regardless of cell type, a cell occurred which had a very characteristic and interesting morphology (Fig. 10). The cell was very large (usually larger than the epithelioid cell) and frequently showed branching. The cytoplasm contained pleomorphic, rounded bodies composed of all forms of melanin, from microgranules to large solid granules. These bodies resembled the inclusions of macrophages seen in other tissues, and particularly those in pigmented tissues. The inclusions were readily visible by light microscopy, some being as large as a red blood cell.

In addition to the forms of melanin, the inclusions contained a particulate material which was less dense than the melanin microgranules. This material was evenly dispersed throughout the inclusion and obscured its limiting membrane.

The cytoplasm of the macrophage differed from that of the epithelioid cells in that it had large mitochondria and few free RNP particles. It would be difficult to distinguish this cell from the spindle B cells on the basis of cytoplasmic content.

**Blood sinuses.** The walls of the blood sinuses were less than 0.2 μ thick and resembled capillaries in structure. They were composed of a thin layer of endothelium, thin basement membrane, and a few collagen fibers. Macrophages were frequently found in the lumen just inside the wall. In one tumor two macrophages were observed entering the sinus through gaps in the endothelial layer.

**Discussion**

The ultrastructure of normally pigmented cells has occupied the attention of many investigators for the past several years. The melanocytes of the skin, is, hands, retina, and uveal tract of human beings have been studied. The melanocyte of the skin of various animals also has been studied by Selby. Comparison of the normal melanocyte with the malignant melanoma cell shows some differences. The nuclei are similar in structure, and, except for the greater size of nucleus in relation to cytoplasm in the epithelioid cell, cannot be distinguished. The nucleolus of the epithelioid cell is outstanding in its size and ropelike pattern. The cell membranes are similar except that in the melanocyte they are more irregular. The membranes of the spindle cell and epithelioid cell also are similar. Some differences are noted between the cytoplasm of the normal and of the malignant cells. The epithelioid cell contains very numerous RNP granules, mostly lying free, in
contrast with the melanocyte and spindle cells, which have RNP in considerable amounts but attached to the membranes of the endoplasmic reticulum. The epithelioid cell also contains large amounts of smooth membranes and vesicles, even in areas remote from the Golgi apparatus.

The melanin granules in the cytoplasm of all the malignant cells vary in amount, size, and composition. Some cells show large granules of uniform density, size, and distribution, similar to those of normal melanocytes. Others show very fine granules of a size beyond the resolution of the light microscope. Still others show small granules which vary in size and distribution, some with a solid structure, or with fine granules or striated particles. The variegated granules more nearly resemble those seen in "immature" melanocytes of the human choroid and those of the developing mouse.

The findings in these specimens show the spindle B cells to resemble more closely those described by Wellings and Siegel in skin melanomas. They found considerable endoplasmic reticulum with attached RNP particles. In contrast with their findings, the Golgi apparatus in our specimens did not appear more prominent than in the normal melanocyte. The smooth vesicles and sacs in the cytoplasm of most malignant cells, however, might be a part of the apparatus, for we could not establish the origin of the melanosomes since they were frequently found in the periphery of the cell at some distance from the Golgi zone. This study and a previous one suggest that the melanin granule arises in vesicles in the cytoplasm as described by Moyer in embryonic mice. An initial formation of fine parallel filaments occurs in the vesicle, followed by alignment of osmiophilic melanin granules, like beads on a string, along these filaments. Cross sections of such vesicles show a fine granulation, and longitudinal sections show them to be oval and to contain the fine filaments with their melanin granules. Eventually, the whole vesicle becomes filled with melanin.

In this respect Wellings and Siegel also found the granules in melanoma cells to be composed of granular and rod-shaped structures.

It is less easy to compare the spindle B cells of our tumors with the two described by Francois and associates. The cloudy swelling of the mitochondria in their second case suggests degenerative changes caused by other disease, or poor fixation. Otherwise, the endoplasmic reticulum with its attached RNA granules and other organelles were similar to the spindle B cells of our tumors. The cytoplasm of some cells in the first case described also showed the curving elements of endoplasmic reticulum which were found in some spindle B cells of our mixed tumors. In addition to their "normal" pigment granules, they observed cytoplasmic spaces which were bound by membranes and contained melanin granules and a less opaque granular material. These spaces are considerably larger and the melanin microgranules are more dispersed than any seen by us or by other investigators.

The macrophages in these tumors, initially described by Dalton and Felix, may be a true reticuloendothelial cell which has gathered pigment liberated from adjacent melanoma cells, or a neoplastic cell which behaves as a phagocyte. The light microscope study of unbleached and bleached sections shows they are indistinguishable from melanoma cells. The electron microscope shows them to be large and to contain pigment and other material in very large masses in the cytoplasm. It is not possible to determine whether these cells are melanoma or reticuloendothelial cells. We have observed the same type of cell in a normal component of the human choroid, which suggests that it is more likely a macrophage than a melanoma cell.

The interstitial tissues and blood spaces of all these tumors were found to be much as described with the light microscope. No nerves were encountered in any of the tumors.
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REFERENCES