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Cell Membrane Structure of Human Giant-Celled Glioblastoma

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Summary. A giant-cell glioblastoma was examined by electron microscopy and by the freeze-fracture technique. The cell membranes bordering the extensive extracellular space often showed complicated undulations and peripheral vacuoles as well as occasional microvilli or filopodia. The undulations were mainly composed of plasmalemmal vesicles as well as of large (400–800 nm in diameter) and small (30–50 nm in diameter) localized protrusions and invaginations of the cell membrane. Deep invaginations of the cell membrane apparently resulted in two separate cytoplasmic portions. Locking of protruded cytoplasmic tongues and adherens junctions were sometimes seen in closely approximated cell membranes. The average number of membrane particles per μm^2 was 630 ± 130 on the P face and 180 ± 30 on the E face. The membrane particles were occasionally aggregated to form clusters about 30 to 150 μm in diameter. Gap junctions were occasionally found, but there were no tight junctions. Large particles about 30 nm in diameter were found in places.

Key words: Giant-celled glioblastoma – Cell membrane structure – Membrane particles – Gap junction – Cell membrane undulation.

Since the initial recognition of a bizarre giant-cell glioma by Meyer in 1913, the nature of the giant cells has been controversial: some consider the tumour to be a variety of glioblastoma (Scherer, 1935; Russel and Rubinstein, 1959; Becker et al., 1967), others a sarcoma (Zülch, 1931, 1940; Zülch and Wechsler, 1968). Several electron microscopic studies have been performed in an attempt to identify the origin of the nature of the giant cells (Fukumitsu, 1964; Lynn et al., 1968; Poon et al., 1971; Hadfield and Silverberg, 1972). The present report attempts to characterize the ultrastructure of the cell membrane in thin sections and by the freeze-fracture technique.

Material and Methods

Representative specimens were taken at surgery from the right frontal tumour of a 49-year-old female before its blood supply was interrupted. They were diced immediately into small pieces and fixed for 2 h at 4°C in 2.5% glutaraldehyde buffered with 0.1 M phosphate (pH 7.4). Some of them were dehydrated in graded alcohol and embedded in Epon. Thick sections stained with toluidine blue and thin sections stained with lead citrate and uranyl acetate were examined. The former showed giant-celled glioblastoma. The other fixed specimens were washed briefly in phosphate buffer and immersed at 4°C for 5 h in a 30% glycerol solution buffered with the phosphate (pH 7.4). They were then mounted on a copper specimen holder, frozen rapidly in liquid Freon 12, transferred into liquid nitrogen, and fractured in vacuo in a HFZ-1 and HUS-4 freeze-etching apparatus. The fracture surface was replicated by platinum and carbon. The replica, after digesting the tissue in a commercial bleaching solution, was washed in distilled water, picked up on grid, and examined with an HU-12 electron microscope.

Results

Thin Sections. Tumour cells were usually large and dispersed throughout extensive extracellular spaces (Fig. 1) in which flocculent material was predominant and in which occasional filaments, about 10 nm thick, were present. No collagen fibrils were found in the extracellular space. The cell membranes bordering the extracellular space were usually irregular and often showed complicated protrusions and invaginations and peripheral vacuoles as well as occasional microvilli or filopodia (Fig. 1). Some tumour cells showed a relatively smooth cell membrane. Close cellular contacts, which were usually formed by an apposition of 2 to 5 tumour cells, often showed few, if any, regular width, largely because of the complicated cell

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membrane undulations and because of the presence of occasional microvilli. Where deep invagination of the cell membrane occurred the cell was strongly constricted at that site, forming apparently two separate cells. The separate cytoplasms were connected with each other through a narrow gap about 250 nm in width (Fig. 2A). Another intercellular relationship is shown in Figure 2B. Here, a protruding tongue of cytoplasm is locked in the invaginated portion of a closely apposed adjacent cell, and its neck is narrowly constricted. Adherentes junctions were occasionally found (Fig. 2C). They were about 50 nm wide and showed fuzzy filaments beneath the cell membrane and an intermediate dense line in the center of the intercellular space.

The tumour cells had a generally dense appearance and were rich in cytoplasmic organelles. Bundles or swirls of filaments, about 8 nm in diameter, were occasionally found in the perikaryon (Fig. 3) or the cytoplasmic processes (Fig. 1). In the latter they occupied most of the cytoplasm, being suggestive of glial origin. Nuclei were usually irregular, often showing irregular invaginations of the nuclear membrane and an occasional nuclear sheet.

Freeze-Fracture. The fracture process exposed an extensive area of cell membrane, with P and E faces. Membrane particles, about 10 nm in diameter, were generally abundant and diffusely scattered on the P face (Figs. 4 and 5), whereas they were reduced in number and scarcely distributed on the E face (Figs. 4, 7, and 8). The exact distribution of the membrane particles varied somewhat from cell to cell, and the average number of membrane particles per unit area of cell membrane was difficult to assess precisely. The average number of membrane particles per μm^2 of cell membrane counted under a dissecting microscope was 630 ± 130 on the P face and 180 ± 30 on the E face. The membrane particles on the P face were occasionally aggregated to form clusters about 30–150 μm in diameter (Fig. 6A). These clusters were rather irregular in shape, usually not closely packed with particles, showed a very irregular centre-to-centre spacing of the particles, and were not associated with any complementary structures on the E face. In addition, the constituent individual particles varied in size from 8–13 nm in diameter. On the other hand, gap junctions as shown in Figure 6B were uncommon. They were polygonal and measured about 200 nm. They were composed of

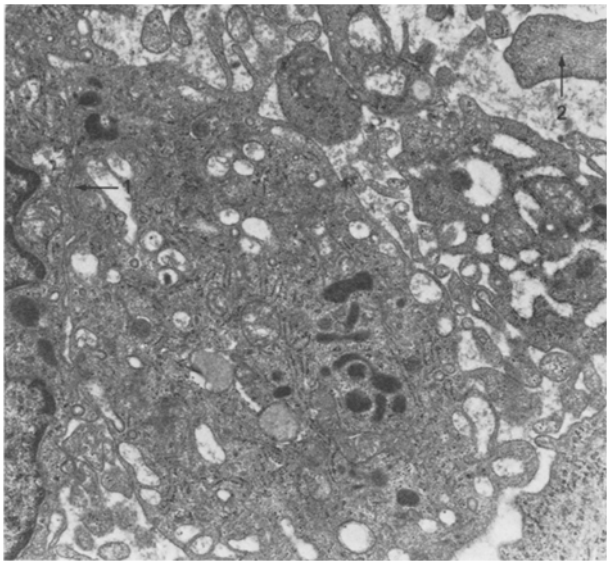
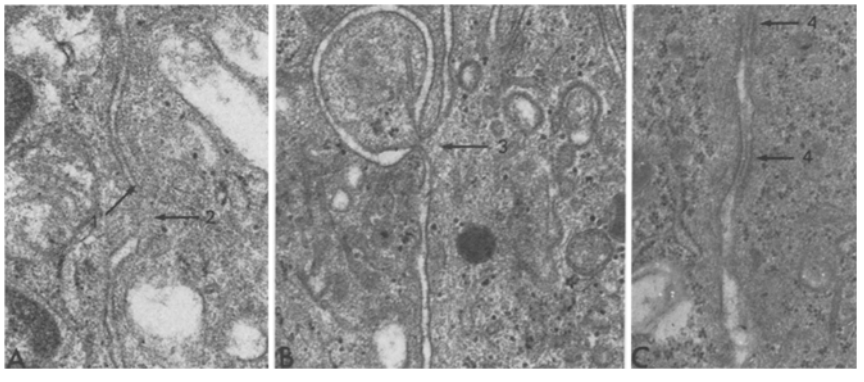


Fig. 1. The cell membranes of the tumour cells are generally irregular and often associated with peripheral vacuoles as well as with occasional microvilli and filopodia. In addition, the cell membrane is deeply invaginated (arrow 1). Bundles of glial filaments are seen in a cytoplasmic process (arrow 2). ×9800



Figs. 2A–C. Three features of apposed cell membranes are shown. Figure **2A** is a high magnification of Figure 1 and exhibits a deep invagination of the cell membrane (arrow 1), forming a narrow cytoplasmic area (arrow 2) between the cell membrane invaginations. In Figure **2B** a protruded cytoplasmic tongue is locked in an invaginated portion of an adjacent cell, and its neck is narrowly constricted (arrow 3). Figure **2C** shows two adherentes junctions (arrows 4). ×26520 in Figure **2A**, ×22400 in Figure **2B**, ×23800 in Figure **2C**

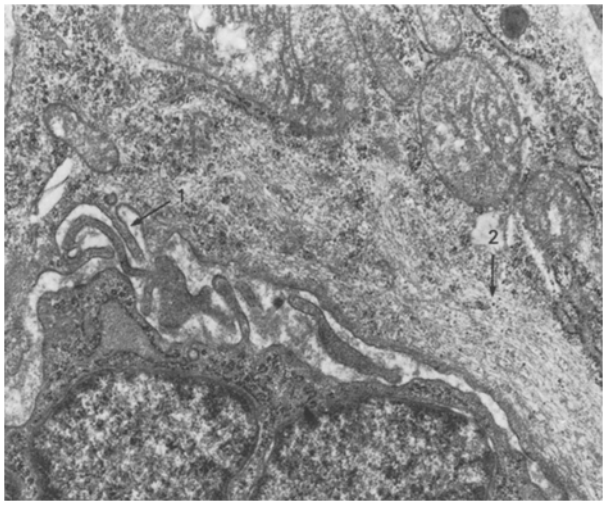


Fig. 3. A tumour cell exhibits microvilli (arrow 1) and bundles of glial filaments (arrow 2). ×14000

closely packed particles about 8 nm in diameter. Large particles in gap junctions appeared to be formed by the union of two or three particles, possibly due to artefact, and a small smooth fracture face was visible in the centre of the gap junctions. No tight junctions were seen. Large particles about 30 nm in diameter, were found in places (Figs. 4, 5, and 7).

A characteristic feature of the cell membrane was the frequent occurrence of rounded holes and mounds, which varied in size. The holes on the P and E faces represented invaginations and protrusions of the cell membrane respectively, whereas the mounds on the P and E faces represented the respective protrusions and invaginations of the membrane. The plasmalemmal vesicles attached to the membrane usually appeared as smooth depressions, holes, or depressed circular plaques on the P face (Figs. 4 and 5) and as domes or broken-off necks on the E face (Figs. 4, 7, and 8), depending on the passage of the fracture plane. Each measured about 60–80 nm in diameter. When the fracture process did not follow the contours of the attached plasmalemmal vesicles, the latter showed as circular constellations of particles. The percentage of surface area differentiated into plasmalemmal vesicles ranged from zero in the cells with a smooth surface to about 3.0% of the cell surface in the most vesiculated cells.

The microvilli or filopodia were demonstrated in the replicas as rounded domes on the P face (Fig. 5) and as rounded holes on the E face (Figs. 4 and 8). Each measured less than 0.1 μm in diameter in the microvilli and more than 0.1 μm in diameter in the filopodia. Both were also associated with a fractured cytoplasm. Large mounds on the P face (Figs. 4 and 5) and large holes on the E face (Figs. 4

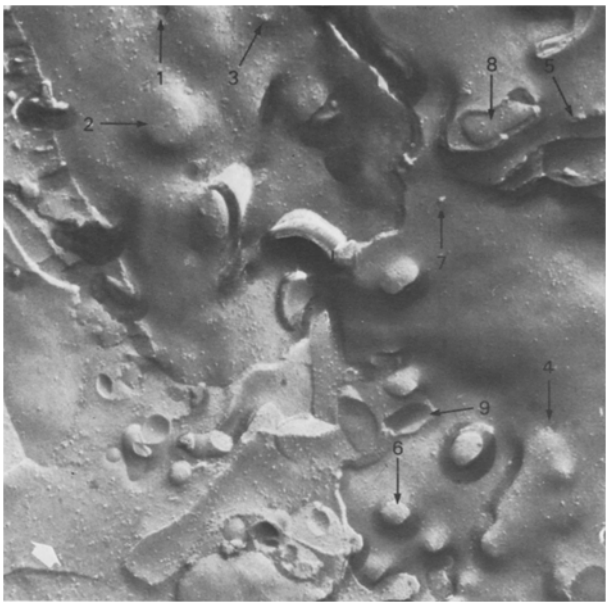


Fig. 4. Membrane particles are abundant on the P face and scarce on the E face. Three attached plasmalemmal vesicles (arrow 1) as well as two large (arrow 2) and one small (arrow 3) protrusions are visible on the P face, and a microvillus (arrow 4) as well as two attached plasmalemmal vesicles (arrow 5) and ten cell membrane invaginations (arrow 6) are evident on the E face. In addition, a large particle (arrow 7) is seen on the E face. Arrow 8 indicates the P face of the adjacent cell membrane, and arrow 9 is a fracture face of a vacuolar membrane associated with the cell membrane. ×27300

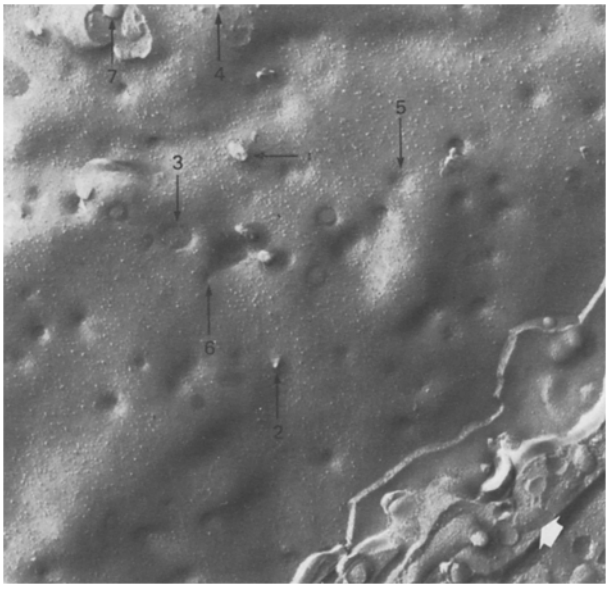


Fig. 5. Four microvilli (arrow 1), one small cell membrane protrusion (arrow 2), five cell membrane invaginations (arrow 3), several large particles (arrow 4), and many attached plasmalemmal vesicles (arrow 5) are found on the P face. The initial stage of formation of plasmalemmal vesicles is shown as a tiny depression (arrow 6). A vacuole (arrow 7) associated with the cell membrane is found in protruded cytoplasm. ×23100

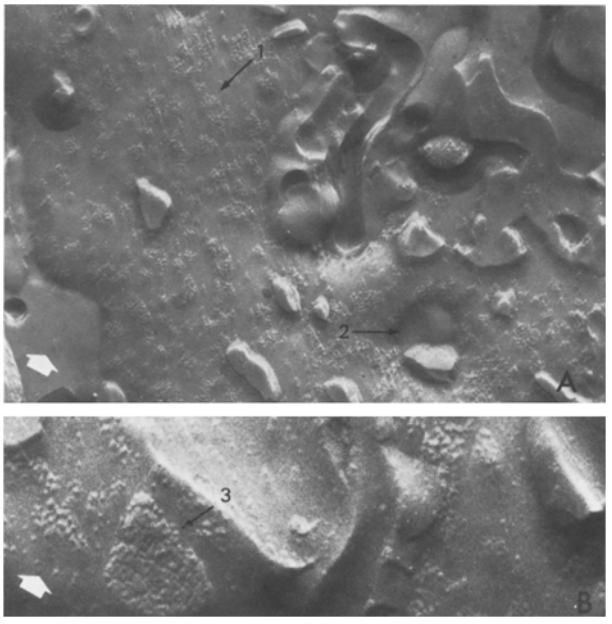


Fig. 6 A and B. Many clusters of membrane particles (arrow 1) are seen on the P face. They are rather irregular in shape and diffusely scattered. Arrow 2 indicates a large depression of the P face associated with a rounded extracellular space. A gap junction (arrow 3) is visible on the same fracture face. ×30800 in Figure **6A**; ×71400 in Figure **6B**

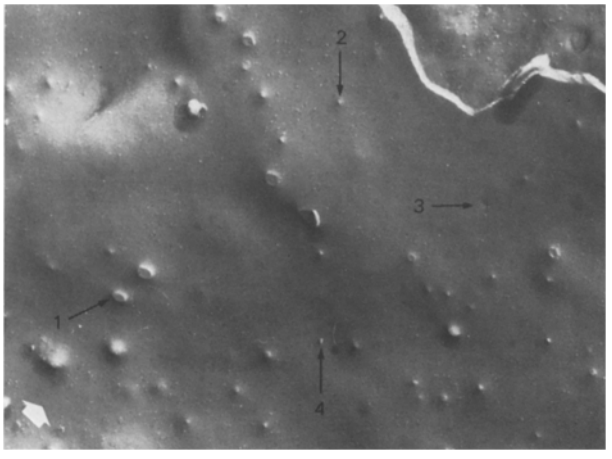


Fig. 7. Many attached plasmalemmal vesicles (arrow 1) and many tiny protrusions (arrow 2) are visible on the E face. The latter suggests an initial stage of formation of the plasmalemmal vesicles. In addition, tiny depression (arrow 3) and large particles (arrow 4) are also found. ×23100

and 8) as well as large holes on the P face (Fig. 6A) and large mounds on the E face (Fig. 4), which varied in size from 400–800 nm in diameter, represented large protrusions and invaginations of the cell membrane respectively, and sometines exhibited a fractured cytoplasm in the protrusion (Figs. 5 and 8) and a

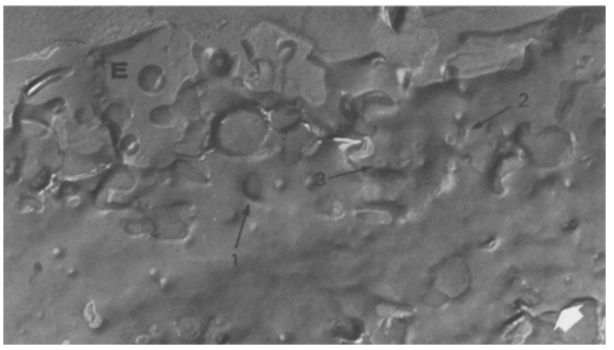


Fig. 8. The E face adjacent to the extensive extracellular space (E) clearly shows an irregular cell membrane invagination and protrusion (arrow 1). In addition, microvilli (arrow 2) and many attached plasmalemmal vesicles (arrow 3) are seen. ×10500

fractured extracellular space in the invagination (Fig. 6A). In addition, a close association of the cell membrane invagination with the corresponding adjacent cell membrane protrusion (Fig. 4), or of the cell membrane protrusion with peripheral vacuoles (Fig. 5), was evident in the replicas. Tiny protrusions of cell membrane, about 40–50 nm in diameter, were seen as small mounds on the P face (Figs. 4 and 5) and as small holes on the E face (Fig. 7), whereas tiny depressions of the cell membrane, about 30–40 nm in diameter, were demonstrated as small holes on the P face (Fig. 5) and as small mounds on the E face (Fig. 7). The tiny cell membrane depressions were suggestive of an initial stage of formation of the plasmalemmal vesicles.

In general, many uneven undulations of cell membrane were beautifully shown in the replicas. A complex arrangement of plasmalemmal vesicles, microvilli, filopodia, large cell membrane protrusions or invaginations was often associated with the cell membrane bordering the extensive extracellular space, as shown in Figure 8.

Discussion

The ultrastructure in thin sections of the present tumour was essentially similar to that of giant-celled glioblastomas reported previously (Fukumitsu, 1964; Lynn et al., 1968; Poon et al., 1971; Hadfield and Silverberg, 1972), i.e. it demonstrated glial filaments, transitions from better differentiated neoplastic astrocytes to giant cells, vascular feet, and lack of collagen. All these features seem to support a glial origin and argue against a mesenchymal origin. The occasional occurrence of reticulin fibers in the tumour may be the result of a desmoplastic reaction.

The average number of membrane particles per μm^2 of cell membrane was 630 ± 130 on the P face

and 180 ± 30 on the E face and among various brain tumours most similar to that of glioblastoma multiforme, which was 800 ± 230 on the P face and 100 ± 24 on the E face (Tani, 1976a). The average numbers of membrane particles per μm^2 of cell membrane in mesenchymal brain tumours have been reported (Tani, 1976a) as 2860 ± 160 on the P face and 670 ± 124 on the E face in a meningocytic meningioma, as 2130 ± 184 on the P face and 760 ± 122 on the E face of a fibroblastic meningioma, as 1590 ± 275 on the P face and 500 ± 75 on the E face of a hemangioblastoma, and as 1280 ± 128 on the P face and 160 ± 21 on the E face of a cerebellar sarcoma. Clusters of membrane particles have been reported in glioblastoma multiforme, medulloepithelioma, medulloblastoma, and ependymoma (Tani, 1976a). They were evident on the P face in the former three tumours, whereas they were visible on the E face in ependymoma. The significance of the occasional large particles measuring about 30 nm in diameter, is unknown at present.

The junctional complexes that were occasionally found in the present tumour consisted of adherens and gap junctions. Adherens junctions have been found in astrocytic tumours, ependymomas, and medulloblastomas, and gap junctions have been found in astrocytic tumours, oligodendrogliomas, ependymomas, medulloepithelioma, meningiomas, hemangioblastoma, cerebellar sarcoma, and germinoma (Tani, 1976a). In addition, tight junctions have been observed in germinoma, medulloepithelioma, oligodendrogliomas, and meningiomas, and rarely in ependymomas, medulloblastomas, and hemangioblastomas (Tani, 1976a). Therefore, the coexistence of adherens and gap junctions and the lack of tight junctions seem to be features of astrocytic tumours.

Many attached plasmalemmal vesicles have been found in replicas of meningioma, germinoma, ependymoma, and astrocytic tumours (Tani, 1976a). The attached plasmalemmal vesicles in astrocytoma were occasionally aggregated in a curvilinear, semicircular, or circular fashion (Tani, 1976a, b). Microvilli and filopodia have usually been found in astrocytic tumours, particularly on the cell membrane bordering the extensive extracellular space (Tani, 1976a, b). The localized, large and small protrusions and the invaginations of the cell membrane in the present tumour, associated with peripheral vacuoles, suggest active cell membrane undulation.

The structure of the cell membrane in the present tumour appears to favour a glial origin, particularly astrocytic, by virtue of the morphological features of its membrane particles, junctional complexes, plasmalemmal vesicles, microvilli, and filopodia. On the other hand, the cell membrane structures of mesenchymal brain tumours, including meningioma, hemangioblastoma, and cerebellar sarcoma, seem to be dissimilar from that of the present tumour. It is indisputable that the present tumour is an independent entity unrelated to other brain tumours. However, it is also true that both its cytoplasmic organization and its cell membrane structure support its glial origin.

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