Title
The Cilia on Cultured Ependymal Cells Examined by Scanning Electron Microscopy

Authors
Dalen, Helge
Schlapfer, Werner T
Mamoon, Abdel-Megid

Publication Date
2023-09-06

ucrl-20845

THE CILIA ON CULTURED EPENDYMAL CELLS EXAMINED BY SCANNING ELECTRON MICROSCOPY

Helge Dalen[1], Werner T. Schlapfer[2] and Abdel-Megid Mamoon
Donner Laboratory, University of California, Berkely, Calif. 94720, USA.

SUMMARY

The activity of cilia and their three dimensional arrangement on the free surface of newborn rat ependymal cells in vitro have been investigated by phase contrast and scanning electron microscopy. According to our findings cilia in different developmental stages were present in the cultures. There were great differences in distribution and number of cilia from cell to cell.

The ciliary movements on the ventricular surface were first described by Purkinje in 1836 (15). The rapid wipe-like strokes seem to be coordinated in a particular direction, and are thought to be important for the circulation of the cerebrospinal fluid in the brain (7, 11, 12, 20). Tissue culture techniques have made it possible to cultivate ependymal cells of normal (8, 9, 10, 14, 17) or malignant (3) origin, so that their development and ciliary activity can be studied over a long period under the phase contrast microscope.

Present addresses: [1]Department of Pathology, Karolinska Sjukhuset, S-104 01 Stockholm 60, Sweden

[2]Dept. of Psychiatry, School of Medicine, University of California, La Jolla, Calif. 92037, USA.

DISCLAIMER

There is an extensive literature describing the ultrastructure of ependymal tissue from many species. For mammals the most comprehensive studies were performed by Brightman and Palay (6) and Kohno and Usui (11) on adult rats, and by Tennyson and Pappas (19) who examined the embryonic and early postnatal stages in the development of ependyma in rabbits.

The purpose of the present report was to attempt to combine the study of ciliary activity under the phase contrast microscope with morphological studies of the same structures in the scanning electron microscope.

MATERIAL AND METHODS

Cultures from cerebellum of newborn rats were prepared on collagencoated coverslips in roller tubes similar to the technique described by Hild (8). Sterilly dissected cerebella were cut sagitally into 6 - 8 pieces. The explants were placed onto 12 x 50 mm Gold Seal # 1 coverslips coated with reconstituted rat tail collagen (4), covered with a few drops of medium (47.5% heat inactivated calf serum, 47.5% Gey's balanced salt solution, 5% chicken embryo extract, with glucose concentration increased to approximately 5 mg/ml) and incubated undisturbed for at least 24 h in plastic petri dishes at 37° C in a humid 5% CO_2 atmosphere. After attachment of the tissue to the collagen, the cultures were transferred to roller tubes containing 2 ml of medium and incubated at 36.5° ± 0.5° C in roller drums rotating at 1/5 rev/min. The medium was changed weekly thereafter.

Living cultures of different ages were examined with the phase contrast microscope and areas of interest were photographed and marked with a diamond scribe attached to the microscope.

Immediately after the microscopic examination the cells were fixed in OsO_4-vapor for 5 min. followed by double fixation in 2% glutaraldehyde for 3 h and 1% OsO_4 for 1 h. Both fixatives were made up in 0.1 M phosphate buffer at pH 7.2 (13, 16). The dehydration was carried out in increasing concentrations of ethanol at 4° C, and the cells were thereafter allowed to air-dry. A thin conducting film of gold was evaporated onto the specimens. The surface morphology of selected cells was examined in a JEOL (Model JSM-1) scanning electron microscope. The instrument had a 45° inclined sample stage and was operated at 25 kV with a specimen current near 3×10^{-11} amp.

RESULTS

In most of the cultures examined under the phase contrast microscope ciliated ependymal cells are present and easily recognizable because of their ciliary activity. The number could vary from a few to many in a well developed epithelial-like sheet. Often ciliated ependymal cells are arranged in rosettes, pools or elongated double rows with the cilia directed toward the center of the pools or rosettes (9). The rapid wipe-like ciliary beatings are coordinated sometimes creating micro-currents in a particular direction in the surrounding medium. This was demonstrated in the directed flow of cellular debris best seen in pools or rosettes.

In cultures where the ciliary activity was arrested by ethanol, or 10% formalin in balanced salt solution, the distribution of these processes was studied with the phase contrast microscope. They could be present from a few to a large number per cell. Very often they were arranged in bundles, so that they appeared as whirl-like structures under the microscope (fig. 1 b).

Detailed examination with the scanning electron microscope was limited to ciliated ependymal cells, that had their ciliated surfaces oriented away from the coverslip toward the medium, since they are best suited to this kind of study.

Compared to the flat image seen in the phase contrast microscope, the scanning electron microscope reveals a detailed three-dimensional view of the surface morphology of the ependymal cells. The mosaic picture in fig. 2 is a representative area of ependymal cells arranged in an epithelial-like pattern where the outline of many of the cells is prominent.

There was a great difference in distribution and number of cilia from cell to cell. The cilia were often arranged in bundles (figs 1 b, 2). Characteristic bud-like structures, representing the early stages of ciliary development, were regularly seen on the ependymal cell surface (fig. 2). Each bud usually contained several ciliary processes (fig. 3).

DISCUSSION

It is well documented that ependymal cells in tissue culture will develop cilia with coordinated beating (8, 9, 10, 14, 17). The microcurrent created by this activity across the ependymal surface was in accordance to our observations. In vivo this mechanism is thought to be important for the circulation of the ventricular fluid (7, 11, 20). The application of the tissue culture technique is most valuable, since the same cells observed in the living state can be prepared for comparative studies of the three-dimensional surface morphology in the scanning electron microscope.

Observations in the transmission electron microscope of the ependymal histogenesis from different animals have demonstrated

that the cilia originate from the centrioles, appearing first as buds on the ventricular surface, and are fully developed at the time of birth (18, 19). In adult animals the cilia are unevenly distributed and the apical surface is rich in minor irregular folds (6, 11). In some cases where the tissue is sectioned parallel to the ventricular surface numerous basal bodies appear in clusters (6), which indicates the possibility that many cilia can sprout simultaneously in groups, and when fully developed appear in bundles on the cell surface. This is clearly confirmed by our observations.

However, further improvements of the preparative technique seem necessary. Artefacts caused by air-drying are obvious. The slender ciliary processes collapse onto the cell surface, and have a tendency to stick together (2). Freeze drying of the specimen certainly would have retained a better spatial distribution of the cilia, but was not used because of the abundance of cell fractures associated with this technique (5). On the other hand future application of the critical point drying method (1) seems most promising, since it has the ability to preserve the life-like pattern of the surface processes with a minimum of artefacts (5).

The in vitro system presented here seems ideal for comparative optical and scanning electron microscopy studies of ciliary genesis and behaviour under different experimental conditions. In addition the investigation of the same cell cultures in the transmission electron microscope would be most important for the interpretation of the fine structural surface morphology.

We thank Prof. Cornelius A. Tobias for his helpful discussions and his financial support. The valuable advice of Dr. T. L. Hayes in the operation of the scanning electron microscope is gratefully acknowledged. Special thanks are expressed to Miss Roseanne Stevens for her great help in the preparation of the cultures for these experiments.

This research was partly supported by the Southwest Center for Advanced Studies, Dallas, Texas, partly by University of California, Lawrence Radiation Laboratory, Berkeley, California, and partly by the U. S. National Aeronautics and Space Administration (NASA P/O # W 12, 972 task 6 to the U. S. Atomic Energy Commission for work at Lawrence Radiation Laboratory at Berkeley, California).

REFERENCES

1. Andersson, TF, Trans NY acad sci ser II 13 (1951) 130
2. Barber, VC & Boyde, A, Z zellforsch 84 (1968) 269
3. Batzdorf, U & Pokress, SM, J neuropath exptl neurol 27 (1968) 333
4. Bornstein, MB, Lab invest 7 (1958) 134
5. Boyde, A & Wood, C, J microscopy 90 (1969) 221
6. Brightman, MW & Palay, SL, J cell biol 19 (1963) 415
7. Cathcart, RS III & Worthington, WC jr, Anat rec 148 (1964) 269
8. Hild, W, Z zellforsch 46 (1957) 259
9. Hild, W, Takenaka, T & Walker, F. Exp neurol 11 (1965) 493
10. Hogue, MJ, Anat rec 99 (1947) 523
11. Kohno, K & Usui, T, Bull tokyo med dent univ 13 (1966) 381
12. Konno, I & Shiotani, Y, Fol psychiatr et neur jap 10 (1956) 1
13. Millonig, G, J appl physiol 32 (1961) 1637
14. Nakai, J & Okamoto, M, Morphology of neuroglia (ed J Nakai) p. 65. Igaku Shoin Ltd, Tokyo & Charles C. Thomas, Springfield (1963).
15. Purkinje, J, Müller's arch anat u physiol 3 (1836) 289
16. Sabatini, DD, Bensch, K & Barrnett, RJ, J cell biol 17 (1963) 19
17. Singer, I & Goodman, SJ, Exp cell res 43 (1966) 367
18. Sotelo, JR & Trujillo-Centóz, O, Z zellforsch 49 (1958) 1
19. Tennyson, VM & Pappas, GD, Z zellforsch 56 (1962) 595
20. Worthington, WC jr & Cathcart, RS III, Science 139 (1963) 221

FIGURE LEGENDS

Fig. 1a — Phase contrast picture of ependymal cells forming an epithelial-like sheet in the outgrowth a 20 days old tissue culture of rat cerebellum. Note the distinct outline of some of the ependymal cells (arrows). The spherical cell (M) is a macrophage.

Fig. 1b — Phase contrast picture demonstrating the ciliary processes (arrows) on top of the ependymal cells from the same area as in fig. 1a. The rapid ciliary beating had been arrested by adding 10% formalin in balanced salt solution to the medium.

Fig. 2 — A representative mosaic scanning electron micrograph from a similar area to the one shown in fig. 1. Note the great variation in distribution and number of cilia from cell to cell. Ciliary arrangement in bundles (B) and characteristic bud-like structures of cilia in development (arrows) are frequent. Due to air-drying of the specimen the processes have a tendency to stick together and have collapsed onto the cell surface.

Fig. 3 — High magnification of a ciliary bud, showing several cilia sprouting simultaneously

I am sorry that no figures are included. Will be sent to you when available.

A. Mamoon

WRITE IT – DON'T SAY IT

TO ~~Baird Whaley~~ Please UCRL # for me

RE: "The Cilia on Cultured Ependymal Cells Examined by Scanning Electron Microscopy" by Dalen, Schlapfer and Mamoon 6-16

~~Regarding the order for reprints from EXPERIMENTAL CELL RESEARCH, we find there is no UCRL number for the paper. Could Dr. Mamoon send us a copy of the manuscript which we could process?~~

Thanks UCRL-20845 assigned 7-15-71

10-7-71

TBW

FROM Mabel Smith

DATE June 4, 1971

RL-2447(REV.4/62)