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On Arachnoid Villi and Meningiomas: Functional Implication of Ultrastructure, Cell Adhesion Mechanisms, and Extracellular Matrix Composition

Tetsumori YAMASHIMA

Department of Neurosurgery, Kanazawa University School of Medicine, Japan

Arachnoid villi or granulations are small projections of the arachnoid barrier layer into the venous sinus and its major tributaries. They are closely related to the absorption of cerebrospinal fluid, and are widely accepted to be the origin of human meningiomas. Arachnoid villi and meningiomas show a number of similarities in ultrastructure, cell adhesion mechanisms, and extracellular matrix composition. Ultrastructurally, both arachnoid and meningioma cells are characterized by interdigitations connected with junctional complexes, and extracellular cisterns related to the fluid transport. Extracellular cisterns and the intercellular space reveal abundant membrane-derived multilamellar phospholipids when a conventional ultrastructural fixative supplemented with tannic acid is used. Both arachnoid and meningioma cells are connected by Ca^{2+}-dependent adhesion molecules: epithelial-cadherins which are concentrated at the adherens junctions. Membrane-cytoskeleton interactions by means of merlin and α-catenin molecules are thought to be crucial in signal transduction resulting in contact inhibition of cell growth in normal arachnoid cells. Impairment of these molecules might be related to meningiomagenesis. Glutathione-independent prostaglandin D_2 synthase [EC 5.3.99.2] responsible for the biosynthesis of prostaglandin D_2 in the central nervous system is also consistently expressed in human arachnoid villi and meningiomas. The multilamellar phospholipids are conceivably related to this arachidonate metabolism. (Pathology Oncology Research Vol 2, No3, 144–149, 1996)

Key words: arachnoid villus, meningioma, E-cadherin, prostaglandin D_2 synthesis, multilamellar phospholipids

Introduction

Since Weed's classic work,[43] many physiological and morphologic studies have been made on arachnoid villi or granulations in experimental animals.[1,4-6,10,16,30,35,36,44-46] These works have focused mainly on the drainage mechanisms of the cerebrospinal fluid (CSF) and particulate matter across the endothelium. However, there are considerable species variations in size, shape and structure of arachnoid villi, and the drainage mechanisms of the CSF in the human are still obscure. Arachnoid villi are known to be much larger and more complicated in the human[14,34,39,47-50,53] than in experimental animals.[1,4-6,10,16,30,35,36,44-46]

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Correspondence: Tetsumori Yamashima, M.D., Ph.D.; Department of Neurosurgery, Kanazawa University School of Medicine, Takaramachi 13-1, Kanazawa City 920, Japan, Tel: (81) (762)-628151; Fax: (81) (762)-344262

Human arachnoid villus[14,34,39,47-50,53] is a protrusion of arachnoid barrier layer through the dural wall of lateral lacunae or the venous sinus into the venous lumen. Most of the villus surface is covered with endothelial lining cells on top of thin fibrous tissue similar to the surrounding sinus endothelium and dura mater, respectively.[39,48-50] Accordingly, studying the features of both the arachnoid barrier layer and the endothelial lining cells might be important in elucidating the mechanisms involved in CSF absorption.

It is widely accepted that meningiomas are derived from arachnoid cell clusters capping arachnoid villi. In 1831, Bright[2] recognized for the first time the similarities between meningioma cells and the cells within arachnoid villi. The currently-favored concept that meningiomas arise from arachnoid cells were later proposed independently by Cleland[3] and Robin.[24] In 1902, Schmidt[28] confirmed histological similarities between normal arachnoid villi and meningiomas which strongly supported the derivation of these tumors from arachnoid cells.

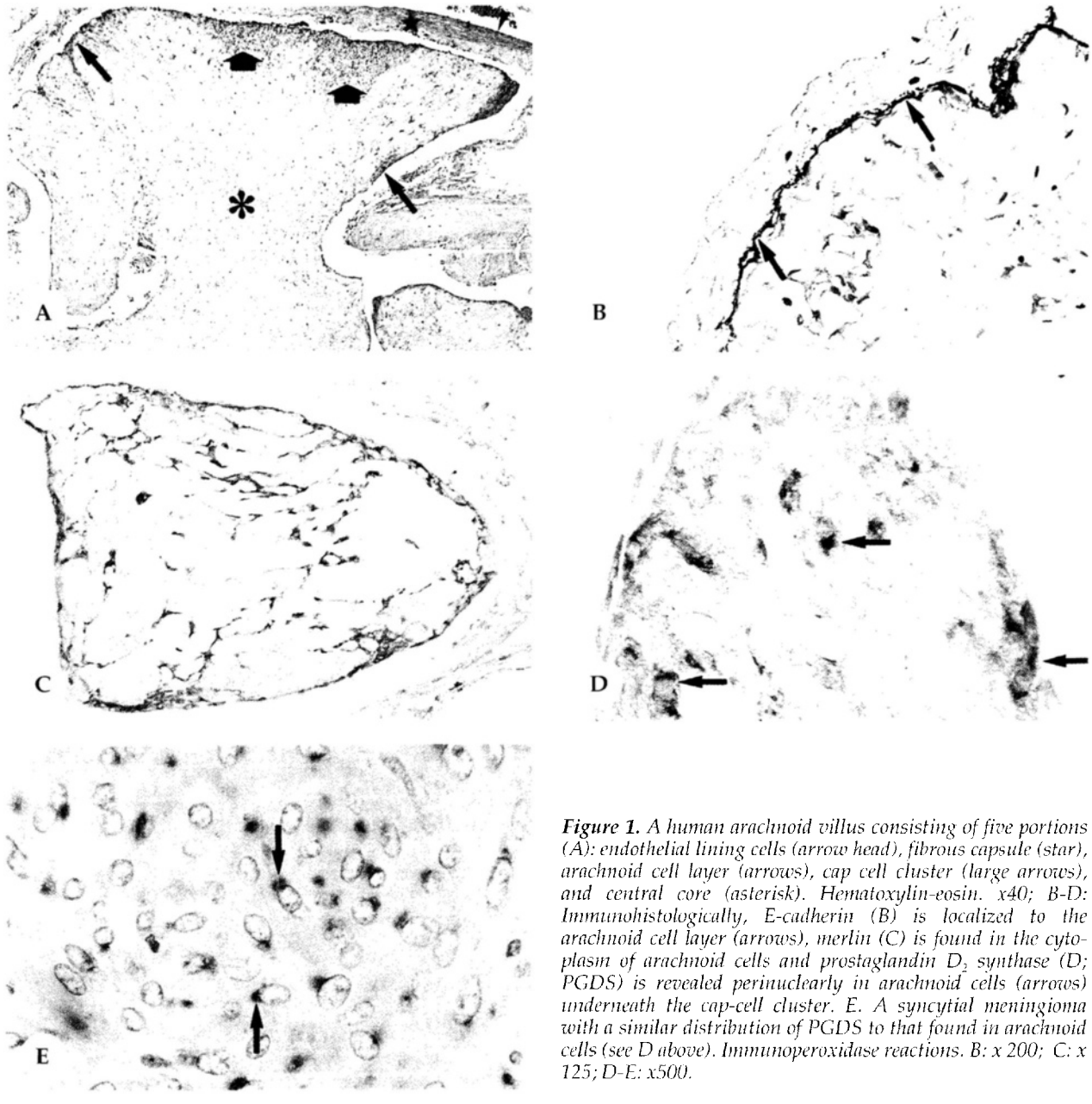

Figure 1. *A human arachnoid villus consisting of five portions (A): endothelial lining cells (arrow head), fibrous capsule (star), arachnoid cell layer (arrows), cap cell cluster (large arrows), and central core (asterisk). Hematoxylin-eosin. x40; B-D: Immunohistologically, E-cadherin (B) is localized to the arachnoid cell layer (arrows), merlin (C) is found in the cytoplasm of arachnoid cells and prostaglandin D_2 synthase (D; PGDS) is revealed perinuclearly in arachnoid cells (arrows) underneath the cap-cell cluster. E. A syncytial meningioma with a similar distribution of PGDS to that found in arachnoid cells (see D above). Immunoperoxidase reactions. B: x 200; C: x 125; D-E: x500.*

Meningiomas constitute approximately 20% of all intracranial tumors.[23] Despite this frequency, the etiology and pathobiology of these tumors are still largely unknown. It is widely accepted that meningiomas of diverse histologic appearance and biologic properties commonly show a loss of a part of chromosome.[2213,23,29,58]

As normal arachnoid cells are thought to have a low rate of cell division, the induction of cell division due to this gene mutation might be an important early step in the transformation of these cells. By considering both the mechanisms of CSF absorption and tumorigenesis, this paper is to review on human arachnoid villi and meningiomas with particular attention paid to the ultrastructure, cell adhesion mechanisms, and prostaglandin D_2 synthase expression.

Functional implications of ultrastructure

Human arachnoid villi basically comprise five portions: endothelial lining cells, fibrous capsule, arachnoid cell layer, cap cell cluster and central core[14,48-50] (*Fig.1A*). The endothelial cells, which play a crucial role in the absorption of CSF, ultrastructurally show numerous micropinocytotic vesicles, intracytoplasmic vacuoles, and microvillous projec-

tions.[14,48-50] The neighboring endothelial cells are connected to each other by tight junctions. Flattened cells quite similar to dural border cells[18,27] are arranged in tiers in the fibrous capsule, and are intermingled with connective tissue fibers.

The arachnoid cell layer of the villus is the continuation of the arachnoid barrier layer surrounding the adjacent subarachnoid space. This is thickened in places to form cap cell clusters which often contain concentric calcified organelles called psammoma bodies. The normal arachnoid cell layer is characterized by numerous extracellular cisterns[14,48-50] which appear electron-optically to be empty, or contain fine granular or fuzzy material and multilamellar phospholipids (*Fig.2A-B*). These extracellular cisterns form outflow channels for CSF from the central core into the venous lumen. In the cap cell cluster, polygonal arachnoid cells are tightly juxtaposed and linked by a series of junctional devices. The central core is in direct continuity with the cranial subarachnoid space, where a loose network of arachnoid cell processes traverses distended extracellular cisterns.

Meningioma cells have less junctional devices than those of arachnoid cells.[53] Meningioma cells otherwise show many ultrastructural features in common with non-neoplastic arachnoid cells within arachnoid villi.[53] The most typical ultrastructural features of arachnoid cells such as conspicuous interdigitations, numerous junctional complexes and cytoplasmic filaments are also present in meningioma cells. At the ultrastructural level syncytial areas of meningiomas can hardly be differentiated from the cap cell cluster of arachnoid villi.[14,48-50] Elongated cells in the fibroblastic type or in the fibrous septum of the syncytial type of meningiomas are quite similar to those in the fibrous capsule of the arachnoid villus. Psammoma bodies are also often formed within meningocytic whorls.

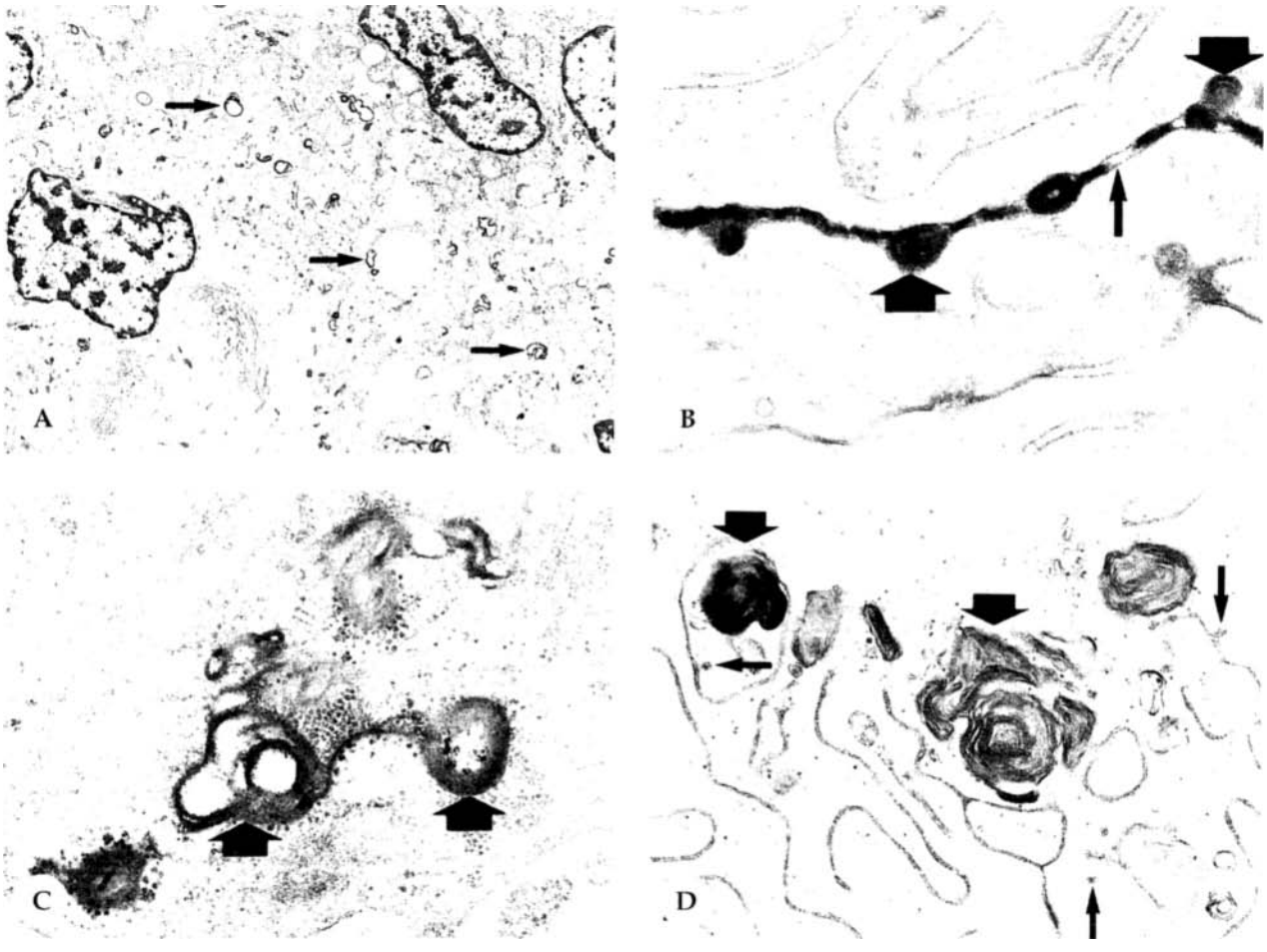

Figure 2. *Ultrastructural details of a human arachnoid villus (A-B) and meningiomas (C-D) fixed in a conventional fixative containing 0.1% tannic acid. A-B: Note the abundance of multilamellar phospholipids in the extracellular cisterns (arrows in A) and intercellular space (thick arrows in B). The outermost lamella is in direct continuity with the plasma membrane (thin arrow in B). C-D: Meningiomas also exhibit fuzzy material (arrows in C), micropinocytotic vesicles (thin arrows in D) as well as multilamellar phopholipids (thick arrows in D) both intra- and extracellularly. A: Uranyl acetate and lead citrate counterstaining. B–D: Lead citrate counterstaining. A: x 3,500; B: 40.000; C: 25,000; D: 20,000.*

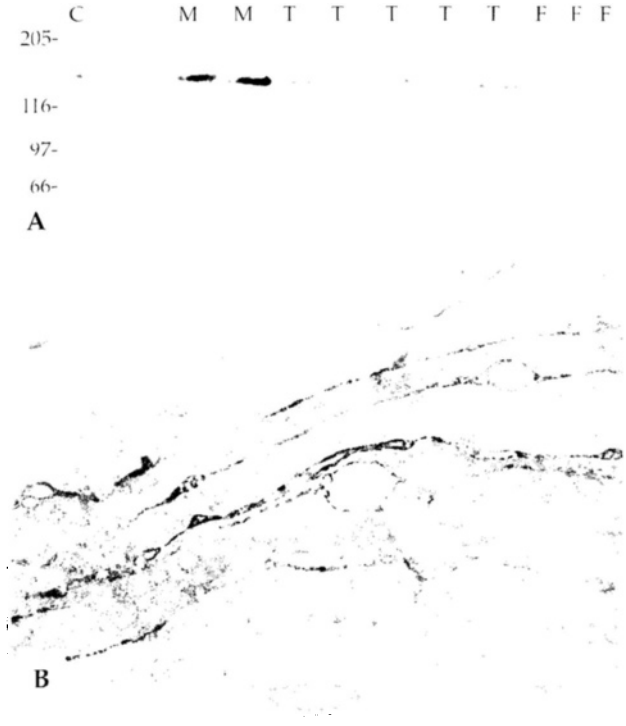

Figure 3. A: Western blot analysis for E-cadherin shows bands at ~124 kDa in the control colon epithelium (lane C), syncytial meningiomas (lanes M), and transitional meningiomas (lanes T). Fibroblastic meningiomas (lanes F) gave negligible staining. B: The same E-cadherin specific antibody stains the cell boundaries in a syncytial meningioma. Ultrastructural immunoperoxidase reaction, counterstained with osmium tetroxide and uranyl acetate. x 9,000

Cell adhesion mechanisms and tumorigenesis

Epithelial (E)-cadherin,[32,55,56] a transmembrane glycoprotein responsible for Ca^{2+}-dependent cell adhesion, is consistently found between arachnoid cells in human arachnoid villi.[53,54] It is distributed especially in the arachnoid cell layer containing numerous extracellular cisterns (*Fig.1B*) rather than in the cap cell cluster with less cisterns.[53] The primary role of E-cadherin is to passively bind adjacent arachnoid cells together in arachnoid villi. The resulting strong adhesive interactions conceivably allow flexible conformational changes of individual cells and extracellular cisterns during the absorption of CSF.[53]

Meningiomas show *en block* expansive growth by usually compressing and rarely invading the adjacent brain. Accordingly, it is suggested that meningioma cells proliferate with maintaining tight cell-cell adhesion. In the syncytial and transitional types of meningiomas, immunoblotting discloses consistent E-cadherin expression (*Fig.3A*). Ultrastructurally, E-cadherin is localized at the cell border[34] where it is concentrated at the adherens junctions (*Fig.3B*).

The cadherin-dependent cell adhesion is known to be mediated by a group of cytoplasmic proteins such as α-catenin, moesin, ezrin, radixin, etc.[7,17,26,31,38] α-catenin is consistently identified in meningiomas by immunoblotting and immunohistochemistry (data not shown). Merlin, a radixin-like protein encoded by the neurofibromatosis type-2 tumor suppressor gene, as well as moesin and ezrin were recently cloned.[25,37] These proteins are localized underneath the plasma membrane to link the membrane protein E-cadherin to the cytoskeletal microfilament.

With immunoblotting and/or immunohistochemistry merlin expression has been found in all arachnoid villi and meningiomas studied. Meningioma cells usually show negligible or weak immunostaining in their cytoplasm as compared to normal arachnoid cells (*Fig.1C*). Merlin is considered to act as a tumor suppressor[33] by linking cell membrane proteins and actin filaments to each other which results in the normal contact inhibition of cell growth. Impairment and mis-localization of mutated merlin gene products might be related to meningioma-genesis.

Multilamellar phospholipids and prostaglandin D_2 synthase production

When prefixed with glutaraldehyde containing 0.1-1% tannic acid and postfixed with osmium tetroxide, both arachnoid and meningioma cells exhibit abundant fingerprint-like multilamellar bodies[51,52] (*Fig.2A-D*). These are found intracytoplasmically as well as in the intercellular space and extracellular cisterns. The number of lamellae in a single multilamellar body ranges from 3 to 20, with a distance of ~5.0 nm between the lamellae. The electron-dense zone measures 3.0 nm while the electron-lucent zone is 2.0 nm. The outermost lamella sometimes shows a direct continuity with the cytoplasmic or mitochondrial membranes of these cells. Furthermore, tannic acid is known to interact with the choline component of phosphatidyl choline,[11,12] which is a major component in multilamellar bodies and is also found in the cell membrane. These suggest that multilamellar bodies are most probably formed from cell membrane-derived phospholipids. Interestingly, these bodies are quite similar ultrastructurally to the deposits of human pulmonary surfactant.[11,12]

Glutathione-independent prostaglandin D_2 synthase (PGDS) is an enzyme [EC 5.3.99.2] responsible for the biosynthesis of prostaglandin D_2 in the central nervous system.[40] PGDS has recently been identified in the rat leptomeninges[41] as well as in human arachnoid villi and meningiomas.[51] In arachnoid villi, PGDS was predominantly seen in the perinuclear cytoplasm of arachnoid cells underneath the cap cell cluster (*Fig.1D*). Meningioma cells show similar PGDS reaction in their perinuclear region and variable extent of diffuse immunostaining in their cytoplasm (*Fig.1E*). RT-PCR reveals an amplified PGDS gene expression in meningiomas, regardless of their histological subtype (*Fig.4*). Therefore, it is sugges-

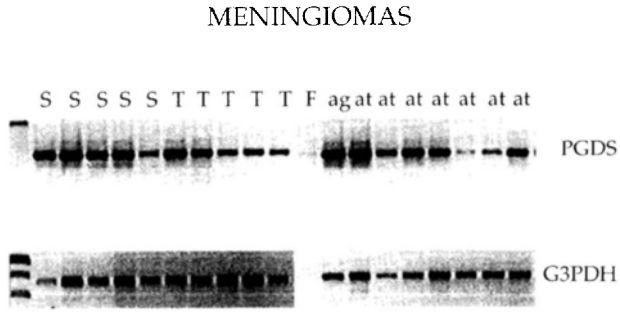

Figure 4. RT-PCR analysis of prostaglandin D_2 synthase gene expression in 19 human meningiomas as compared to the internal control G3PDH gene. S: syncytial, T: transitional, F: fibroblastic, ag: angiomatous, and at: atypical meningiomas.

ted that human arachnoid and meningioma cells can produce PGDS and synthesize prostaglandin D_2.

In 1973, Olsson et al.[20] demonstrated that approximately 40% of ^{125}I-labeled β-trace, which was recently demonstrated to be identical with PGDS,[8,15,42,57] is excreted in the urine if injected into the lumbar subarachnoid space of human volunteers. In the CSF of healthy men 8μg/ml PGDS was detected. In contrast, in the CSF of patients suffering from hydrocephalus, extremely low levels (<0.4μg/ml) of PGDS were found.[9] A homology search on the primary structure of this protein revealed that PGDS is a member of the lipocalin superfamily,[19,21] a group of proteins consisting of a variety of secretory proteins that bind and transport small lipophilic molecules.[22] It is thought that PGD_2 is synthesized by PGDS from multilamellar bodies through arachidonate cascades and PGDS as a carrier protein may contribute to the absorption of CSF-related substances from arachnoid villi into the venous sinus. If so, it is quite reasonable for hydrocephalus patients to show significantly less PGDS levels than healthy individuals.

Conclusions

Concerning their ultrastructure, cell adhesion mechanism, and synthesis of multilamellar phospholipids or PGD_2, meningioma cells show remarkable similarities with normal arachnoid cells. The less organized ultrastructure, reduced merlin expression and genetic mutations result in an impaired differentiation and function in meningiomas. Further studying the consequences of neoplastic transformation may assist in elucidating the molecular mechanisms involved in the function of normal arachnoid villi.

References

1. *Alksne JF and Lovings ET*: Functional ultrastructure of the arachnoid villus. Arch Neurol 27:371-377, 1972.
2. *Bright R:* Reports of medical cases, selected with a view of illustrating the symptoms and cure of disease by a reference to morbid anatomy. Longman, London. 2:p342, 1831.
3. *Cleland J:* Description of two tumours adherent to the deep surface of the dura-mater. Glasgow Med J 11:148-159, 1864.
4. *Gomez DG, Potts DG, Deonarine V and Reilly KF:* Effects of pressure gradient changes on the morphology of arachnoid villi and granulations of the monkey. Lab Invest 28:648-657, 1973.
5. *Gomez DG, Potts DG and Deonarine V:* Arachnoid granulations of the sheep: Structural and ultrastructural changes with varying pressure differences. Arch Neurol 30:169-175, 1974.
6. *Gomez DG and Potts DG:* The surface characteristics of arachnoid granulations: A scanning electron microscopic study. Arch Neurol 31:88-93, 1974.
7. *Hirano S, Kimoto N, Shimoyama Y, Hirohashi S et al:* Identification of a neural-catenin as a key regulator of cadherin function and multicellular organization. Cell, 70:293-301, 1992.
8. *Hoffmann A, Conradt HS, Gross G, et al:* Purification and chemical characterization of β-trace protein from human cerebrospinal fluid: its identification as prostaglandin D synthase. J Neurochem 61:451-456, 1993.
9. *Hoffmann A, Grabenhorst E, Nimtz M and Conradt HS:* Characterization of human β-trace protein from natural body fluids, from patients and from recombinant sources. Prostaglandins 51:284, 1996.
10. *Jayatilaka ADP:* An electron microscopic study of sheep arachnoid granulations. J Anat 99:635-649, 1965.
11. *Kalina M and Pease DC:* The preservation of ultrastructure in saturated phosphatidyl cholines by tannic acid in model systems and type II pneumocytes. J Cell Biol 74:726-741, 1977.
12. *Kalina M and Pease DC*: The probable role of phosphatidyl cholines in the tannic acid enhancement of cytomembrane electron contrast. J Cell Biol 74:742-746, 1977.
13. *Katsuyama J, Papenhausen PR, Herz F, et al:* Chromosome abnormalities in meningiomas. Cancer Genet Cytogenet 22:63-68, 1986.
14. *Kida S, Yamashima T, Kubota T, Ito H, et al:* A light and electron microscopic and immunohistochemical study of human arachnoid villi. J. Neurosurg. 69:429-435, 1988.
15. *Kuruvilla AP, Hochwald GM, Ghiso J, et al*: Isolation and amino terminal sequence of β-trace, a novel protein from human cerebrospinal fluid. Brain Res 565:337-340, 1991.
16. *Levine JE, Povlishock JT and Becker DP:* The morphological correlates of primate cerebrospinal fluid absorption. Brain Res 241:31-41, 1982.
17. *Luna EJ and Hitt AL:* Cytoskeleton-plasma membrane interactions. Science 258:955-964, 1992.
18. *Nabeshima S, Reese TS, Landis DMD and Brightman MW:* Junctions in the meninges and marginal glia. J Comp Neurol 164:127-170, 1975.
19. *Nagata A, Suzuki Y, Igarashi M, et al:* Human brain prostaglandin D synthase has been evolutionarily differentiated from lipophilic-ligand carrier proteins. Pro Natl Acad Sci USA 88:4020-4024, 1991.
20. *Olsson JE, Link H and Nosslin B:* Metabolic studies on [^{125}I] beta-trace protein within the nervous system. J Neurochem 21:1153-1159, 1973.
21. *Peitsch MC and Boguski MS:* The first lipocalin with enzymatic activity. Trends Biochem Sci 16:363, 1991.
22. *Pervaiz S and Brew K:* Homology and structure-function correlations between α-acid glycoprotein and serum retinol-binding protein and its relatives. FASEB J 1:209-214, 1987.
23. *Rachlin JR and Rosenblum ML:* Etiology and biology of meningiomas. In: Meningiomas. (Ed: Al-Mefty O), Raven Press, 1991, pp. 27-35
24. *Robin C:* Recherches anatomiques sur l'epithelioma des sereuses. J Anat 6:239-288, 1869.
25. *Rouleau GA, Merel P, Lutchman M, et al:* Alteration in a new gene encoding a putative membrane-organizing protein causes neurofibromatosis type 2. Nature 363:515-521, 1993.

26. *Sato N, Funayama N, Nagafuchi A, et al:* A gene family consisting of ezrin, radixin and moesin. Its specific localization at actin filament/plasma membrane association sites. J Cell Sci 103:131-143, 1992.
27. *Schachenmayr W and Friede RL:* The origin of subdural neomembranes. I. Fine structure of the dura-arachnoid interface in the man. Am J Pathol 92:53-68, 1978.
28. *Schmidt MB:* Ueber die Pacchioni'schen granulationen und ihr verhaltniss zu den sarcomen und psammomen der dura mater. Virchows Arch [Pathol Anat] 170:429-464, 1902.
29. *Seizinger BR, de la Monte S, Atkins L, et al:* Molecular genetic approach to human meningioma: loss of genes on chromosome 22. Proc Natl Acad Sci USA 84:5419-5423, 1987.
30. *Shabo AL and Maxwell DS:* The morphology of the arachnoid villi: A light and electron microscopic study in the monkey. J Neurosurg 29:451-463, 1968.
31. *Shimoyama Y, Nagafuchi A, Fujita S, et al:* Cadherin dysfunction in a human cancer cell line: Possible involvement of loss of α-catenin expression in reduced cell-cell adhesiveness. Cancer Res., 52:5770-5774, 1992.
32. *Takeichi M:* The cadherins: cell-cell adhesion molecules controlling animal morphogenesis. Development (Camb.) 102:639-655, 1988.
33. *Takeshima H, Izawa I, Lee PSY, et al:* Detection of cellular proteins that interact with the NF2 tumorsuppressor gene product. Oncogene 1994; 9:2135-2144, 1994
34. *Tohma Y, Yamashima T and Yamashita J:* Immunohistochemical localization of cell adhesion molecule epithelial cadherin in human arachnoid villi and meningiomas. Cancer Res 52:1981-1987, 1992.
35. *Tripathi BJ and Tripathi RC:* Vacuolar transcellular channels as a drainage pathway for cerebrospinal fluid. J Physiol (Lond) 239:195-206, 1974.
36. *Tripathi R:* Tracing the bulk outflow route of cerebrospinal fluid by transmission and scanning electron microscopy. Brain Res 80: 503-506, 1974.
37. *Trofatter JA, MacCollin MM, Rutter JL, et al:* A novel moesin-, ezrin-, radixin-like gene is a candidate for the neurofibromatosis 2 tumor suppressor. Cell 72:791-800, 1993.
38. *Tsukita S, Tsukita S, Nagafuchi A et al:* Molecular linkage between cadherins and actin filaments in cell-cell adherens junctions. Current Opin Cell Biol 4:834-839, 1992.
39. *Upton ML and Weller RO:* The morphology of cerebrospinal fluid drainage pathways in human arachnoid granulations. J Neurosurg 63:867-875, 1985.
40. *Urade Y, Fujimoto N and Hayaishi O:* Purification and characterization of rat brain prostaglandin D synthase. J Biol Chem 260:12410-12415, 1985.
41. *Urade Y, Kitahama K, Ohishi H, et al:* Dominant expression of mRNA for prostaglandin D synthase in leptomeninges, choroid plexus, and oligodendrocytes of the adult rat brain. Pro Natl Acad Sci USA 90:9070-9074, 1993.
42. *Watanabe K, Urade Y, Mäder M, et al:* Identification of β-trace as prostaglandin D synthase. Biochem Biophys Res Comm 203:1110-1116, 1994.
43. *Weed LH:* Studies on cerebro-spinal fluid: III. The pathways of escape from the subarachnoid spaces with particular reference to the arachnoid villi. J Med Res 31:51-91, 1914.
44. *Weed LH:* The absorption of cerebrospinal fluid into the venous system. Am J Anat 31:191-221, 1922.
45. *Welch K and Friedman V:* The cerebrospinal fluid valves. Brain 83:454-469, 1960.
46. *Welch K and Pollay M:* Perfusion of particles through arachnoid villi of the monkey. Am J Physiol 201:651-654, 1961.
47. *Yamashima T, Kida S, Kubota T, et al:* The origin of psammoma bodies in the human arachnoid villi. Acta Neuropathol (Berl) 71:19-25, 1986
48. *Yamashima T:* Ultrastructural study of the final cerebrospinal fluid pathway in human arachnoid villi. Brain Res. 384:68-76, 1986.
49. *Yamashima T:* Functional ultrastructure of cerebrospinal fluid drainage channels in human arachnoid villi. Neurosurg. 22:633-641, 1988.
50. *Yamashima T, Kida S and Yamamoto S:* Ultrastructural comparison of arachnoid villi and meningiomas in man. Mod Pathol, 1:224-234, 1988.
51. *Yamashima T, Yamashita J and Takahashi T:* Pulmonary surfactant-like multilamellar bodies in human arachnoid villi. J Himforsch 31:795-802, 1990.
52. *Yamashima T and Yamashita J:* Histological, ultrastructural and chromatographical discrimination of phospholipids in meningiomas. Acta Neuropathol (Berl) 80:255-259, 1990.
53. *Yamashima T, Tohma Y and Yamashita J:* Expression of cell adhesion molecule E-cadherin in human arachnoid villi. J Neurosurg, 77:749-756,1992.
54. *Yamashima T, Sakuda K, Tohma Y, et al:* Prostaglandin D synthase in human arachnoid, arachnoid villi and meningiomas. Prostaglandins 51:287-288, 1996
55. *Yoshida C and Takeichi M:* Teratocarcinoma cell adhesion: identification of a cell-surface protein involved in calcium-dependent cell aggregation. Cell 28:217-224, 1982.
56. *Yoshida-Noro C, Suzuki N and Takeichi M:* Molecular nature of the calcium-dependent cell-cell adhesion system in mouse teratocarcinoma and embryonic cells studied with a monoclonal antibody. Dev Biol 101:19-27, 1984.
57. *Zahn M, Mäder M, Schmidt B, et al:* Purification and N-terminal sequence of β-trace, a protein abundant in human cerebrospinal fluid. Neurosci Lett 154:93-95, 1993.
58. *Zang KD:* Cytological and cytogenetical studies on human meningioma. Cancer Genet Cytogenet 6:249-274, 1982.