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Lecture

Choroid plexus

—— with special reference to neuroprotective function ——

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Abstract

Choroid plexus (CP) produces the cerebrospinal fluid (CSF) that fills the ventricles and subarachnoidal space, and infiltrates the intercellular spaces of CNS parenchyma. CP transplantation enhances axonal outgrowth in the spinal cord lesion. Cultured choroid plexus epithelial cells (CPECs) secret neurotrophic factors into the medium. CP undergoes cytological changes in diseases such as Alzheimer and Huntingon's disease. The ischemia-injured infraction due to middle cerebral artery occlusion is suppressed by transplantation of CPECs into the CSF in the rat. Allo- or xenotransplantation of encapsulated CP has been studied for the treatment of experimental Huntington's disease. CP can be regarded as the neurotrophic center of the CNS, regulating and maintaining the normal brain function via CSF.

Key words: trophic function, nerve protection, transplantation, culture, in situ hybridization

Introduction

The choroid plexus (CP) is located in ventricles, including lateral, third and fourth ventricles. The CP is composed of a simple epithelium and underlying connective tissue. The choroid plexus epithelial cells (CPECs) are a continuation of the ventricular ependymal cells, and the connective tissue is part of pia mater covering the surface of the cerebral hemisphere. CPECs are modified ependymal cells having basal laminae at the basal side facing the pia mater. Unlike the ventricular ependymal cells having many cilia on the ventricular surface, CPECs have only occasional cilia, instead, they have numerous microvilli on the ventricular surface. CPECs contain numerous mitochondria in the cytoplasm. Numerous clear vesicles are located in the apical zone of the cytoplasm (Fig. 1).

Originally CPECs are the neural epithelium of the neural tube that produces the cerebral parenchyma in development. The neural epithelium of the neural tube changes into ependymal cells after ceasing the production of the cerebral parenchymal tissues, while that of the CP remains as a single cell layer without producing any cerebral parenchyma in development. Therefore, CPECs form a simple epithelium directly contacting with the pia mater. The pia mater at the CP is highly vascularlized, with numerous sinusoidal blood vessels beneath the CPEC basal lamina.

The CP is the tissue responsible for producing the cerebrospinal fluid (CSF). The CSF volume is 80–150 ml in human, and the volume of the newly produced CSF is approximately 500 ml per day. In rats, considering the difference in body weight, the CSF volume might be 400–750 μl, and the new production might be 2.5 ml per day. The turnover of CSF is approximately 3–4 times per day, taking 6-8 h for one turnover in rats. Younger rats take

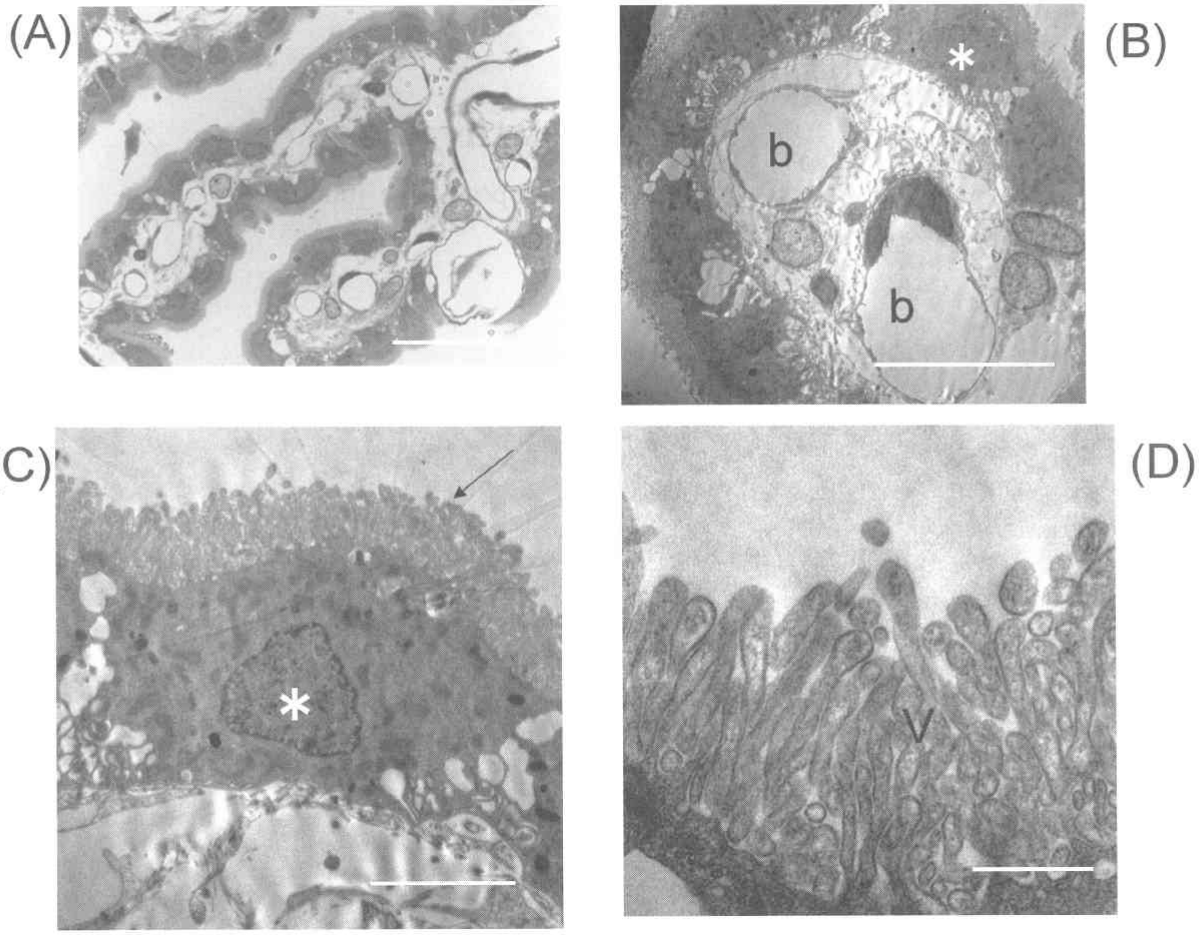

Fig. 1

(A) Choroid plexus of the rat 4th ventricle. The choroid plexus consists of epithelial cells and underlying connective tissue containing an abundance of blood vessels. The connective tissue is a continuation of pia mater covering the surface of cerebral hemisphere. Scale bar: 50 μm

(B) Low magnification of electron microscopy of choroid plexus. Cross section of a villous projection of choroid plexus. Choroid plexus epithelial cells (*) surround the connective tissue containing blood vessels (b). Scale bar: 20 μm

(C) Moderate magnification of a choroid plexus epithelial cell. The choroid plexus epithelial cell (*) has numerous microvilli (arrows) on the ventricular surface. Scale bar: 5 μm

(D) High magnification of microvilli of choroid plexus epithelial cells. These microvilli are grouped into small bundles that have a common root emerging at the epithelial cells surface. Scale bar: 1 μm

shorter time (2-3 h) for one turnover. In human, CSF production rate is 0.41 ml/min at 28 years of age, and 0.19 ml/min at 77 years (Preston et al, 2001). The CSF contains lower concentration of glucose, potassium, calcium bicarbonate and amino acid than blood. In contrast, sodium, chloride, magnesium and folate are maintained at a higher concentration than in blood. Transthyretin is one of the most abundant proteins of the CSF. The rich vasculature in the CP is needed to supply a sufficient amount of blood for the production of CSF (Serot et al. 2003).

The CP has been extensively studied from the point of view of CSF production. The CSF flows through the ventricles and fills the subarachnoidal space. Considering that the CSF infiltrates the intercellular spaces of neurons and glial cells, CSF plays a critical role in maintaining the normal and regular function of neurons and glial cells in the CNS. This indicates that the CP, as the CSF-producing organ, plays an important role in regulating the functions of neurons and glial cells. The CSF components are directly related to the conditions of the neural tissues of CNS. Bioactive molecules, which include neurotrophic factors play an important role in maintaining neural tissues and in regulating their normal functions in the CNS.

Regarding the functions of CP and CPECs, Chodobski and Szmydynger-Chodoboska, (2001) described that choroidal expression of several polypeptides is observed during brain development and in various CNS disorders, including traumatic brain injury and ischemia. It is proposed that the CP plays an integral role not only in normal brain functioning, but also in the

recovery from injury. In the same respect, Johanson and Palm (2000) described as follows: "Mammalian CPECs synthesize and secrete to regions of the cerebroventricular system many growth factors and other peptides that are of trophic benefit following injury. For example, several growth factors are upregulated in choroid plexus after ischemic and traumatic insults to the CNS. The presence of numerous types of growth factor receptors in choroid plexus allows growth factor mediation of recovery processes by autocrine and paracrine mechanisms."

In this article, we will present various kinds of CP functions in terms of neurotrophic effect in the CNS, and CP responses to changes in neurodegenerative diseases and traumatic injuries.

CPECs have a strong effect on neurite extension

First, we examined neurotrophic functions of CPECs in vivo and in vitro. The first in vivo experiment was performed in 2001. In this experiment, the CP was transplanted into the injured spinal cord of a rat (Ide et al. 2001). The CP was minced into small fragments before transplantation and engrafted into the dorsal funiculus of the spinal cord at the C2 level. Several days later, it was found that numerous regenerating axons had extended into the graft (Fig. 2). Electron microscopy and immunohistochemistry showed that growing axons were intimately associated with grafted CPECs. In order to know whether growing axons extending in the caudo-rostral direction within the lesion derived from the dorsal root or from intirinsic spinal cord neurons, immunohistochemistry for CGRP (calcitonine-gene related polypeptide) - positive fibers were performed, since CGRP is a marker for the sensory axons coming from the dorsal roots. CGRP-positive axons were contained in the growing axons, being partly in contact with engrafted CPECs. Similarly, HRP that had been injected into the sciatic nerve labeled growing axons within the lesion. Numerous axons were labeled, and some of them further extended beyond the rostral border of the lesion. This study revealed for the first time that the CP, if grafted into the spinal cord, has a unique function of enhancing axonal regeneration of sensory nerves. The findings of this study suggest that the CPECs might exert neurotrophic functions by secreting some diffusible molecules, and/or might express specific adhesion molecules on the cell surface.

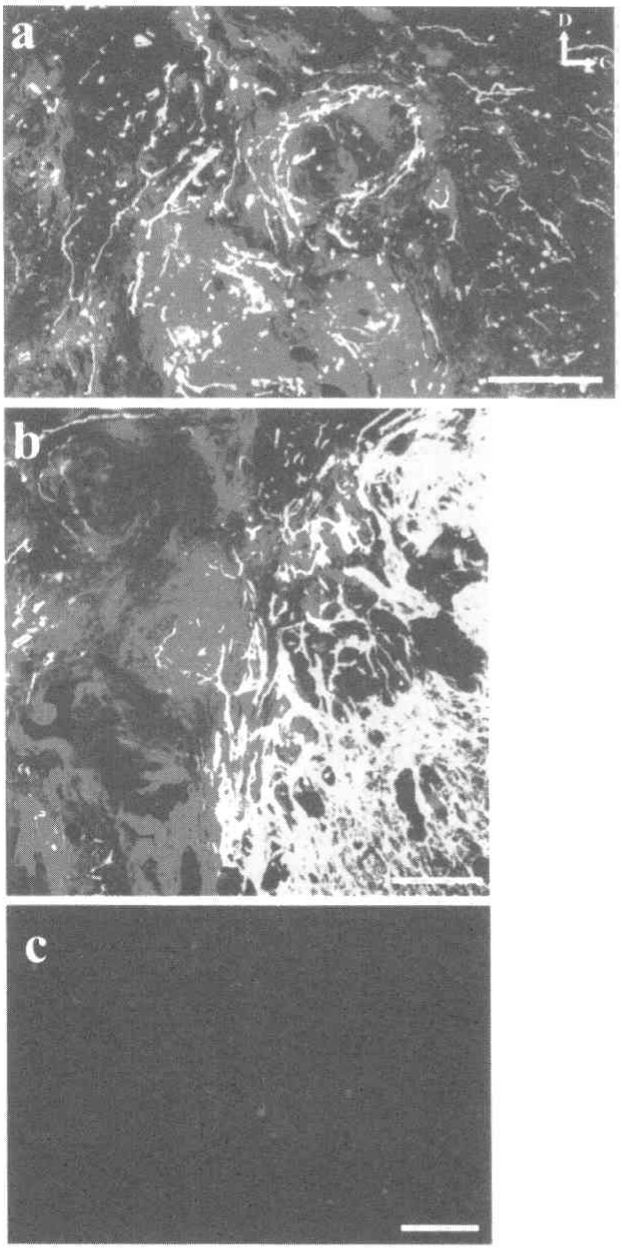

Fig. 2

(a) One month after grafting of choroid plexus into the rat cervical spinal cord. The choroid plexus was minced into small fragments before grafting. Grafted choroid plexus is faintly stained in the background, and regenerating axons are clearly seen as whitish strings. In the original micrograph, choroid plexus was stained red, while regenerating axons were green. There are numerous regenerating axons, many of which are in close association with the choroid plexus. Scale bar: 50 μm

(b) This micrograph shows the interaction of choroid plexus with astrocytes. Choroid plexus was originally stained red, and astrocytes are green. It is shown that choroid plexus are interacted with astrocytes. Scale bar: 50 μm

(c) This micrograph was obtained from the spinal cord in which freeze-treated choroid plexus was grafted. No choroid plexus remained in the spinal cord. Scale bar: 100 μm

(from Ide et al., 2001)

In order to address these problems, we next performed an in vitro experiment to examine whether the CPECs have specific adhesion molecules on the cell surface that enhance axonal outgrowth (Chakrabortty et al. 2000). CPECs of the 4th ventricle of mice were cultured in a monolayer. Neurons were isolated from the dorsal root ganglia of newborn mice, and co-cultured on the monolayer culture of CPECs. It was demonstrated that neurons from dorsal root ganglia extended many long neurites with elaborate branches on the surface of CPECs. The total length of neurites per neuron was compared with that of neurons co-cultured on astrocytes or cultured on the laminin-coated plates. The neurite length was much greater when neurons were co-cultured with CPECs than when cultured on astrocytes or on the laminin-coated plates. It was impressive to see neurons extending long neurites with branches on the surface of CPECs in vitro (Fig. 3).

Neurons used in this study were peripheral ones derived from dorsal root ganglia. Considering that the CP has influences primarily on the

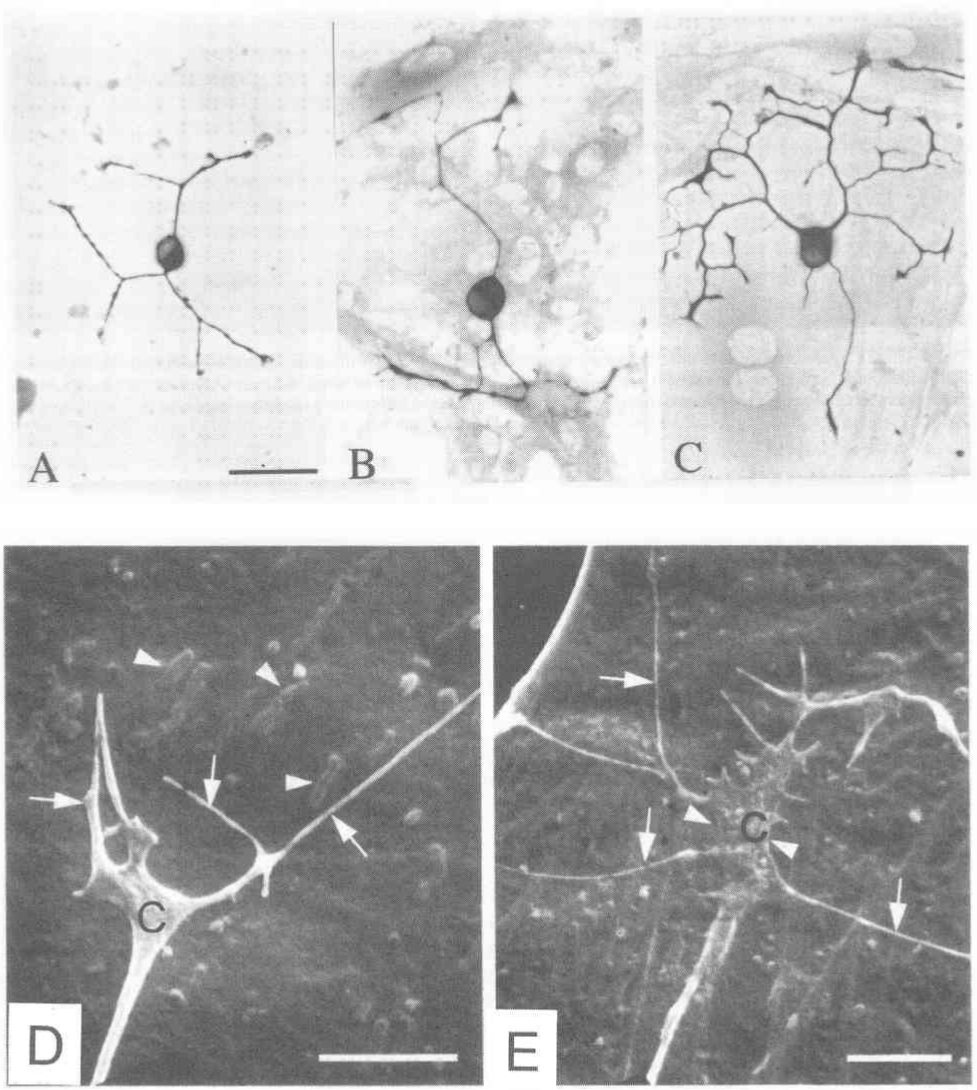

Fig. 3 Comparison of neurite outgrowth from dorsal root ganglion neurons cultured in the following different conditions

A: The culture on the laminin substrate. Neurites are relatively short and straight, and branching with clear-cut angles.

B: The culture on the astrocyte monolayer. Neurites are longer and with more branches than on the laminin substrate.

C: The culture on the choroid plexus epithelia cell monolayer. There are elaborate neurite extensions from a neuron on a choroid plexus epithelial cell. Neurites are thicker and longer with more branches than on the astrocyte monolayer. Scale bar: A-C, 50 μm

D and E: Scanning electron micrographs of neurite outgrowth from the dorsal root ganglion neurons on choroid plexus epithelial cells. The tip of growing axon has a growth cone (C) with several long filopodia (arrows). There are many microvilli (arrowheads) on the surface of the choroid plexus epithelial cells.

Scale bar: D, 6 μm; E, 5 μm

(from Chakrabortty et al., 2004)

CNS neurons, we performed an experiment similar to the one above by using hippocampal neurons. Neurons were obtained from hippocampus of embryonic day-18 mice, and co-cultured on the monolayer of CPECs. For comparison, hippocampal neurons were co-cultured with astrocytes or cultured on the poly-L-lysine (PLL)-coated dishes (Kimura et al, 2004). In this experiment, the cultured CPECs in monolayer were treated with ethanol before co-culturing with hippocampal neurons. This treatment is done to kill CPECs, and thus eliminate the influence of molecules secreted into the culture medium by living CPECs. This in vitro experiment demonstrated that CPECs have an ability to enhance the outgrowth of neurites from neurons that were attached to the surface of CPECs. It was shown that CPECs expressed N-CAM, N-cadherin, laminin, fibronection and p75 (a low affinity neurotrophin receptor) on their surface. In addition, N-cadherin is expressed on the CPECs at the site of contact between CPECs and neurites (Fig. 4). These in vitro studies show that CPECs produce specific molecules, including cell adhesion molecules, extracellular matrix components and receptors of neurotrophic molecules on the cell surface.

As the next step, we examined the effect of conditioned medium of cultured CPECs. The CP was obtained from the lateral and 4th ventricle of 4-week-old rats, and cultured in a monolayer for 12 days according to the method by Zheng and Zhao (2002). In parallel, neurons from the hippocampus were cultured for 24 hours in the

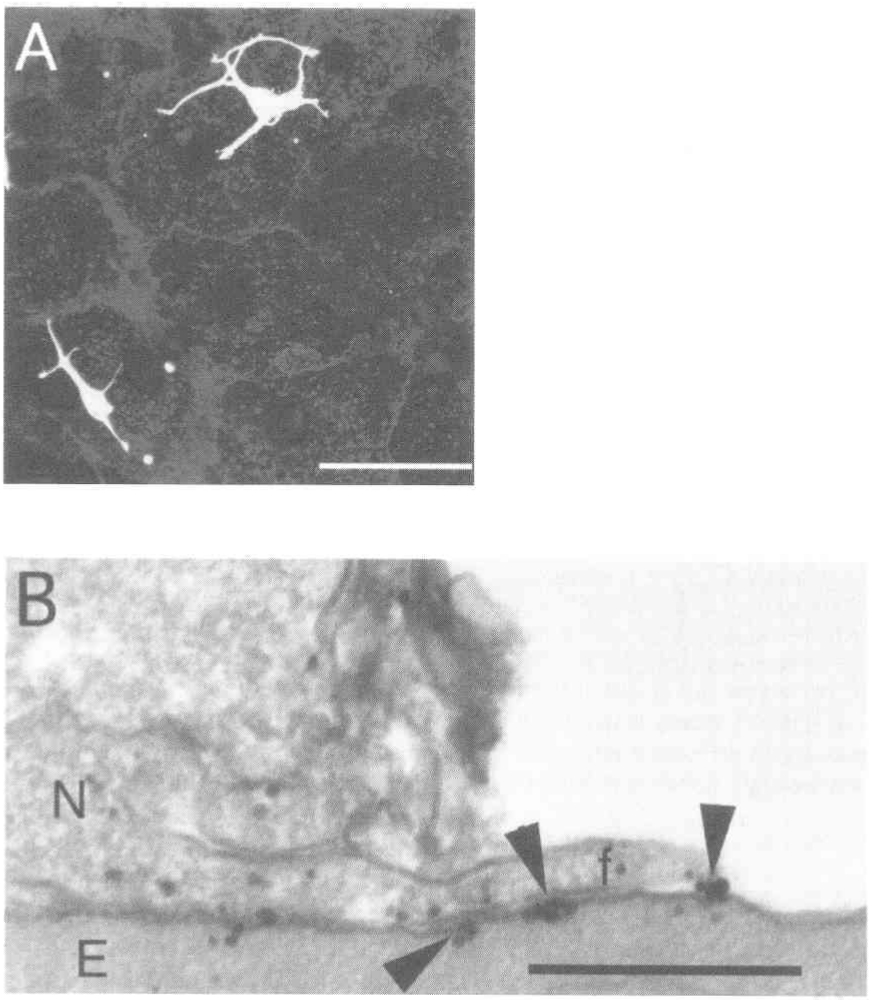

Fig. 4 Hippocampal neurons were cultured on the choroid plexus epithelial cells in rat

A: Neurons were stained for β-tubulin III, and choroid plexus epithelial cells were stained for the N-cadherin cell-cell adhesion molecule. Choroid plexus epithelial cells are seen in the background, and two neurons are seen as whitish bodies. Scale bar: 100 μm

B: Neurites and choroid plexus epithelial cells were cut perpendicular to the culture dish. Cadherin is immunohistochemically marked with gold particles. There is cadherin (arrowheads) at the attachment between neurites (N) and choroid plexus epithelial cell (E). Filopodia extend from neurite along the surface of choroid plexus epithelial cell. Scale bar: 1 μm

(from Kimura et al., 2004)

neurobasal medium (NB) or in the neurobasal supplemented with B27 (NB/B27). The conditioned medium of the CPEC culture was prepared and added to the culture of hippocampal neurons. Neurons were fixed 24 hours after treatment, and the neurite extension was measured. The longest neurite length and the individual neurite lengths were greater in the conditioned medium than in the control. This experiment demonstrated that the conditioned medium enhanced neuron survival and neurite extension (Watanabe et al. 2005). Immunohistochemistry showed that CPECs express trophic factors, including HGF, IGF-II, bFGF, and NT-4. It is considered that many kinds of trophic factors might work in a synergistic fashion for the survival of neurons and for neurite extension from neurons. However, any antibodies against these trophic factors suppressed neither the survival nor the neurite extension activity of neurons in vitro.

CPEC transplantation rescues ischemic damage of the brain

The effect of CPECs on ischemic injury in vivo was examined in the transplantation experiment, in which the middle cerebral artery (MCA) of rat was occluded to produce ischemic lesions in the rat brain, followed by injection of CPECs into the CSF via the 4th ventricle (Matsumoto et al. 2010). This experiment showed that CPEC transplantation markedly reduced the ischemic area of the brain. The occlusion of the middle cerebral artery (MCAO) was performed using a 4-0 nylon monofilament with, at the tip, a small paraffin ball approximately 0.25 mm in diameter. The monofilament was inserted from the external carotid artery (ECA) into internal carotid artery (ICA), and advanced cranially until the advancement of the filament was resisted at the origin of the MCA from the common carotid artery (CCA). In this way, the MCA was occluded with the paraffin ball located at the tip of the monofilament. Thirty minutes after occlusion, cultured CPECs ($6x10^6$) in 60 μl of Hanks' balanced salt solution (HBSS) was stereotaxically injected into the 4th ventricle through the hole made at 3.8 mm caudal to the lambda suture in the midline of the skull. Thirty or 90 minutes later, the MCA was reperfused by removing the monofilament. The MCA perfusion area of the brain was kept in an ischemic condition for 60 to 120 minutes before reperfusion. For cell culture, CP tissues were excised from the lateral and 4^{th} ventricles of 4-week-old rats, dissociated with pronase, and cultured in DMEM containing fetal bovine serum. Five days later, the medium was changed for serum-free DMEM, and after culturing for an additional 7 days, cells were used for transplantation. Cultured CPECs expressed trophic factors, including bFGF, NT-4, HGF, and GDNF.

For the measurement of infarction volume, coronal sections (1.5-mm-thick) of the brain were stained with 2 % solution of 2, 3, 5 -triphenyltetrazolium chloride (TTC) for 20 min. The infarction volume was calculated by multiplying the infarcted areas by the depth of the infarction and presented as a ratio to the volume of the corresponding contralateral cortex or striatum.

The infarcted area is demonstrated as the TTC-negative area. Rats of the CPEC-transplanted group with reperfusion after 60 min of ischemia had smaller infarcted areas in the stratum (43% versus 70%) and cerebral cortex (9 % versus 33%) than those of the vehicle-injected group. Similarly, rats of the CPEC-transplanted group with reperfusion after 120 min had smaller infarcted areas in the sriatum (60% versus 80%), and cerebral cortex (35% versus 70%) than those of the vehicle-injected group (Fig. 5).

Behavioral assessment was performed 24 hours after MCA occlusion using the neurological severity score (6-point scale). Neurological outcome in the CPEC-transplanted group was 2.7 versus 1.2 points in the 60-min MCA occlusion rat, and 3.5 versus 2.4 points in the 120-min MCA occlusion, compared to those of the vehicle-injected rats.

TUNEL-positive apoptotic cells were decreased in number in the cerebral cortex in the CPEC-transplanted group. The proportion of caspase 3-positive cells in the cerebral cortex was decreased in the CPEC-transplanted group (14.0% versus 5.4%).

CPEC injection up-regulated several molecules including CREB (cAMP response element binding protein) and bcl-2, while down-regulating IL-1β (interleukin-1β), iNOS, and TNFα(tumor necrotic factor α). There was extensive infiltration of OX-42 positive cells in the cerebral cortex in vehicle injected rats, while the CPEC injection distinctly decreased the number of OX-42 cell in the brain including cerebral cortex and corpus callosum. These CPEC functions of anti-apoptotic properties and the suppression of proinflammatory molecules contributed to the cell survival in the ischemia-injured brain tissue.

Transplanted cells were located within the lateral and 4^{th} ventricles and on the brain surface. No transplanted CPECs were found at the sites of

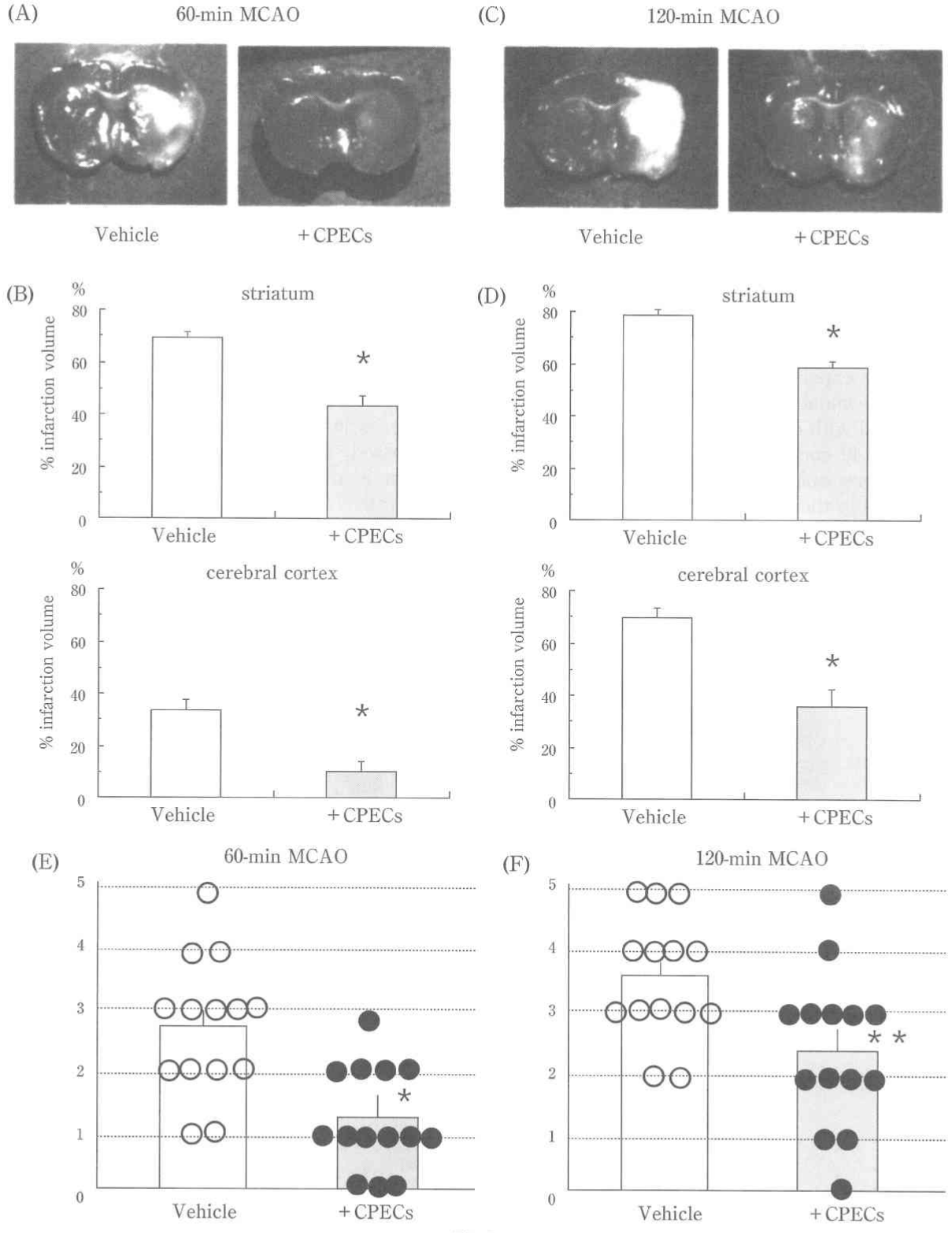

Fig. 5

Rat MCA was occluded for 30 min before injection of BMSCs (5×10^6, 60 μl in HBSS) into the 4th ventricle. Rats were re-perfused after 30 min or 90 min (i. e., 60 or 120 min after induction of ischemia). Twenty-four hours after ischemia, the brain was obtained and sectioned into thin coronal sections. These coronal brain slices were stained with TTC. The infarction volume was markedly reduced in rats with CPEC transplantation (A and C) Representative TTC-stained sections from the vehicle injected group and the CPEC-transplanted group 24 h after 60-min MCAO (A) and 120-min MCAO (C). The white regions of the brain are infracted areas. (B and D) Infarction volumes are significantly smaller in CR\EC-transplanted rats than in vehicle-injected rats, both in the striatum and cerebral cortex (*$p<0.01$). (E and F) Neurological deficits 24 hours after MCAO by 6-point scale method. Open and closed circles the neurological scores of rats from the vehicle-injected and CPEC-transplanted group, respectively. *$p<0.01$, **$p<0.05$ (from Matsumto et al., 2010)

ischemic injury. This suggests that transplanted CPECs did not migrate to the infracted area to rescue neural cells therein, but exerted their effects by releasing diffusible factors that reached the lesion through the CSF.

Specific molecules produced in the CPECs

Matsumoto et al. (2003) isolated genes predominantly expressed in the mouse CP using suppression subtractive hybridization. By subtraction with cerebral cortex cDNA, transthyretin and transphsophodiesterase 1α were predominant in the adult mouse CP.

In other experiments, cDNA derived from the CP of neonatal (postnatal day 5) mice was subtracted with cDNA from adult mouse CP. In this way, 49 complimentary DNA (cDNA) fragments were isolated, in which 43 were known sequences in the data base, and the remaining 6 were novel. The expression of these genes in CPECs was examined using RNA blots and/or in situ hybridization, and the abundant expression of gelsolin, phospholipid transfer protein (PLPT) (Fig. 6), ATP-binding cassette transporter subfamily A member 8 (ABCA8), androgen-inducible aldehyde reductase (AIAR), and Na/sulfate cotransporter SUT-1was confirmed. One novel gene (FS 88) was expressed in the CP from neonatal mice. These findings indicate that CP is involved in the such processes as prevention of fibrillization of amyloid β protein (transthyretin and gelsolin), and lipid metabolism (PLPT and ABCA 8) and detoxication (AIAR).

Gonzalez et al. (2011) reported that augulin is secreted by CPECs. Augulin is the protein encoded by Ecrg 4 (esophageal cancer-related gene) known as a tumor-suppressor gene. When augurin is increased, the cancer proliferation is suppressed, and, therefore, the production of augurin is usually kept at a low level in the proliferative cancer. In their paper, Gonzalez et al. describe the results as follows: "The CPECs are the major source of Ecrg4 in the CNS, and

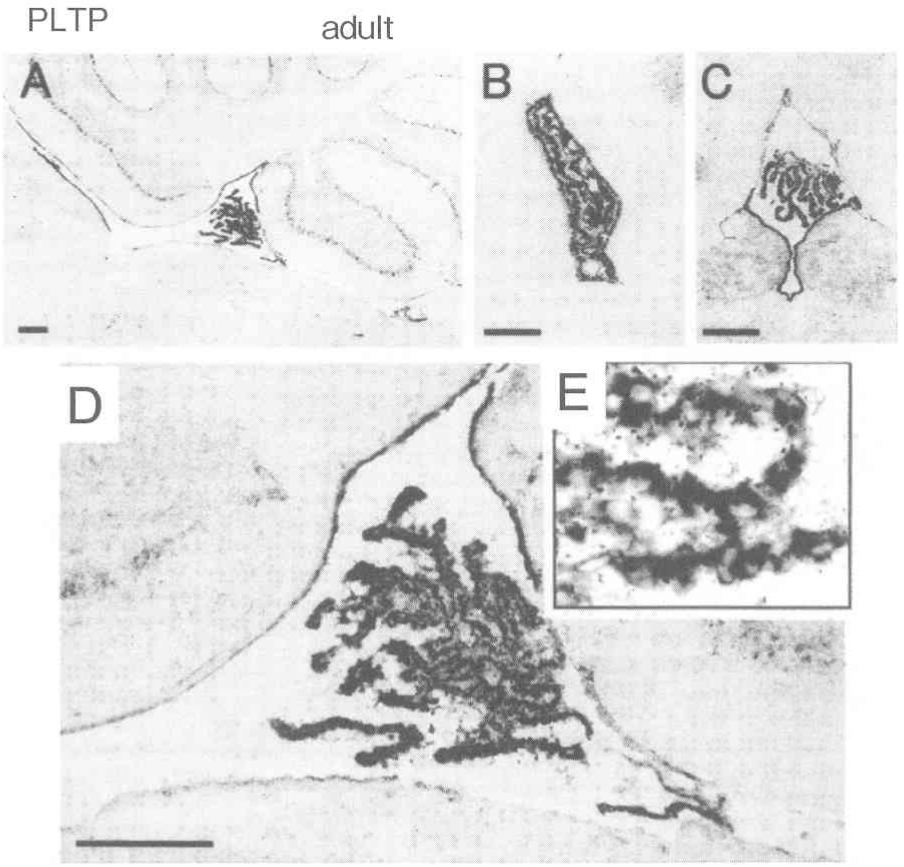

Fig. 6

These micrographs show the expression of phospholipids transfer protein (PLTP) in CPECs. Sagittal (A, D, E) or coronal (B, C) sections of the brain were shown, and CP in the 4th ventricle (A), lateral ventricle (B) and 3rd ventricle (C) were stained by in situ hybridization. The CP in (A) was magnified in (D), and a part of the CP in (D) was further enlarged in (E). mRNA expression was detected by in situ hybridization. The expression of mRNA of PLTP is restricted to the epithelial cells, with no hybridization at the underlying connective tissues. Scale bar: 200 μm
(from Matsumoto et al., 2003)

Ecrg 4 mRNA is predominantly localized to CPECs, ventricular and central canal ependymal cells. After a stab injury into the brain, both augurin staining and Ecrg 4 gene expression decreased precipitously." Based on the results, they concluded as follows: "An unusually elevated expression of the Ecrg4 gene in the CPECs implies that its product augurin plays a role in CP-CSF-CNS function. The results are all consistent with a model whereby an injury-induced decrease in augurin dysinhibits target cells at the ependymal-subependymal interface." They speculate that the ability of CPECs and ependymal cells to alter the progenitor cell response to CNS injury may be mediated in part by Ecrg 4.

CPECs are characterized by containing a high concentration of transthyretin (TTR). Therefore, TTR is a reliable immunohistochemical marker for CP. TTR is a binding protein of thyroxine (T4) and retinol (vitamin A). The name "transthyretin" comes from the property of binding thyroxine and retinol. TTR is synthesized also in the liver. The fact that CPECs produce a large amount of TTR at the site of the blood-CSF barrier means that CP might play an important role in transporting T4 and retinol from the blood flow into the CSF. TTR is also related to the detoxification of the CSF by binding toxins, including PVB. TTR is also important in keeping the protein in a normal condition. Abnormal proteins change into amyloid β, which is in most cases deposited in the brain parenchymal tissue, thus causing Alzheimer's disease. TTR function as detoxification by binding abnormal protein to remove it from the CNS. This indicates that TTR is profoundly related to the development of Alzheimer's disease.

Choroid plexus in diseases

The CP tissues underwent varying kinds of changes in many diseases including Alzheimer's disease (Serot et al. 2003), and amylotrophic lateral sclerosis (Uchino et al. 1994). In Alzheimer's disease, CPECs become atrophic, i. e., epithelial cells decrease in height and contain a large number of lipofuschin vacuoles. The basal lamina of the epithelial cells becomes irregular and thickened. Microvilli are thickened, and their stroma become fibrotic. The wall of vessels becomes thick, and there are deposits of IgG and IgM along the epithelial basal lamina. These features suggest the decrease of functional capacities of CPECs, leading to the delayed turnover of CSF. The CSF turnover rate is usually 6–8 h, however, it becomes delayed to 36 h in Alzheimer's disease (Serot et al. 2003). The level of CSF transthyretin in ALS is lower than normal. Transthyretin, which is contained abundantly in CPECs, is normally associated with amyloid protein to form complexes to inhibit amyloid protein from undergoing fibrillotransformation (Serot et al. 1997). In rats, the clearance of intraventricularly injected amyloid $\beta$is 10.4 /min at 3 months of age, and it is 0.71 μl /min at 30 months of age. This means that the brain content of amyloid peptide increases in the CSF of old animals (Preston, 2001). In addition, vitamin B_{12} and folate were decreased in CSF of old animals, implying the down-regulation of methylation process of phospholipids, nucleic acids, catecholamine, and acetylcholine in the CNS of aged animals (Ikeda et al. 1990; Serot et al. 2001). Ascorbic acid and α-tocopherol, free radical scavengers were also decreased, indicating the increase of oxidative stress of Alzheimer's disease (Tohgi et al. 1994; Schippling et al. 2000).

It is reported that the beta amyloid permeability across cells was greater from the CSF-facing membrane than from the blood-facing membrane of CPECs. Similarly, cellular accumulation of beta amyloid was greater from the CSF-facing membrane. This shows a bias for efflux. CP selectively cleanses beta amyloid from the CSF by an undetermined mechanism, potentially reducing beta amyloid from normal brains and the brains of Alzheimer's disease patients (Crossgrove et al. 2005).

Huntington's disease will be referred to below.

Concerning the detoxification of the CSF, CP contains a high concentration of glutathione, cysteine and methallothioneins, agents to sequester toxic components by conjugating toxic agents in the CSF. The CP also contains superoxide dismutase, glutathione transferase, and reductase, enzymes to digest free radicals. In addition, it contains an organic ion transport system for exportation of noxious compounds from the CSF.

The above descriptions indicate that CPECs have critical influences on various aspects of functions for maintaining the CNS condition at the normal level.

Transplantation of encapsulated CPECs

As was described above, CPECs have neurotrophic effects on damaged CNS tissues when transplanted into the CSF or into the spinal cord in rats. The fact that, although they do not reach the injured site, CPECs transplanted into the CSF

exert profound effects in CNS indicates that CPECs might release biologically active neurotrophic molecules into the CNS. This suggests that if CPECs are protected from immunological rejection, allogeneic or even xenogeneic transplantation of CPECs might be useful for clinical treatment. This thought leads to a new transplantation method of CPECs, in which clusters of porcine CPECs are encapsulated in an alginate- and poly-ornithine-coated capsule, and transplanted in the vicinity of the lesion in the CNS. This encapsulation protects CPECs from immunological reaction, while CPEC-secreted molecules can be released through the capsule into the surrounding tissue space (Skinner et al. 2009).

Thanos et al. (2007) showed the stability of conventional alginate formulation encapsulated using a commercially available technique and apparatus, which they describe as follows: "The microcapsules were produced, characterized, and implanted into the brain, peritoneal cavity and subcutaneous space of rats. After 14–215 days, capsules were explanted and the surface was analyzed using Fourier-transform infrared spectroscopy and scanning electron microscopy. The surface morphology examined by these techniques indicates that the encapsulated techniques and formulation produced a stable biocapsule capable of survival in all sites, including the harsh peritoneal environment for at least 215 days".

Encapsulated CPECs were transplanted into the ischemia-injured brain in rat. In the experimental acute stroke occlusion of MCA for 1 h in a Wistar rat, encapsulated CPECs were transplanted immediately after occlusion in the predicted core of the cerebral infarction. Three days later, treated animals had better recovery of neurological function than did control animals, and the volume of the infarction was significantly decreased in the encapsulated CPEC-transplanted rats (Borlongan et al. 2004). They also showed that the conditioned medium of CP culture can rescue serum-deprived neurons cultured from rat embryonic brain cortex.

The effects of encapsulated CPECs were examined in the experimental Huntington disease (Borlongan et al. 2004). In the abstract of the paper they report as follows: "CP from neonatal pig was encapsulated in alginate microcapsule into the striatum. Three days later, the same animals received a unilateral injection of quinolinic acid (225 nmol) into the site 2 mm from the transplant of the ipsilateral striatum. At an early stage, the GABAergic/encephalinergic projection to the external globus pallidus was preferentially affected. Cholinacetyltranspherase, substance P, cholecystokinin and angiotensin-converting enzyme were decreased. After surgery, animals were tested for motor function using the placement test. Choroid plexus transplants ameliorated the weight loss and motor impairments resulting from quinolinic acid injection. Histological analysis (Nissl staining) demonstrated that choroid plexus transplants significantly reduced the volume of striatum damage produced by quinolinic acid and protected choline acetyltransferase (ChaT) - but not NADPH-diaphorase-positive neurons." These studies imply that the transplantation of porcine CPECs can be used to protect striatal neurons from excitotoxic damage and that the pattern of neuroprotection varies across specific neuronal populations.

In the study of Huntington's disease using primates, Cynomolgus monkeys were transplanted with encapsulated porcine CPECs into the caudate and putamen. This is xenogeneic transplantation. One week later, quinolinic acid was injected into the caudate and putamen. Stereological counts of NeuN-positive neurons showed that striatal neurons were distinctly protected by CPEC transplants, and that the striatal volume was well preserved (Emerich et al. 2006)

Stem cells in the CPECs

Itokazu et al. (2006) reported that CPECs contain progenitor cells that can differentiate into neurons and glial cells. They reported as follows: "Cell spheres were produced in the culture of the CP of the 4th ventricle of rats 1 day, 7 days and 8 weeks after birth. Neurons (positive for βtubulin class III), astrocytes (positive for GFAP) and oligodendrocytes (positive for O4) were differentiated from the cell spheres in the differentiation medium." Further they examined whether neuroprogenitor cells are contained in the CPECs. First, they produced a monoclonal antibody 3E6 that specifically labels the microvilli of the CPECs. Using this monoclonal antibody, CPECs were isolated from the CP of postnatal-8-week rats by FACS (fluorescence-activated cell sorting). Immunohistochemistry confirmed that there was no contamination from fibroblasts, endothelial cells, macrophages, or Schwann cells in the FACS-isolated cells. Cell spheres formed in the cultures of these 3E6-labeled CPECs. After expansion, these cell spheres gave rise to Tuj-1-(5 %), GFAP-(45%) and O4- positive cells (0.16%). The remaining cells (45%) were unlabeled neural or glial markers. Some CPECs were immunohistochemi-

cally stained with lineage-associated markers of Musashi-1 and epidermal growth factor-receptor (EGF-R). In addition, infusion of EGF of fibroblast growth factor-2 (FGF2) into the ventricle increased the number of BrdU-positive (mitotic) cells among CPECs. This study shows that CP contains neuroprogenitor cells.

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