hiBMEC/kyas, A NEWLY IMMORTALIZED HUMAN BRAIN MICROVASCULAR ENDOTHELIAL CELL LINE, IS A PROMISING TOOL FOR AN IN VITRO BLOOD-BRAIN BARRIER MODEL
Satoshi Kishida, Tomomi Furihata, Atsuko Kamiichi, Yuki Ohta, Kosuke Saito and Kan Chiba
Laboratory of Pharmacology and Toxicology, Graduate School of Pharmaceutical Sciences, Chiba University. 1-8-1 Inohana, Chuou-ku, Chiba-shi, Chiba 260-8675, Japan

[Purpose] In drug development, an in vitro BBB model is an important tool for evaluation of drug penetration into the brain. However, many of current in vitro BBB models consist of animal cells, which may not be able to adequately predict brain permeability in humans. Therefore, the purpose of the present study was to establish and characterize a novel immortalized human brain microvascular endothelial cells (hiBMEC) that can be used in vitro BBB model.

[Methods] Temperature-sensitive simian virus 40 large T antigen and human telomerase reverse transcriptase were transduced to human primary BMEC (hprBMEC), termed hiBMEC/kyas. mRNA expression profiles and functions of tight junctions (TJs) and transporters were examined by RT-PCR, sucrose permeability assay and transport assay.

[Results and Discussion] hiBMEC/kyas exhibited the spindle-like morphology similar to that of hprBMEC and had high proliferation ability in a temperature-dependent manner. mRNA expression profiles of the TJ genes and the transporter genes in hiBMEC/kyas were also similar to those of hprBMEC. The results of permeability assay showed that permeability coefficient for sucrose was $1.02 \pm 0.0725 \times 10^{-3}$ cm/min, suggesting that functional TJs were formed between the cells. Amount of rhodamine 123, substrate of P-glycoprotein, transported from brain side to blood side was 1.5-fold higher than blood side to brain side, suggesting the function of P-glycoprotein at the apical side of the cells.

[Conclusions] Our results showed that hiBMEC/kyas had many functional characteristics to control drug penetration. Together with a novel culture method, establishment of which is currently ongoing, hiBMEC/kyas is thought to be a highly promising cell line for an ideal in vitro BBB model.

SYSTEM L AMINO ACID TRANSPORTER 1 (LAT1) IS A POSSIBLE TARGET FOR COMBINATION THERAPY WITH ANTIPROLIFERATIVE AMINOPEPTIDASE INHIBITORS IN HUMAN OVARIAN CANCER CELLS
Takeo Nakanishi1, Douglas D Ross2, Hiroshi Arakawa1, Ikumi Tamai1
1Dept. of Membrane Transport and Biopharmaceutics, Faculty of Pharmacy, Kanazawa University, Kakumacho, Kanazawa 920-1192, Japan, and 2University of Maryland School of Medicine Baltimore, Maryland

[Purpose] Amino acids activate nutrient signaling via the mTOR, we therefore evaluated the relationship between amino acid transporter gene expression and proliferation in human ovarian cancer cell lines. [Methods] Expression of three cancer-associated amino acid transporter genes, LAT1, ASCT2 and SN2, was measured by qRT-PCR and Western blot. The effects of silencing the system L amino acid transporter LAT1 gene and its inhibitor BCH on cell growth were evaluated by means of cell proliferation and colony formation assays. [Results and Discussion] LAT1 was upregulated in human ovarian cancer SKOV3, IGROV1, A2780, and OVCAR3 cells, compared to normal ovarian epithelial IOSE397 cells, whereas ASCT2 and SN2 were not. BCH reduced phosphorylation of p70S6K, a downstream effector of mTOR, in SKOV3 and IGROV1 cells, and decreased their proliferation by 30% and 28%, respectively. Although proliferation of SKOV3 (S1) or IGROV1 (I10) cells was unaffected by LAT1-knockdown, plating efficiency in colony formation assays was significantly reduced in SKOV3(S1) and IGROV1(I10) cells to 21% and 52% of the respective plasmid transfected control cells, SKOV3(SC) and IGROV1(IC), suggesting that LAT1 affects anchorage-independent cell proliferation. Finally, BCH caused 10.5- and 4.3-fold decrease in the IC50 value of bestatin, an antiproliferative aminopeptidase inhibitor, in IGROV1 and A2780 cells, respectively, suggesting that the combined therapy is synergistic. [Conclusion] Our findings indicate that LAT1 expression is increased in human ovarian cancer cell lines; LAT1 may be a target for combination therapy with antiproliferative aminopeptidase inhibitors to combat ovarian cancer.