**A human immortalized cell-based blood-brain barrier model: characterization as a tool for exploring brain-permeable AAV vectors**

**Ryuto Isogai1**, Hanae Morio1, Mei Fukuda2, Yoshinori Tanaka3, Sachiko Okamoto3, Tomomi Furihata1.

Lab Adv. Drug Dev. Sci, Sch Pharm, Tokyo Univ Pharm & Life Sci.1, Hachioji, Tokyo, Japan;

Lab Clin Pharm & Exp Therapeut, Sch Pharm, Tokyo Univ Pharm & Life Sci.2, Hachioji, Tokyo, Japan;

Takara Bio Inc.3, Kusatsu, Shiga, Japan.

**Background and aims.** Adeno-associated virus (AAV) vectors with high blood-brain barrier (BBB) permeability have attracted growing interest in gene therapy for brain diseases. To facilitate the development of such vectors, *in vitro* human BBB models that can accurately assess their permeability are required. In this study, we focused on the human immortalized cell-based BBB model (hiBBB model) established in our laboratory1) to characterize its performance as a platform for identifying BBB-permeable AAV vectors.

**Results and Discussion.** Using the hiBBB models constructed from three types of human immortalized BBB cells, we first evaluated the BBB permeability of AAV9 and AAV2, which are known as BBB-permeable and non-BBB-permeable serotypes, respectively. The results showed that AAV9 exhibited higher BBB permeability than AAV2 (6.0-fold on average), consistent with previous report2). The effect of AAV9 and AAV2 on the barrier integrity was simultaneously evaluated using FITC-labelled dextran. As a result, neither AAV9 nor AAV2 affected the barrier function, thereby indicating that the BBB permeability difference observed between AAV9 and AAV2 is likely to attributable to their intrinsic virological properties. We then used a barcode-labeled library of 18 AAV vectors (wild-type serotypes, AAV9 variants, and AAV2 variants), to examine whether the hiBBB model could screen for BBB-permeable AAV vectors. As a result, AAV9 showed the highest BBB permeability among the wild-type serotypes. Meanwhile, several AAV variants showed slightly higher or lower permeability than AAV9 and AAV2, respectively.

**Conclusion.** Our results indicate that the hiBBB model is a valuable tool for evaluating BBB permeability of AAV vectors. Therefore, this model is expected to serve as a useful tool to accelerate the research and development of novel AAV vectors for the treatment of brain diseases.

**References:**

(1) Ito et al. Mol Pharm. 2019;16:4461-4471.

(2) Merkel et al. J Neurochem. 2017;140:216-230.