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Holy grails and *in vitro* blood–brain barrier models

The neuropharmaceutical industry has been searching for nearly 20 years for the holy grail of rapid screening of blood–brain barrier (BBB) transport – an *in vitro* BBB model. Such a model would provide the anchor for a CNS drug delivery program, which could be merged with ongoing HTS systems devoted to CNS drug discovery.

Why discover CNS drugs if you can not deliver them across the BBB? The pharmaceutical industry needs to know if their CNS drug candidates cross this barrier. The search for an *in vitro* BBB model has had a long and checkered history but now there has been a breakthrough with a new *in vitro* BBB model developed by Terasaki and co-workers [1].

The BBB permeability properties are determined by the capillary endothelial cell and *in vitro* BBB models attempt to re-create the BBB with the cell culture of brain capillary endothelium. The singular reason that the creation of a functional *in vitro* BBB model has been so difficult over the years is that the tissue-specific gene expression within the brain capillary endothelial cell is so severely down-regulated when the endothelial cells are taken out of brain and grown in culture.

The genes expressing the numerous BBB transporters (and enzymes) are repressed in cell culture and the permeability properties of most cultured brain endothelia are closer to that of cultured fibroblasts than to the BBB *in vivo*. If industry used conventional *in vitro* BBB models to screen for L-DOPA transport across the BBB, they would conclude that this amino acid drug did not cross the BBB [2]. This, of course, would be a mistake because L-DOPA does cross the BBB owing to its affinity for the LAT1 large neutral amino acid transporter at the BBB.

However, LAT1 gene expression is down-regulated in brain endothelial cell culture about 100-fold [3]. There are exceptions to the rule but the majority of BBB-specific genes are turned off in cell culture. This situation generally does not change with endothelial/astrocyte co-cultures, although endothelial/astrocyte mixed cultures do seem to induce BBB gene expression to some extent [4]. However, it is not feasible to measure drug transport across a mixed-culture system. What the field needs is an *in vitro* BBB system where BBB specific gene expression is not turned off in cell culture. This has now been accomplished [1].

Transgenic mice expressing a temperature sensitive (ts) SV40 large tumor antigen (T-ag) were developed at Tohoku University (http://www.tohoku.ac.jp) over 10 years ago [5]. The SV40 T-ag is an oncogene that suppresses the expression of tumor suppressor genes and enables cell proliferation. The ts variant of the T-ag is not expressed at physiological temperatures (37°C) but is expressed at permissive temperatures (33°C), which favors cell growth and which has pleiotropic effects on the expression of many genes. Consequently, genes that are normally suppressed in cell culture are now turned on.

Terasaki and colleagues hypothesized that brain endothelial cell lines originating from the ts SV40 large T-antigen transgenic mice (or transgenic rats) might be ideal candidates for an *in vitro* BBB model, wherein brain endothelial specific gene expression remains high in culture. They were right. There is high gene expression of BBB specific transporters in these in transgenic *in vitro* BBB models [1].

The search for the Holy Grail continues, however. What we still do not know is why BBB genes are turned off in culture. Perhaps clues to this will be forthcoming with the application of genomics methodology to the study of BBB gene expression.

References

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