BBB-Genomics: creating new openings for brain-drug targeting

'Blood–brain barrier genomics provides the platform for the future discovery of novel brain-drug targeting technologies.'

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Pharmaceutical genomics identifies novel potential targets for both drug action and drug toxicity. However, a third application of genomics that has not received much attention, as yet, is its use in the discovery of new pathways for drug delivery and targeting. The brain is arguably the most complex organ within which to target drugs; the blood–brain barrier (BBB) prevents the uptake of nearly all large-molecule drugs and >98% of small-molecule pharmaceuticals. The existence of the BBB causes the majority of CNS drug development programs to result in termination, because modern CNS drug discovery is practised in the absence of a parallel program in CNS drug delivery and targeting. If this situation were to change, and formal programs in CNS drug targeting were developed, then progress could be accelerated with a 'BBB-genomics' research strategy.

Standard brain genomics begins with RNA isolated from the whole brain. By contrast, BBB-genomics begins with RNA isolated from purified brain capillaries, which form the BBB *in vivo* (Fig. 1). Brain genomics programs are currently being used to aid the discovery of new targets of drug action in the brain. Given the existing investment in this area, one might ask why it is necessary to start again with a BBB-genomics effort. The current sensitivity of existing gene microarrays is 1 in 10,000 mRNA molecules (Ref. 1). However, the volume of the capillary endothelium in the brain is <1 µl g^{-1} (10^{-3} parts per brain)[2]. Therefore, only the most abundant transcripts produced within the brain capillary endothelium will be detected in a whole-brain gene microarray. However, virtually any gene that is selectively expressed within the brain microvasculature can be detected using a 'BBB-genomics' or 'brain-vasculature genomics' program that targets exclusively the capillary endothelium, rather than the whole brain.

Recently, a polymerase chain reaction (PCR)-based subtraction-cloning method – suppression-subtractive hybridization – was used to clone BBB-specific genes from 'tester' cDNA generated from the polyA$^+$ RNA of purified rat-brain capillaries (i.e. the target tissue)[3]. The tester cDNA was subtracted using 'driver' cDNA generated from a pool of rat liver and kidney polyA$^+$ RNA (by subtracting out mRNA common to both brain tissue and kidney and liver tissues). Screening of only 5% of the subtracted tester cDNA library resulted in the identification of 50 gene products, and >80% of these were selectively expressed at the BBB, as shown by northern blot analysis[3]. These BBB-specific genes included: (1) genes not previously known to be expressed at the brain microvasculature; (2) expressed sequence tags (ESTs); and (3) novel sequences not found in any databases.

The discovery of novel gene sequences that are selectively expressed at the BBB, and not found in current brain EST databases, is not surprising given the small volume of the brain that is occupied by the BBB. Genes that were not known to be selectively expressed at the BBB include: tissue plasminogen activator, insulin-like growth factor-2, PC-3 gene product, myelin basic protein, regulator of G-protein signaling-5, utrophin, IκB, connexin-45, the class I

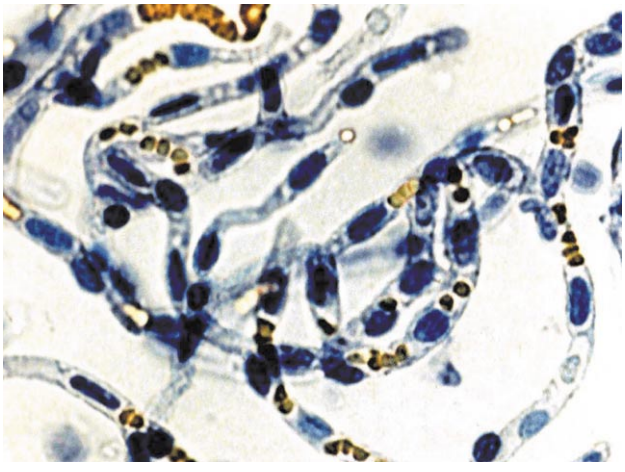

Figure 1. Isolated brain capillaries × 600. These structures contain the RNA products of blood–brain barrier-specific genes. The cells trapped in the lumen of the capillaries are erythrocytes.

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major histocompatibility complex and rat homologues of the transcription factors hbrm or EZH1 (Ref. 3). This work also led to the discovery of novel drug transporters at the BBB including organic anion transporting polypeptide type 2 (Oatp2) and BBB-specific anion-transporter type 1 (BSAT1)[3].

The need for brain-drug targeting platforms

Genes that are selectively expressed in neurons and glial cells are targets for CNS drug discovery, whereas genes that are selectively expressed at the brain capillary endothelium are targets for CNS drug delivery. The rationale for a 'BBB-genomics' program is derived from a pre-existing strategy in molecular BBB research and development. The need for building molecular brain-drug targeting programs is demonstrated in Fig. 2, which illustrates present and future approaches to BBB drug transport and CNS drug delivery. The present day approach is shown in (a), and this chemistry-driven process is focused solely on lipid-mediated small-molecule transport, and the usual properties such as MW, hydrogen bonding, lipid solubility and plasma-protein binding. Small molecules can cross the BBB if: (1) the MW is <400 Da and (2) the drug is lipid soluble and forms <10 hydrogen bonds[2]. These molecular characteristics are absent in >98% of small-molecule drug candidates and in virtually all large-molecule drugs. Consequently, the majority of new drug candidates do not cross the BBB, which hinders the development of new drugs for intractable CNS diseases such as Alzheimer's disease, Parkinson's disease, brain cancer and cerebral AIDS.

A new approach to CNS drug development is shown in Fig. 2b, and this biology-driven process focuses on endogenous BBB transporters as novel entry points for drugs using brain-drug targeting technology[2]. Large-molecule drugs can enter the brain via receptor-mediated transport (RMT) systems, such as the insulin or transferrin receptor. Small-molecule drugs can cross the BBB via carrier-mediated transport (CMT) systems, and several of the CMT systems at the BBB have been cloned (Fig. 2). In addition, active efflux transporters (AET) are expressed at the BBB[4], and these include P-glycoprotein, Oatp2 and BSAT1. Co-drugs that inhibit AET systems could increase the brain uptake of drugs that are excluded from the brain by such mechanisms.

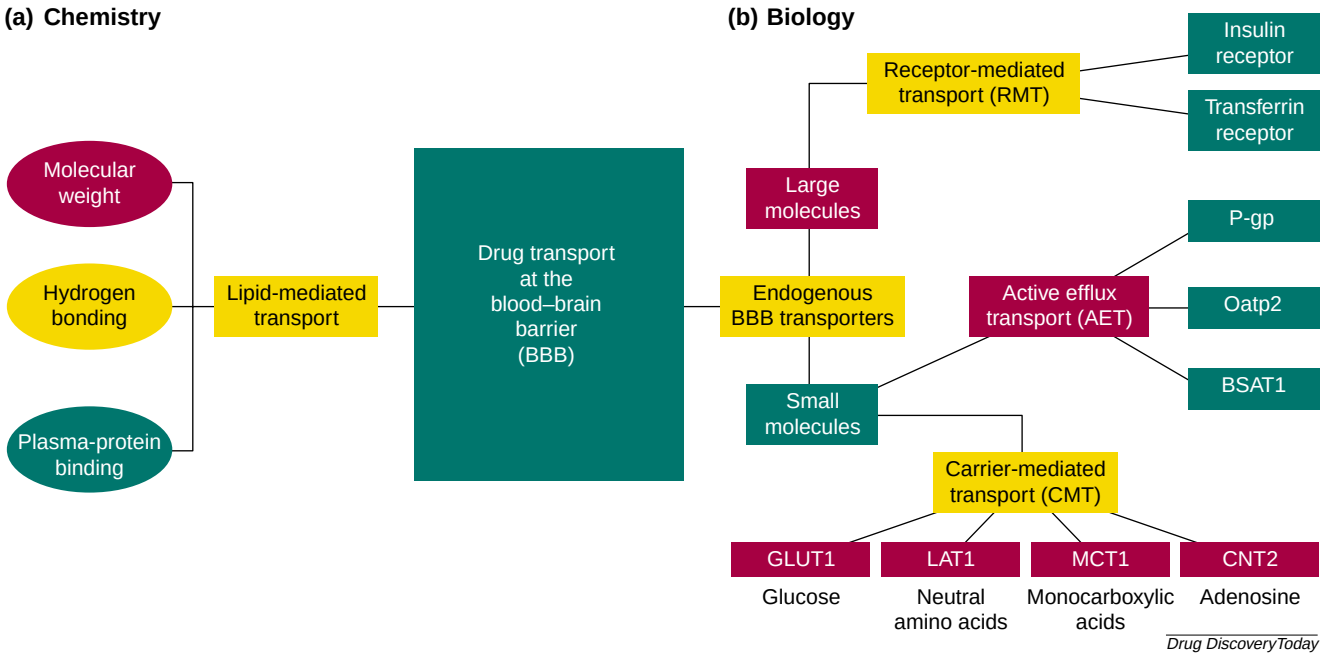

Figure 2. (a) The classical approach to the analysis of drug transport at the blood–brain barrier (BBB) is a chemistry-driven platform that emphasizes small-molecule drug candidates, molecular weight, hydrogen bonding, lipid solubility and plasma-protein binding. **(b)** shows the expansion of brain-drug targeting technology into a biology-driven platform that focuses on endogenous BBB transporters. The expanded brain-drug targeting platform enables the reformulation of either large-molecule or small-molecule drug candidates and targets these drugs to the brain via one of several endogenous BBB transporters. These transporters fall into three general categories: receptor-mediated transport (RMT) systems, carrier-mediated transport (CMT) systems and active efflux transport (AET) systems. Abbreviations: P-gp, P-glycoprotein; Oatp2, organic anion transporting polypeptide 2; BSAT1, BBB-specific anion-transporter type 1; GLUT1, glucose transporter isoform 1; LAT1, large amino acid transporter 1; MCT1, monocarboxylate transporter 1; CNT2, concentrative nucleoside transporter 2.

There are additional RMT, CMT and AET systems at the BBB that remain to be discovered, and these could prove to be novel new conduits for drug targeting to the brain. The discovery of these transporters, and the development of new brain-drug targeting technologies, could be accelerated by a BBB-genomics program. However, there must first be a change in our approach to the problem of poor drug transport across the BBB. We must move away from relying on the chemistry-driven platform that focuses on molecular weight and lipid solubility (Fig. 2a). Although these issues are, of course, crucial in the design of clinically effective neuropharmaceuticals, this classical approach must be expanded to include a biology-driven platform that focuses on endogenous BBB transport systems (Fig. 2b). These endogenous transporters can be used as portals of drug entry to the brain, based on principles of brain-drug targeting technology. Once this shift in thinking takes place, future progress in brain-drug targeting will only be limited by the discovery of novel endogenous BBB transporters and, therefore, the application of BBB-genomics in the discovery of such transporters will prove to be crucial.

References

1 Schena, M. *et al.* (1995) Quantitative monitoring of gene expression patterns with a complementary DNA microarray. *Science* 270, 467–470

2 Pardridge, W.M. (2001) *Brain Drug Targeting: The Future of Brain Drug Development*, Cambridge University Press

3 Li, J.Y. *et al.* (2001) Blood–brain barrier genomics. *J. Cereb. Blood Flow Metab.* 21, 61–68

4 Kusuhara, H. and Sugiyama, Y. (2001) Efflux transport systems for drugs at the blood–brain barrier and blood–cerebrospinal fluid barrier (Part 1). *Drug Discov. Today* 6, 150–156

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What do YOU think about blood–brain barrier genomics?

How do you think we could improve CNS drug development?

How do you think we should approach the problem of poor drug transport across the blood–brain barrier?

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