

**Figure 3.17** Cytocentrifuged human brain capillaries were dual-labeled with antibodies to pglycoprotein (Pgp) (panels A, D), glial fibrillary acidic protein (GFAP, panels B, G), or the *Glut1* glucose transporter (panels E, H). The overlap of panels A and B is shown in panel C; the overlap of panels D and E is shown in panel F; and the overlap of panels G and H is shown in panel I. Reprinted from *Brain Res.*, **819**, Golden, P.L. and Pardridge, W.M., P-glycoprotein on astrocyte foot processes of unfixed isolated human brain capillaries, 143–6, copyright (1999), with permission from Elsevier Science.



**Figure 4.7** (A and B) Freshly isolated, unfixed rat brain capillaries are incubated with rhodamine-labeled pegylated immunoliposomes before confocal analysis. The immunoliposomes contain 20–24 molecules of either the mouse IgG<sub>2a</sub> isotype control (A) or the OX26 monoclonal antibody (MAb) (B) conjugated at the tip of the polyethylene glycol strands. (C) Cross-section through the rat brain capillary shown in (B) obtained by computer-aided three-dimensional construction of a series of consecutive optical sections. Colors were used to illuminate the capillary lumen (blue/black), luminal and abluminal endothelium plasma membranes (purple), and endothelial cytoplasm (yellow). (D, E, F) Double-labeling of unfixed rat brain capillaries using the OX26 MAb with a fluorescein conjugated secondary polyclonal antibody and a plasma membrane marker, rhodamine-phosphatidyl ethanolamine (PE). The signals for rhodamine-PE alone or OX26 MAb alone are shown in (D) and (E), respectively, and the overlay of these two images is shown in (F). From Huwyler and Pardridge (1998) with permission.



**Figure 7.11** (A, C, E) Experimental U87 brain tumors were grown in nude rats for 16 days. The brain was removed and frozen sections were immunostained with a mouse monoclonal antibody to the human epidermal growth factor (EGF) receptor; these studies used a biotinylated horse antimouse immunoglobulin G (lgG) secondary antibody that had been preabsorbed with rat immunoglobulin. The study shows abundant expression of the immunoreactive EGF receptor in the U87 experimental tumors in brains of nude rats. (B, D, F) Film autoradiography of frozen sections of brain obtained from U87 tumor-bearing nude rats injected intravenously with 100  $\mu$ Ci of either [<sup>111</sup>In]diethylenetriaminepentaacetic acid (DTPA)-EGF-polyethylene glycol (PEG)<sup>3400</sup>-biotin conjugated to OX26/streptavidin (B and D) or [<sup>111</sup>In]DTPA-EGF-PEG<sup>3400</sup>-biotin without conjugation to the BBB-targeting system (F). The panels on the right (B, D, F) are labeled as brain scan in living animals because the radiolabeled EGF chimeric peptide was administered in vivo and frozen sections were subsequently developed by quantitative autoradiography (QAR), as opposed to in vitro QAR, where the labeled peptide is applied to tissue sections in vitro. From Kurihara and Pardridge (1999) with permission.



**Figure 9.7** (A)  $\beta$ -galactosidase histochemistry in brain at 48 h after intravenous injection of the  $\beta$ galactosidase gene packaged inside the OX26 pegylated immunoliposomes. hippo, hippocampus; LV, lateral ventricle; III, third ventricle; son, supraoptic nuclei. (B) Control brain from rats receiving no gene administration. (C) Punctate gene expression in intra-parenchymal capillaries is shown and may represent gene expression in either the endothelium or microvascular pericytes. (D) Gene expression in the epithelium of choroid plexus is shown. The lumen (L) of the capillary of the choroid plexus is labeled. The absence of  $\beta$ galactosidase gene product in the capillary lumen demonstrates the  $\beta$ -galactosidase enzyme activity in the brain does not arise from enzyme in the plasma compartment. (E) The thalamic (thal) nuclei below the choroid plexus of the third ventricle are shown. Magnification bars: (A) 1.5 mm, (B) 2.2 mm, (C) 57  $\mu$ m, (D) 23  $\mu$ m, and (E) 230  $\mu$ m. Panels A and B were not counterstained. From Shi, N. and Pardridge, W.M. (2000). Antisense imaging of gene expression in the brain in vivo. *Proc. Natl Acad. Sci. USA*, **97**, 14709–14. Copyright (2000) National Academy of Sciences, USA.