GPIHBP1 expression in gliomas promotes utilization of lipoprotein-derived nutrients

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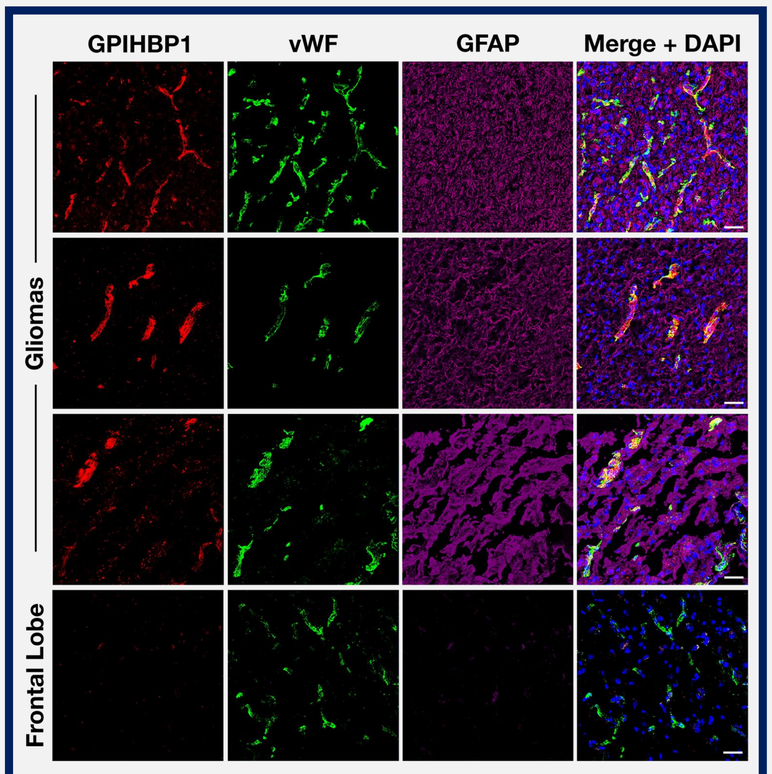
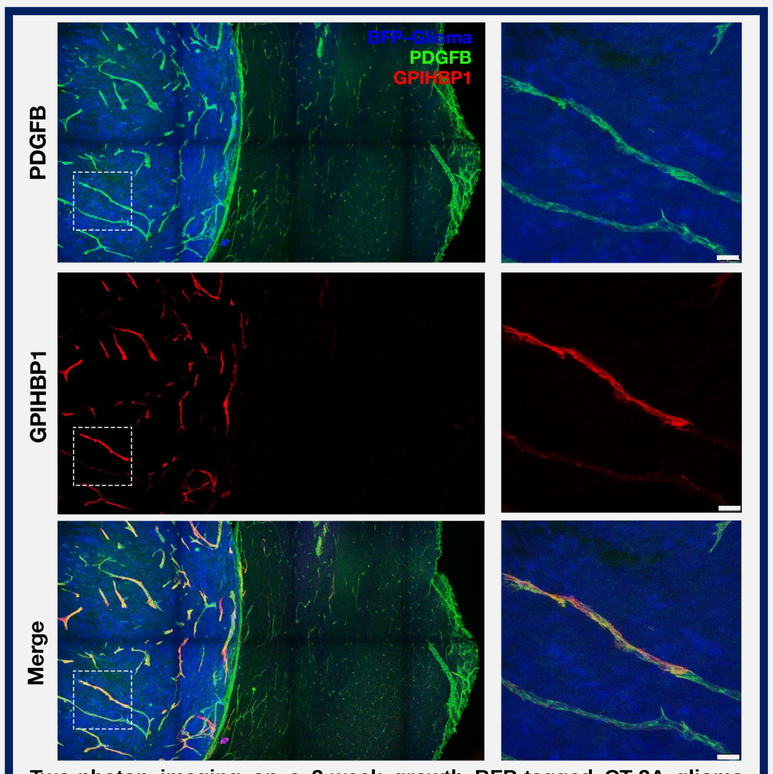
Abstract—GPIHBP1, a glycolipid-bound protein of capillary endothelial cells, binds lipoprotein lipase (LPL) in the interstitial spaces (where it is secreted by parenchymal cells) and shuttles it to the capillary lumen. Within capillaries, the GPIHBP1–LPL complex is required for the margination of triglyceride-rich lipoproteins (TRLs), allowing triglyceride hydrolysis to proceed. This process, intravascular lipolysis, releases fatty acids for utilization by adjacent parenchymal cells. GPIHBP1 is expressed in capillaries in peripheral tissues but not in the brain, which relies on glucose for fuel. We suspected that GPIHBP1 might be expressed in gliomas, where capillaries are morphologically abnormal and where the blood–brain barrier is often defective. Using immunohistochemical studies, we found that GPIHBP1 is easily detectable in capillaries of mouse and human gliomas but was absent in surrounding brain tissue. We also observed LPL expression in gliomas, suggesting that we might find evidence for TRL margination and TRL processing in gliomas. Indeed, by NanoSIMS imaging, we observed margination of TRLs along glioma capillaries but not capillaries of the normal brain. We also found robust uptake of TRL-derived nutrients in gliomas but not in normal brain. We suspect that the capacity of gliomas to process TRLs contributes to tumor growth.

1. GPIHBP1 is present in endothelial cells of human gliomas, but not in normal brain.



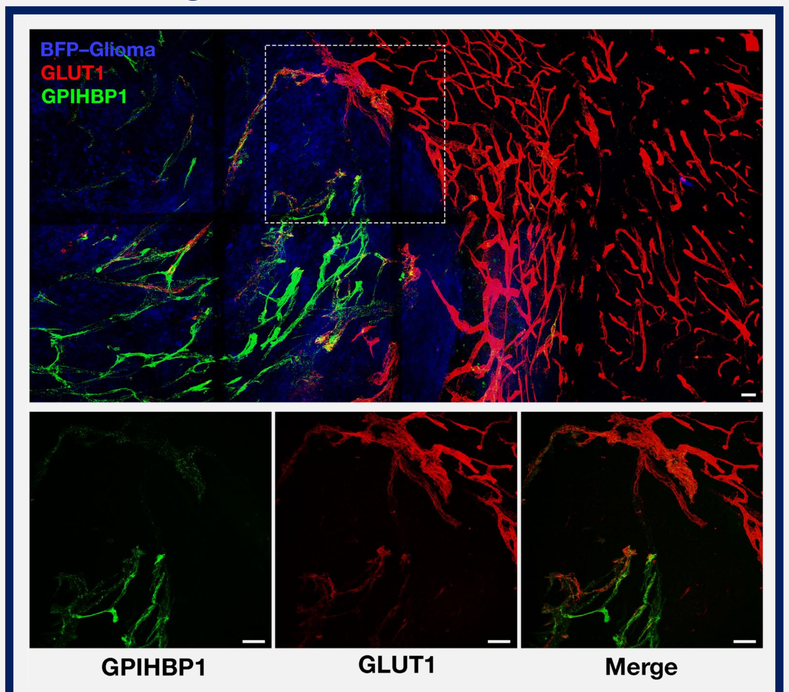
Immunohistochemical studies on surgically resected gliomas and non-diseased human frontal lobe, revealing expression of GPIHBP1 in gliomas but not in normal brain. GPIHBP1 (*red*) colocalized with von Willebrand factor (vWF, a marker for endothelial cells; *green*) but not with glial fibrillary acidic protein (GFAP, a marker for astroglial cells; *magenta*). DNA was stained with DAPI (*blue*). Scale bar, 50 μm.

2. GPIHBP1 is expressed in endothelial cells of mouse gliomas but is absent in normal brain.



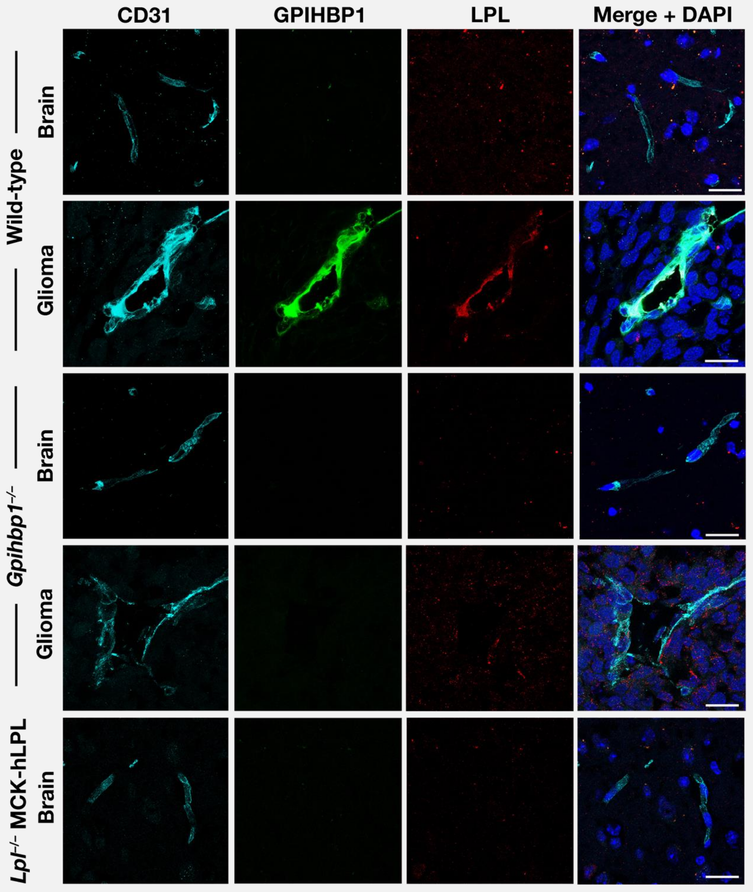
Two-photon imaging on a 3-week growth BFP-tagged CT-2A glioma implanted in a mouse brain, demonstrating colocalization of GPIHBP1 (*red*) with PDGFB (a marker for endothelial cells; *green*) in glioma (*blue*) but not normal brain. High magnifications of the boxed area are shown on the right. Scale bar, 50 μm.

3. GPIHBP1 expression is associated with decreased GLUT1 expression in mouse gliomas.



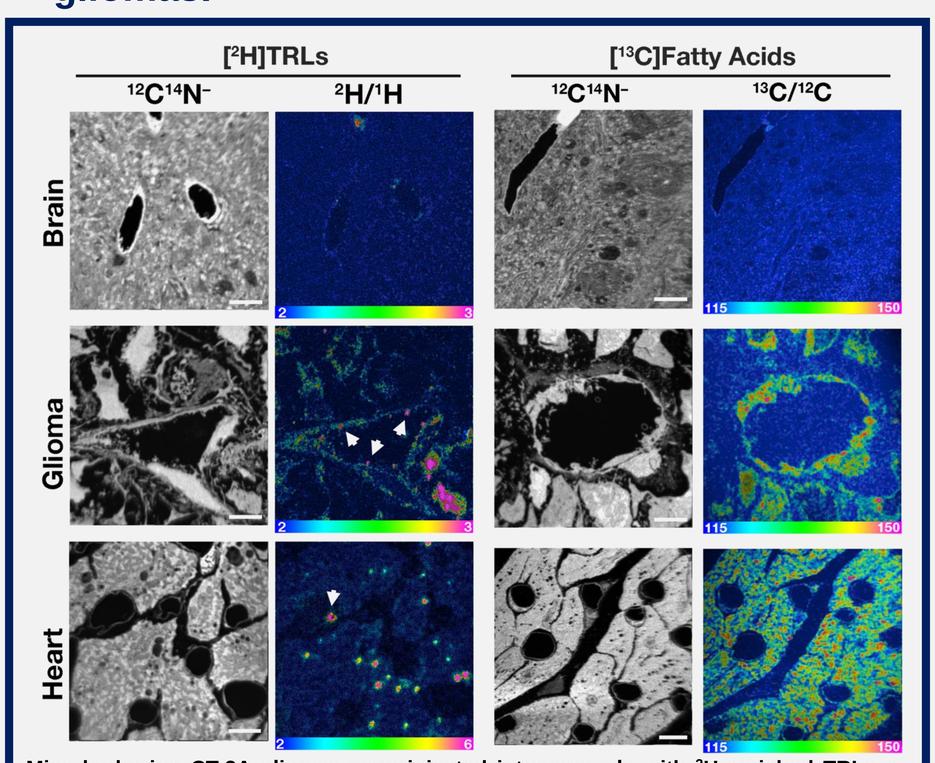
Immunohistochemical staining on a BFP-tagged CT-2A glioma implanted in a mouse brain, showing high levels of GPIHBP1 (*green*) and low levels of GLUT1 (*red*) in capillaries of glioma (*blue*). High magnifications of the boxed white region are shown below. Scale bar, 50 μm.

4. Lipoprotein lipase colocalizes with GPIHBP1 in capillaries of gliomas.



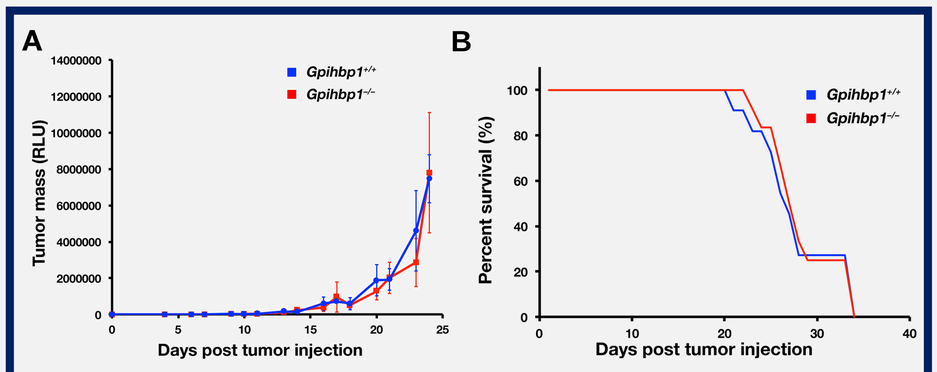
Confocal fluorescence microscopy studies on sections of brain and glioma from wild-type and *Gpihbp1*$^{-/-}$ mice and brain from *Lpl*$^{-/-}$ mice rescued with human LPL driven by a muscle creatine kinase promoter (*Lpl*$^{-/-}$ MCK-hLPL). LPL (*red*) colocalized with GPIHBP1 (*green*) and CD31 (*cyan*) in capillaries of gliomas, but not in normal brain. GPIHBP1 and LPL were not detected in capillaries of gliomas in *Gpihbp1*$^{-/-}$ mice. DNA was stained with DAPI (*blue*). Scale bar, 20 μm.

5. NanoSIMS imaging reveals TRL margination and high levels of fatty acid uptake in mouse gliomas.



Mice harboring CT-2A gliomas were injected intravenously with ^{2}H-enriched TRLs or given ^{13}C-labeled fatty acids by gastric gavage. Tissues were then prepared for NanoSIMS imaging. $^{12}C^{14}N^{-}$ images show tissue morphology; $^{2}H/^{1}H$ ratio images depict uptake of [^{2}H]TRLs-derived nutrients into surrounding tissue after 30 min; $^{13}C/^{12}C$ ratio images depict uptake of ^{13}C-labeled fatty acids into the surrounding tissue. Scale bars, 4 μm.

6. Glioma studies in *Gpihbp1*$^{+/+}$ and *Gpihbp1*$^{-/-}$ mice.



CT-2A glioma cells that were stably transfected with a Gaussia luciferase reporter were injected intracranially into *Gpihbp1*$^{+/+}$ (blue) and *Gpihbp1*$^{-/-}$ (red) mice (n = 11/group). (A)Tumor growth in live animals was assessed by measuring luciferase activity in the blood. (B) Survival curves of mice (mice were euthanized when they lost > 20% of their body weight). No significant differences were observed in either study. A Student's *t*-test was performed, assuming both equal variance and unequal variance. Both tests yielded a p-value of 0.33. Means ± SDs are shown.

7. Conclusions

- GPIHBP1 is expressed in capillary endothelial cells of mouse and human gliomas, but not normal brain.
- Expression of GPIHBP1 in glioma vasculature is associated with decreased expression of GLUT1.
- Lipoprotein lipase is present in capillary endothelial cells of mouse gliomas, but not in normal brain.
- The expression of GPIHBP1 in capillaries was associated with increased uptake of fatty acids into glioma cells.
- There were no significant difference in tumor growth and survival between *Gpihbp1*$^{+/+}$ and *Gpihbp1*$^{-/-}$ mice.