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Novel cryo-imaging of the glioma tumor microenvironment reveals migration and dispersal pathways in vivid three-dimensional detail

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Abstract

Traditional methods of imaging cell migration in the tumor microenvironment include serial sections of xenografts and standard histological stains. Current molecular imaging techniques suffer from low resolution and difficulty in imaging through the skull. Here we show how computer algorithms can be used to reconstruct images from tissue sections obtained from mouse xenograft models of human glioma and can be rendered into 3-D images offering exquisite anatomic detail of tumor cell dispersal. Our findings identify human LN-229 and rodent CNS-1 glioma cells as valid systems to study the highly dispersive nature of glioma tumor cells along blood vessels and white matter tracts *in vivo*. This novel cryo-imaging technique provides a valuable tool to evaluate therapeutic interventions targeted at limiting tumor cell invasion and dispersal.

Keywords

Brain/central nervous system cancers; imaging of tumor progression and metastasis; animal models of cancer; glioma; dispersal; cell migration; glioblastoma multiforme

Introduction

Glioblastoma multiforme (GBM) is a devastating disease characterized by necrotic primary tumor centers with robust neovascularization (1) surrounded by pseudo-palisades of migrating cells (2). In fact, infiltration of tumor cells as far away as 4cm from the primary tumor has been observed (3). Migrating or dispersing GBM cells follow characteristic pathways along secondary structures including perivascular, perifascicular, perineural and neuronophagic, subpial or “surface”, and intrafascicular growth (4). Perivascular growth is

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the most common individual form observed, while the most common combination of structures observed in a single tumor is perivascular, neuronophagic and subpial growth (4). The type and location of the dispersed cells are likely influenced by the location of the primary neoplasm itself. Given that migration of tumor cells appears to be a primary feature of GBM neoplasia, a better understanding of the regulation of this migration is in order.

The current cellular theory of GBM dispersal into the surrounding tissue involves cellular detachment from the primary tumor, attachment to and degradation of the extracellular matrix (ECM), and finally migration (5). A number of molecules have been implicated in regulating these processes in *in vitro* experiments, including receptor tyrosine kinases and phosphatases, cell adhesion molecules, and proteases (5, 6).

To identify the molecular regulation of GBM cell infiltration *in vivo*, accurate models of GBM dispersal need to be developed. To date, the best animal models that recapitulate the main features of GBM are spontaneous brain tumors observed in dogs (7). Spontaneous, transgenic, xenograft and syngeneic tumor models in rodents have also been characterized histologically (7–9). From these studies, various cell lines have been identified that mimic elements of human GBM pathology. For instance, human tumor-derived U-87 MG cells injected intracranially into C57/B6 mice are highly angiogenic (7), while rat tumor-derived CNS-1 cells injected into Lewis rats mimic the pseudopalisading and hemorrhaging of GBM tumors in addition to being infiltrative (7, 9).

In this article, we describe the analysis of mouse orthotopic xenograft tumors using a novel 3-D imaging technique developed in the laboratory of Dr. David Wilson at Case Western Reserve University. The Case Cryo-Imaging System acquires high-resolution anatomic color, cellular and molecular fluorescent images then three-dimensionally reconstructs a tissue or an entire organism, such as a mouse. Cryo-imaging is unique among all *in vivo* and microscopic techniques in that it allows micron-level resolution and information-rich contrast over large 3-D fields of view. We used the system to analyze the 3-D extent of cell migration and dispersal from orthotopic glioma tumors along blood vessels and white matter tracts within the brain.

We recently described the development of algorithms to reconstruct the 3-D architecture of blood vessels and tumor cell dispersal within the mouse brain (10). We used those algorithms to characterize how commonly used human (Gli36Δ5, U-87 MG, LN-229) and rodent (CNS-1) glioma cell lines disperse in the mouse brain. In this manuscript, we provide a complete 3-D analysis of the dispersal and migration of these four tumor cell lines on blood vessels and white matter tracts. Our studies suggest that LN-229 and CNS-1 are effective cell lines to use to study the dispersive nature of tumor cells along both blood vessels and white matter tracts, while Gli36Δ5 cells are not dispersive *in vivo* and instead stay associated with the primary tumor. U-87 MG cells showed limited dispersal only along blood vessels. Our data suggest that either the human LN-229 cell line or the rat CNS-1 cell line in mouse xenograft models of glioma are the most appropriate for future studies investigating the molecular regulation of tumor cell dispersal along particular anatomical structures within the brain. These xenograft systems evaluated with the Case Cryo-Imaging System will allow for future testing of therapeutics aimed at blocking GBM tumor cell dispersal.

Materials and Methods

Orthotopic xenograft intracranial tumors

Human LN-229 and U-87 MG glioma cells were obtained from American Type Culture Collection (Manassas, Virginia). CNS-1 rodent glioma cells were obtained from Mariano S.

Viapiano (11). Human Gli36Δ5 glioma cells constitutively overexpress the vIII mutant forms of the EGFR gene (12) and were obtained from E.A. Chiocca. The CNS-1 and Gli36Δ5 cell lines were authenticated by Research Animal Diagnostic Laboratory at the University of Missouri (Columbia, MO) for interspecies and mycoplasma contamination by PCR analysis. 5–8 week old NIH athymic nude mice (20–25 g each) were housed in the Athymic Animal Core Facility at Case Western Reserve University according to institutional policies. All animal protocols were approved by the IACUC.

Gli36Δ5, U-87 MG, CNS-1 or LN-229-GFP cell lines were infected with GFP encoding lentivirus, harvested for intracranial implantation by trypsinization and concentrated to 1×10^5 cells per μL of PBS. Mice were anesthetized by intraperitoneal administration of 50mg/kg ketamine/xylazine and fitted into a stereotaxic rodent frame (David Kopf Instruments, Tujunga, California). Cells were implanted at AP= +0.5, ML= −2.0 from bregma at a rate of 1 μL /minute in the right striatum at a depth of −3 mm from dura. For examination of dispersal along white matter tracts, cells were implanted at a depth of −2 mm from dura for tumor formation in close vicinity to the corpus callosum. 50,000 to 200,000 cells were implanted per mouse.

Mice were sacrificed 7–38 days after implantation, based on tumor burden. Dissected brains were embedded in Tissue-Tek OCT compound (Sakura Finetek U.S.A., Inc. Torrance, CA), frozen in a dry ice/ethanol slurry and transferred to the stage of the cryo-imaging device.

Cryo-imaging of tissue samples

Three brains implanted per cell type with either LN-229, CNS-1, U-87 MG or Gli36Δ5-GFP were analyzed. Frozen brains were alternately sectioned and imaged using the cryo-imaging system at a section thickness of 15–40 μm and a resolution of $11 \times 11 \times 15 \mu\text{m}$ or $15.6 \times 15.6 \times 40 \mu\text{m}$. The cryo-imaging system consists of a mouse-sized stage on a motorized cryostat with special features for imaging, a modified bright-field/fluorescence microscope and a robotic *xyz* imaging-system positioner, all of which are fully automated. The system images fluorescent agents or cells at very high resolution and sensitivity. Brightfield and fluorescence images were acquired for each of the brains using a low light digital camera (Retiga Exi, QImaging Inc., Canada), GFP fluorescence filters (Exciter: HQ470/40x, Dichroic: Q495LP, Emitter: HQ500LP, Chroma, Rockingham, VT) and an epi-illumination fluorescent light source (XCite 120PC, EXFO, Canada).

Image processing algorithms for visualization of tumor cells and vasculature

We recently developed methods for segmentation and visualization of the vasculature, main tumor mass, and dispersing cells (10). To segment the main tumor mass, we used a 3-D seeded region growing algorithm recently developed (10). Results were reviewed in individual slice images and manually edited if necessary. To segment dispersed tumor cells and clusters, we applied a high pass filter and thresholded the result, excluding the binary volume consisting of the main tumor. To create a 3-D volume of the brain vasculature, brightfield images from each brain specimen were processed using algorithms developed for vessel edge detection and volume rendering (10). These algorithms resulted in a 3-D reconstruction of the brain vasculature. The lower limit for the blood vessel detection algorithm was ~ 30 μm diameter vessels. 3-D pseudo-colored volumes were created which included the main tumor (green), dispersing cells (yellow), and vasculature (red). The location of dispersed cells was visually inspected by rotation of the composite 3-D volume within Amira (Visage Imaging Inc., San Diego, CA) to confirm a distinct non-fluorescent region separating dispersing cells from the main tumor and to identify cells in close proximity to blood vessels.

Dispersal on white matter

The corpus callosum white matter was manually segmented using brightfield cryo-images and Amira Software. The segmented region was reconstructed as a 3-D volume that was merged with the 3-D tumor and dispersed cell volumes. The white matter was pseudo-colored gray, and the dispersed cells in contact with the white matter were pseudocolored magenta, whereas all other dispersing cells were pseudocolored yellow.

Results

Orthotopic xenograft models of glioma cells in rodents are useful for assessing tumor growth characteristics and response to therapeutics. Gli36Δ5 is a human glioma cell line that grows rapidly to form a large tumor within two weeks (12). However, the tumors become encapsulated and cell dispersal from Gli36Δ5 tumors was never observed, as shown in 2-D histological sections (Fig. 1 a, b). Xenografts of U-87 MG human glioma cells also grow rapidly as closely associated cells with defined margins (Fig. 1 c, d). The LN-229 human glioma cell line exhibits slower growth characteristics and examination of orthotopic xenografts in histological sections revealed that LN-229 cells disperse from the main tumor at 4–6 weeks post-implantation (Fig. 1 e, f). The CNS-1 glioma cell line was developed in the inbred Lewis rat and exhibits very rapid growth (11). Within one week following implantation, these tumors were highly vascularized, the cells of the tumor were loosely associated and individual cells had dispersed from the entire perimeter of the main tumor (Fig. 1 g, h). By ten days of growth *in vivo*, the dispersed cells were observed throughout the entire frontal lobe of the hemisphere surrounding the main tumor (data not shown).

Perivascular growth is the most common form of glioma dispersal (4). To examine whether these cell lines dispersed along blood vessels, histologic sections of GFP-expressing tumor xenografts of U-87 MG (Fig. 2 a–d), LN-229 (Fig. 2 e–h) and CNS-1 (Fig. 2 i–l) cells were immunolabeled with the endothelial cell specific antibody CD-31. U-87 MG tumor cells dispersed along blood vessels in close proximity to the main tumor (Fig. 2 a–d), however the extent of dispersal was minimal. In contrast, LN-229 (Fig. 2 e–h) and CNS-1 (Fig. 2 i–k) cell dispersal occurred primarily along blood vessels.

The 2-D histologic sections provided a high-resolution snapshot of a single plane through the tumor of interest. However, 3-D reconstruction of histology images is time-consuming and prone to tissue shrinkage and errors in image alignment. To overcome these obstacles the Case Cryo-Imaging System was used to analyze tumor cell dispersal from orthotopic xenografts of the four glioma cell lines in 3-D at high resolution. The technique utilized bright-field images of the block face for overall brain anatomy and fluorescent images to detect the GFP-expressing glioma cells (10). 3-D volumes were created for the vasculature (pseudocolored red), white matter (pseudocolored gray) and the main tumor mass (pseudocolored green). Glioma cells that were no longer physically connected in any dimension to the main tumor were pseudocolored yellow to indicate tumor cell dispersal. Multiple xenograft specimens of each cell line were analyzed using this technique, and representative examples are shown. The comparison with standard histology images highlights the tumor biology that can be visualized using this novel technique.

Gli36Δ5 cells are non-dispersive

The growth of Gli36Δ5 xenografts was rapid, resulting in a large encapsulated mass by 2 weeks after implantation (arrow in Fig. 3 a). 3-D cryo-image analysis of Gli36Δ5 tumors showed average tumor volume was 40.57 mm³ (n=3). Tumor growth resulted in the compression of surrounding brain structures (Fig. 3 a). In addition, 3-D reconstruction of the brain vasculature indicated that although the tumors were highly vascularized, cell dispersal

was never observed (Fig. 3 b, c), which supported previous observations in 2-D histologic sections (Fig. 1a, b).

U-87 MG cells are marginally dispersive along blood vessels

In U-87 MG xenografts, tumor cells dispersed along blood vessels in close proximity to the main tumor. This dispersal pattern was observed in both 2-D histologic sections (Fig. 2 a–d), and 3-D tumor reconstructions from cryo-image analysis (Fig. 3 e–f). Analysis of the 3-D tumors indicated the average dispersed cell volume was 0.0028 mm^3 , and average tumor volume was 1.86 mm^3 ($n=3$). Therefore, the dispersed cells represented only 0.15% of the total tumor cell population. However, the cells dispersed up to three hundred microns from the main tumor (Supplementary Fig. 1). Although U-87 MG tumors exhibit somewhat limited dispersal, they may be good models to analyze effects of chemotherapeutics on reduction of tumor load or migration on blood vessels.

LN-229 is a dispersive human glioma cell line

In comparison with Gli36Δ5 or U-87 MG, the LN-229 xenografts grew at a slower rate to form an average tumor load of 2.76 mm^3 ($n=3$) after 4–6 weeks. 2-D histologic analysis revealed cell dispersal along the length of the tumor (Fig. 1 f), often in association with blood vessels (Fig. 2 e–h). Analysis of 3-D cryo-image volumes illustrated that LN-229 cells frequently disperse as connected strands for several hundred microns along blood vessels (Fig. 4 c, d). It is unclear whether these vessels were pre-existing or due to tumor-mediated angiogenesis. Small populations of cells released all along the main tumor to migrate through the brain parenchyma (Fig. 4 b, f) and (10). These cells may also be dispersing along blood vessels that are below the limits of detection with our analysis algorithms ($<30 \text{ }\mu\text{m}$ diameter). Overall, the average dispersed cell volume was 0.035 mm^3 , which represents 1.26% of the total tumor cell population. LN-229 cells were observed to reach the lateral ventricle from the main tumor in some cases, resulting in spread to distant regions of the brain via passive movement in the cerebrospinal fluid (data not shown). In those instances, the cells became imbedded in and spread along the meninges covering the brain.

The rat glioma cell line, CNS-1, is highly dispersive in vivo

The CNS-1 cell line spread aggressively to infiltrate distant regions of the brain from the tumor within 7–10 days post-implantation. The main tumor consisted of loosely associated, pseudopallisading cells (Fig. 1 g, h), with features of hemorrhage and necrosis (arrow in Fig. 5 a, e). Due to the rapid dispersal of CNS-1 cells, the volume of the main tumor at 7 days was only 1.49 mm^3 ($n=3$). However, the dispersed cell volume was 0.18 mm^3 , which represents 10.6% of the total tumor cell population detected by 3-D cryo-image analysis. CNS-1 cells migrated extensively along blood vessels, as shown in the 3-D reconstructions from seven-day tumors (Fig. 5 d, h). In addition, CNS-1 cells readily dispersed through the brain parenchyma (Fig. 1 h). Supplementary video 1 shows an example of CNS-1 cell dispersal around the entire perimeter of the main tumor. Dispersed cells are clustered on blood vessels in close proximity to the tumor edge as well as streaming along vessels at a distance.

We analyzed the dispersal distance of CNS-1, LN-229 and U-87 MG cells using a 3-D morphologic distance algorithm that detects the presence of voxels containing fluorescent cells in a series of dilations from the tumor edge outward (10). The voxel size was $11 \times 11 \times 15$, approximately the size of single cells. The first two dilations closest to the tumor were discarded to reduce nonspecific error. The results from this analysis indicate that LN-229 and CNS-1 xenografts generate thousands of dispersive cells (Supplementary Fig. 1), which represent a 13 to 28-fold increase in total number of dispersed cells respectively when compared to the U-87 MG xenografts. The maximum distance traveled by LN-229

cells was 562 μ m away from the tumor edge, whereas CNS-1 cells dispersed more than 3 mm from the tumor (Supplementary Fig. 1).

LN-229 and CNS-1 cell dispersal along white matter tracts

One of the pathways used by human glioma cells for dispersal is intrafascicular growth on white matter. We analyzed the ability of the four cell lines to utilize white matter for dispersal. Neither Gli36 Δ 5 nor U-87 MG cells dispersed on the corpus callosum, a large white matter structure in close proximity to the main tumors. U-87 MG cells within the main tumor were observed to realign along the longitudinal axis of the corpus callosum (Fig. 6 a, b). In all three xenografts examined by 3-D cryo-image analysis, the U-87 MG tumor bulged out onto the corpus callosum (arrow in Fig. 6 g). Thus, these cells appear to respond to cues on the surface of the myelinated fibers. However, individual U-87 MG tumor cells did not disperse on the white matter (Fig. 6 g).

LN-229 and CNS-1 cell lines dispersed along the corpus callosum as single cells or small cell clusters, as shown in histologic sections (Fig. 6 c–f). Individual cells were observed to exit the main tumor to disperse directly onto the white matter, evidenced in the 3D tumor volumes by the magenta cells at the tumor-white matter transition zone (Fig. 6 h, i). Supplementary video 2 provides a detailed view of cell dispersal on white matter from a CNS-1 tumor. Together, these results provide further support that both LN-229 and CNS-1 cell lines are dispersive in the brain, showing dispersal characteristics comparable to humans, and thus may be good model systems for testing therapeutic efficacy.

Angiogenesis is correlated with tumor growth and dispersal rate

Angiogenesis is a hallmark feature of tumor growth. To determine whether differences in angiogenesis existed between the four glioma cell lines, we used the 3-D cryo-image blood vessel reconstruction volumes to quantitate blood vessel density within each tumor. The most significant increase in blood vessel density occurred in tumors with the fastest growth rate such as Gli36 Δ 5 and U-87 MG, as well as in the most extensively dispersing cells, CNS-1 (Supplementary Table 1).

Discussion

The most useful animal models of human disease exhibit characteristics that are highly representative of those observed in humans. In this manuscript, we characterized the *in vivo* growth and dispersal of four different glioma cell lines using orthotopic xenografts in athymic nude mice. The cryo-imaging and 3-D reconstruction methods described here provide extraordinary insight into tumor growth characteristics within the complex architecture of the brain at single cell resolution that is a significant advancement over standard methods such as histologic sections. Our results also indicate that the best cell lines for studying migration and dispersal in the context of GBM are the LN-229 and CNS-1 cell lines.

Of note, the four cell lines we evaluated in this manuscript showed a gradation of migration within the brain. Gli36 Δ 5 cells do not migrate, U-87 MG cells disperse marginally only along blood vessels, LN-229 cells migrate significantly along blood vessels and white matter tracts, and CNS-1 cells are the most migratory along both these secondary structures. Given that GBM tumor cells show a great range of distances and substrates for dispersal (1, 4), it would be ideal to have a range of cell lines to evaluate migration *in vivo*. Our findings that U-87 MG cells are not highly dispersive *in vivo* are supported by other recent data (13), and suggest that despite its popularity as a GBM tumor model *in vitro*, U-87 MG cells are not the best *in vivo* model.

Dispersal of LN-229 and CNS-1 cells along blood vessels suggests that the cells respond to migration promoting cues present on the surface of blood vessels. A number of migration promoting molecules have been identified in dispersive GBM cells *in vivo*. Stromal cell-derived factor 1 α (SDF-1 α) and its receptor CXCR4 (14), Na⁺/H⁺ exchanger regulatory factor 1 (NHERF-1) (15), ephrin B2, ephrin B3 and the EphB2 receptor (16–18) mRNA and/or protein are all elevated in dispersive GBM cells versus core GBM tumor cells. Additional molecules, such as the cleaved extracellular fragment of the receptor tyrosine phosphatase, PTP μ (19), the Type I receptor of the TNF superfamily TNFRSF19/TROY (20), Neuropilin 1 and its ligand Sema3A (21), and the vitronectin receptor, Nectin5 (22) are also elevated in GBM tumor tissue, although not necessarily in dispersive cells. Only SDF-1 expression has specifically been localized to GBM secondary structures, while its receptor CXCR4 is expressed on migrating glioblastoma cells themselves (14).

All of the aforementioned molecules regulate GBM cell migration *in vitro*, as demonstrated in matrigel invasion or 2-D migration assays (14–18, 20, 21, 23, 24), or 3-D matrix spheroid assays (15, 22). *Ex vivo* models evaluating human GBM cell migration on rodent brain slices have also been employed to investigate the function of specific molecules in GBM dispersal (16–18, 20, 23). In addition to the different *in vitro* and *ex vivo* assays performed, a wide variety of GBM cell lines were evaluated in these studies, including, but not limited to, A172 (21, 22), U-87 MG (14, 16–18, 20, 21, 24), T98G (15, 16, 20) and SNB19 cells (16, 20). The reason for selecting these different cell lines differs, but most often is based on the expression level of a gene or protein of interest.

Both cellular and molecular differences exist between the tumor cells found within the main GBM tumor (or “core”) and those cells that have migrated away from the core (or “edge”) (25). Given this fact, it is important for us to develop means of studying dispersal as a separate event from the primary tumor growth and survival. The methodology that we present here and in Qutaish et al. allows for the evaluation of migrating and dispersing cells *in vivo* at single cell resolution. Tumor models such as the ones described here will be increasingly important to achieve the goal of understanding cell dispersal at a molecular level and evaluation of therapeutics targeting GBM dispersal.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Glioblastoma xenograft tumor growth characteristics in 2-D. Histologic sections of GFP-expressing tumor xenografts of Gli36 Δ 5 (a, b), U-87 MG (c, d), LN-229 (e, f) or CNS-1 cells (g, h) were stained with hematoxylin and eosin to demarcate the tumor borders within the brain parenchyma. Gli36 Δ 5 (a, b) and U-87 MG (c, d) cells remain tightly associated within encapsulated tumors. Cells disperse along the length of LN-229 tumors (e, f). CNS-1 tumors consist of more loosely associated cells that migrate readily through the brain parenchyma (g, h). Scale bar represents 200 μ m.

Figure 2.

Tumor cell dispersal on blood vessels in 2-D. Histologic sections of GFP-expressing tumor xenografts of U-87 MG (a–d), LN-229 (e–h) and CNS-1 (i–l) cells were immunolabeled with the endothelial cell specific antibody CD-31 (c, g, k). Brightfield images from the same tissue sections stained with hematoxylin and eosin are shown in (a, e, and i). U-87 MG cells (a–d) dispersed along blood vessels that were in close proximity to the main tumor. In contrast, LN-229 (e–h) and CNS-1 (i–l) cells dispersed as streams of cells along vessels, often great distances from the main tumor mass. Scale bar represents 100 μm.

Figure 3.

3-D reconstruction of Gli36Δ5 and U-87 MG tumors indicates limited cell dispersal. Mouse brains containing xenografts of GFP-expressing Gli36Δ5 (a–c) or U-87 MG (d–f) cells were cryo-imaged and reconstructed in 3-D to show the main tumor mass (pseudo-colored green), dispersed tumor cells (pseudo-colored yellow) and vasculature (pseudo-colored red). 2-D block face images are shown in (a, d), with the tumor indicated by an arrow. 3-D reconstruction of the same brain specimens shows that no cell dispersal was observed from the Gli36Δ5 tumor despite its large size (b, c). In contrast, cells from the U-87 MG tumor dispersed on a nearby blood vessel (e, f). Scale bars represent 500 μ m.

LN-229

Figure 4.

3-D reconstruction of LN-229 tumors demonstrates cell dispersal on blood vessels in the tumor microenvironment. Mouse brains containing xenografts of GFP-expressing LN-229 glioma cells were cryo-imaged and reconstructed in 3-D to show tumor (pseudo-colored green), dispersed tumor cells (pseudo-colored yellow) and vasculature features (pseudo-colored red). Examples of 4 week (a–d) and 6 week (e–h) LN-229 tumors are shown. 2-D block face images are shown in (a, e), with the tumor indicated by an arrow. 3-D reconstruction of the same specimens illustrates cell dispersal from several regions of the tumors (b, f). Cell dispersal primarily occurred along major blood vessels in close proximity to the tumors (c, g). Panel D shows a higher power view of the tumor shown in (c). Panel H shows a higher power view of the top of the tumor shown in (g). Note the clustering of dispersed cells on multiple blood vessels. Scale bars represent 500 μm in (b, f) and 100 μm in (d, h).

Figure 5.

3-D reconstruction of CNS-1 tumors demonstrates they are highly dispersive along blood vessels. Mouse brains containing seven day xenografts of GFP-expressing CNS-1 glioma cells were cryo-imaged and reconstructed in 3-D to show the main tumor mass (pseudo-colored green), dispersed tumor cells (pseudo-colored yellow) and vasculature (pseudo-colored red). Two examples of CNS-1 tumors are shown (a–h). 2-D block face images are shown in (a, e), with the tumor indicated by an arrow. Vascular density and hemorrhaging within the tumor are visible (a, e). 3-D reconstruction of the same specimens illustrates dramatic cell dispersal from all surfaces of the tumors (b, f). CNS-1 cells were typically clustered on blood vessels near the tumor surface, and dispersed along vessels (c, g, h), to great distances away from the main tumor mass (d). Scale bars represent 500 μm in (b, f) and 100 μm in (d, h). See also Supplementary video 1 for reconstruction of CNS-1 cell dispersal around the entire perimeter of the main tumor.

Figure 6.

3-D reconstruction of U-87 MG, LN-229 and CNS-1 cell dispersal on white matter tracts in the brain. Histologic sections from mouse brains containing xenografts of GFP-expressing U-87 MG (b) LN-229 (d) or CNS-1 (f) glioma cells were examined for cell dispersal. Brightfield images from the same tissue sections stained with hematoxylin and eosin are shown in (a), (c) and (e), respectively. U-87 MG cells within the main tumor realigned along the longitudinal axis of the corpus callosum (a, b), a major white matter tract in the brain. However, individual cells did not disperse onto the white matter. LN-229 cells separated from the main tumor to disperse as single cells on the corpus callosum (d). The cells of a CNS-1 tumor were more loosely associated, but were observed to reorient to align with the corpus callosum for dispersal (f). Mouse brains containing xenografts of GFP-expressing U-87 MG (g), LN-229 (h), or CNS-1 (i) glioma cells were cryo-imaged and reconstructed in 3-D to show the main tumor mass (pseudo-colored green), dispersed tumor cells (pseudo-colored yellow) and white matter of the corpus callosum (pseudo-colored gray). Dispersed cells in contact with white matter were pseudo-colored magenta (h, i). Scale bars represent 500 μ m. See also Supplementary video 2 for a detailed view of cell dispersal on white matter from a CNS-1 tumor.