Imaging (glioma) tumor microvasculature

Cyril Petibois, Yeukuang Hwu ...etc.
CNRS UMR 5248 CBMN, University of Bordeaux, France
Institute of Physics, Academia Sinica, Taiwan R.O.C.
Glioma tumors need angiogenesis to grow over a few mm$^3$

**Glioma = most lethal cancer in humans (with PDAC & HCC)**
- Angiogenesis, tumor growth & diffusion still impossible to control by chemo
- Numerous & tortuous blood vessels, but BBB maintained

**Microvasculature morphology aberrations are characteristic of glioma tumors**
Glioma tumors need fibrous matrix for growth

Normal brain tissue

- Very low collagen amount in ECM of brain parenchyma
- Significant presence = only in BM of capillaries

Glioma Tumor tissue

- Tumor cells produce collagens
- Accumulation in tumor areas
- Plays important role in tumor growth
- Type I present in newly formed tumors
- Type IV = basic component of BM

Collagens are molecular markers for differentiation of brain tumors vs. healthy tissues
**Collagens in human glioma tumors**

<table>
<thead>
<tr>
<th>Collagen</th>
<th>Gene expression in gliomas</th>
<th>Ref</th>
<th>Protein expression in gliomas* a(b)c:</th>
<th>** Fold induction</th>
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<tbody>
<tr>
<td>COL1A1</td>
<td>Up-regulated in 80 % GBM</td>
<td>[18, 153]</td>
<td>7(7) 11 a(b)c</td>
<td>&gt;10</td>
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<td>COL1A2</td>
<td>Up-regulated in glioma, associated with survival</td>
<td>[153, 154]</td>
<td>&gt;2</td>
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<td>COL2A1</td>
<td></td>
<td></td>
<td>&gt;NC</td>
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<tr>
<td>COL3A1</td>
<td>Up-regulated in glioma, associated with survival</td>
<td>[153, 154]</td>
<td>12(0)12</td>
<td>&gt;15</td>
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<td>COL4A1</td>
<td>Up-regulated in glioma, associated with survival</td>
<td>[13, 19, 153, 154]</td>
<td>6 (6) 12</td>
<td>&gt;3</td>
</tr>
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<td>COL4A2</td>
<td>Up-regulated on glioma progression, associated with survival</td>
<td>[13, 19, 153, 154, 164, 165]</td>
<td>11 (1) 12</td>
<td>&gt;5</td>
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<td>COL4A6</td>
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<td>&lt;0.30</td>
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<td>COL5A1</td>
<td>Highly expressed in primary GBM</td>
<td>[19]</td>
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<td>COL6A1</td>
<td>Up-regulated according to glioma grade</td>
<td>[153]</td>
<td>4 (4) 12</td>
<td>3</td>
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<td>Highly expressed in glioma</td>
<td>[19, 24, 166]</td>
<td>10 (10) 11</td>
<td>3</td>
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<tr>
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<td>Increased expression in glioma</td>
<td>[153]</td>
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<td>3</td>
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<td>7 (7) 12</td>
<td>&gt;2</td>
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<td>&gt;5</td>
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<td>COL11A1</td>
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<td>NC</td>
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<td>COL12A1</td>
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<td></td>
<td>2 (2) 12</td>
<td>No report</td>
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<td></td>
<td>&gt;3</td>
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<td></td>
<td>4 (4) 12</td>
<td>0.12</td>
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<tr>
<td>COL16A1</td>
<td>Up-regulated in GBM and linked to glioma invasion</td>
<td>[119, 128]</td>
<td>NC</td>
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<tr>
<td>COL18A1</td>
<td></td>
<td></td>
<td>1( 1) 9</td>
<td>0.3</td>
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Several collagens are over-produced in gliomas of different grades
**Objective**

Unraveling the relationships between fibrous matrix network and micro-vasculature in the development of glioma tumors

Angiogenesis → unique vasculature organization / phenotype / organ

Vascular density & fenestration = key-features for tumor Dvpt

Vascular BM is modified / controlled by tumor cells

Tumor cell ECM = bed for division and invasion

ECM = defense barrier for tumor cells

**Interface between BM and ECM = matrix barrier in tumor micro-environment**
What do we need?

3D imaging of vascular network organization coupled to molecular/chemical imaging of tumor ECM and BM components
3D imaging of microvasculature: Why X-Ray tomography?

No technique able to describe the full 3D organization of glioma tumor microvasculature in situ or in vivo

For both experimental setups, the vasculature imaging is obtained only at short penetration depth from brain surface, limited to a maximum of 1 mm.

Top: Multiphoton microscopy of a mouse brain microvasculature after removing the skull of the animal and labeling of vascular endothelial cells (500 µm deep).

Down: Photoacoustic microscopy imaging of cortex vasculature after removal of skull (1 mm deep).
3D imaging of microvasculature: Why X-Ray tomography?

True 3D confocal imaging technique with Au-NP as imaging contrast agents

**Main performances:**
- ZP = 100 nm spatial resolution
- FOV = 1 cm$^3$
- SR = monochromatic X-Rays
- CCD = imaging

**Example of an implanted solid tumor:**
2D patch X-Ray images of mice head (top-left) are 2*3 cm (66 Mpix) allow zooming on the tumor area for further 3D X-Ray tomography imaging (images selected at different angles: 0 to 160°) of the tissue interface between healthy and tumor volumes.

Final zooming on a tomography view (bottom right) shows functional and anatomical details of tumor and healthy tissues vasculature.

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3D imaging of microvasculature: Multimodal Au-NP as imaging contrast agents

For combining/comparing results from X-Ray tomography, histology, & FTIR imaging

Multimodal Au-NP for imaging tumor microvasculature. From left to right: size distribution of Au-NP; epifluorescence image of cells incubated with Au-NP; epifluorescence image of implanted tumor (after dissection); and FTIR image of tumor tissue section. Biotechnol Adv 2012

Tumor models: solid, diffuse, and infiltrative gliomas

Histopathology of 3 glioma phenotypes after xenotransplantation of U87ctrl, U87dn and U87dn+IL-6 (IRE1 dominant negative + ectopic expression of IL-6) cells in the mouse brain.
3D imaging of microvasculature: Solid tumor analysis
3D imaging of microvasculature: X-Ray tomography of diffuse glioma tumors

Example on diffuse model of glioma tumor

(a and b) Immunofluorescent labeling using anti-vimentin (tumor cells). (Scale bars: 200 μm.) (c and d) Edges of growing tumors, as depicted by detection of vimentin and of CD31 (endothelial cells). (Scale bars: 50 μm.) (e–g) Detail of U87dn cell invasion alongside blood vessels. (Scale bars: 50 μm.)

Other models available: solid, diffuse & mixed; with GFP (tumor) and RFP (vascular endothelial cells)

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3D imaging of microvasculature: Functional features of glioma tumor microvasculature

Determining morphological quantities to characterize each glioma tumor phenotype

Morphological characterization per phenotype
**3D imaging of microvasculature: X-Ray tomography of micro-tumors**

**Imaging the smallest glioma tumors in mice models (solid & diffuse)**

For animal biology research, new way to characterize micro-tumors at unprecedented analytical performances

**Quantitative analysis of vasculature**

![Graphs showing tumor volume growth over time](image)

Typical limit for μ-PET
Summary: X-Ray tomography of glioma tumors

Allows determining glioma tumor microvasculature with spatial resolution < 1 µm

Small animal imaging achieved with full 3D rendering of brain

3D image reconstruction allows extracting pertinent information on micro-vasculature

Micro- to large glioma tumors can be imaged = kinetic studies on tumor growth

Perspectives:

In vivo imaging of glioma tumors?

Kinetic studies on vascular perfusion of tumors

Must be coupled to molecular imaging for functional studies
Imaging of collagens: Analyzing collagens in tissues

Basic features
Fibrillar molecules, non water-soluble, network organization (several types), no specificity in histology, ubiquitous in most tissues = analytical challenge

- **Histological examination**
  - No information about the concentration of molecular contents
  - Depends on labels specificity (very poor between collagen types)
  - BV shape is also tortuous – 2D histology rendering not always adequate

- **Microscopic techniques**
  - E.M.
  - A.F.M.
  - 2-photon microscopy

Can provide information about collagens organization or morphology, but not able to differentiate between collagen types
Imaging of collagens: Analyzing collagen types according to their secondary structure

Triple helix = 3 α-chains

Most abundant collagen types present in tissues

Collagens can be differentiated based on their secondary structure = suitable for vibrational spectroscopy-based techniques (FTIR & Raman)
**Imaging of collagens: Discriminating collagen types by FTIR spectroscopy**

**Differentiation between collagen types**

<table>
<thead>
<tr>
<th>Collagens</th>
<th>α-helix</th>
<th>Triple helix</th>
<th>β-sheet</th>
<th>β-turn</th>
<th>U.S.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I</td>
<td>24.1 ±1.1</td>
<td>21.7 ±1.2</td>
<td>24.7 ±1.5</td>
<td>21.5 ±0.6</td>
<td>8.0 ±0.6</td>
</tr>
<tr>
<td>Type III</td>
<td>22.1 ±1.3</td>
<td>21.5 ±1.1</td>
<td>26.4 ±1.6</td>
<td>21.8 ±1.2</td>
<td>8.2 ±0.5</td>
</tr>
<tr>
<td>Type IV</td>
<td>21.1 ±1.1</td>
<td>19.1 ±1.6</td>
<td>28.1 ±1.5</td>
<td>21.9 ±1.8</td>
<td>9.8 ±0.9</td>
</tr>
<tr>
<td>Type V</td>
<td>23.4 ±1.7</td>
<td>21.6 ±1.2</td>
<td>23.8 ±1.1</td>
<td>24.1 ±1.3</td>
<td>7.1 ±0.7</td>
</tr>
<tr>
<td>Type VI</td>
<td>22.2 ±1.3</td>
<td>14.3 ±0.8</td>
<td>32.6 ±1.2</td>
<td>20.2 ±1.4</td>
<td>10.7 ±0.6</td>
</tr>
</tbody>
</table>

Similar IR spectra but different secondary structure profiles discriminate collagen types.

Anal Bioanal Chem 395 (2009) 829-837
Imaging of collagens: Discriminating glioma tumors based on capillary BM components

Analysis of glioma tumor capillaries (BM) with FTIR imaging

Visible (A) and full spectral intensity (B) images of transversal blood capillaries in grade III (left) and grade IV (right) human glioblastoma. The selected BM FT-IR spectra are averaged before spectral curve-fitting (C) (1800–1500 cm$^{-1}$) to determine the secondary structure of proteins.

FTIR imaging of BM allows grading glioma tumors (humans & animal models)
Imaging of collagens: Analyzing extravascular diffusion with FTIR imaging

Analysis of extravascular diffusion based on Au-NP perfusion (mice model)

➢ Solid tumor perfused with Au-NP (neoangiogenesis)

➢ Diffuse tumor perfused with Au-NP (co-option angiogenesis)
Imaging of collagens: Analyzing collagen contents in glioma tumors with FTIR imaging

Spectral curve fitting of amide I region (1720-1600 cm\(^{-1}\) )

Difference of absorption intensity for triple helix (1637 cm\(^{-1}\) ) band in healthy tissues
Imaging of collagens: Collagens distribution in glioma tumors

Based on the 1637 cm$^{-1}$ absorption band of triple helix, collagen biodistribution can be determined in mice tumor models.

![Image showing healthy tissue, solid tumor, and diffuse tumor with visible and infrared images.](image)

Allows delimitating tumor volume, thus potentially useful for pathologists.
Imaging of collagens: Collagens types in glioma tumors

Differentiation between solid and diffuse tumor tissues

Several parameters discriminate S & D tumors
Imaging of collagens: Collagens types in glioma tumors: multifactorial approach

Distribution of tissues vs. secondary structure parameters

Healthy tissues opposite to triple helix parameter = no collagen content
Imaging of collagens: Collagens types in glioma tumors: multifactorial approach

Distribution of tumors vs. secondary structure parameters

Symmetric plot (axes F1 and F2: 87.00%)

- F1 (76.32%)
- F2 (10.68%)

Solid tumors – coll type 1, 3, 5
Diffuse tumors – coll type 4 & 6

Most abundant types / tumor model
Summary: Multimodal imaging of glioma tumor development

- Au-NP and XRT for tumor localization
- μPET with Avastin treatment
- FTIR imaging of collagens
- FTIR imaging of Au-NP
Conclusion & perspectives

Combining X-Ray tomography & FTIR imaging (and further X-Ray fluorescence microscopy) sheds a new light on glioma tumor development. Allows addressing fundamental issues of cancer biology.

Interface between fibrous matrix and vasculature is the micro-environment of glioma tumors, with functional control of BBB for blood nutrients supply

- this functional interface also exists for PDAC and HCC (other lethal cancers in humans), but with differences (fenestration, vessels density, fibrous matrix...etc.)

Depicting fibrous matrix development for each glioma tumor phenotype

- useful for further proposing adapted anti-angiogenic strategies targeting given collagen type(s)

Multimodal imaging of tumor development (glioma, PDAC, HCC) of the scientific core of the coming international laboratory between France and Taiwan (Exp. starting 2013).