Hepatic sinusoid and tumour microvessels imaging by micro computed tomography in a hepatic metastasis mouse model

Poster No.: C-1949
Congress: ECR 2013
Type: Scientific Exhibit
Keywords: Radiation physics, Abdomen, Anatomy, Experimental, Physics
DOI: 10.1594/ecr2013/C-1949

Any information contained in this pdf file is automatically generated from digital material submitted to EPOS by third parties in the form of scientific presentations. References to any names, marks, products, or services of third parties or hypertext links to third-party sites or information are provided solely as a convenience to you and do not in any way constitute or imply ECR's endorsement, sponsorship or recommendation of the third party, information, product or service. ECR is not responsible for the content of these pages and does not make any representations regarding the content or accuracy of material in this file.

As per copyright regulations, any unauthorised use of the material or parts thereof as well as commercial reproduction or multiple distribution by any traditional or electronically based reproduction/publication method is strictly prohibited.

You agree to defend, indemnify, and hold ECR harmless from and against any and all claims, damages, costs, and expenses, including attorneys' fees, arising from or related to your use of these pages.

Please note: Links to movies, ppt slideshows and any other multimedia files are not available in the pdf version of presentations.

www.myESR.org
Purpose

Microanatomy of hepatic lobule

The liver consists of variable microstructures, the functional unit of which is a conical microvascular subunit of the classic lobules named 'hepatic microvascular subunits'. Classically, this functional unit of the liver is the hexagonal lobule with the terminal hepatic vein (central vein) surrounded by 6 portal tracts. Currently, hepatic lobules are considered a complicated group that includes hepatocytes, macrophages, central vein, portal triads, and other factors. These lobules and their microvascular subunit structures are dependent on hepatic sinusoids.

Key events in the process of hepatic metastasis

Hepatic sinusoids are the main targets for liver metastasis

Tumor cells approach the liver tissue through the finer branches of the portal vein, where they are trapped in either the finest branch, or in the portal hepatic sinusoids

Specific adhesion of tumor cells within the hepatic microcirculation and active extravasation of the surviving cancer cells through the damaged hepatic endothelium occurs

Purpose

This study was designed to demonstrate the feasibility of micro CT imaging for the identification of tumor vessels in hepatic sinusoid in a hepatic metastasis mouse model
Fig. 1: Hepatic Lobules (hexagonal units) 1. Hepatocytes & Macrophages Hepatocytes: liver cells, cuboidal epithelium, radiating plates Macrophages (Kupffer cells) line the sinusoids, are phagocytic 2. Sinusoids, capillaries with open walls, allowing proteins to enter the blood from hepatocytes 3. Central vein middle of lobule, drains the sinusoids 4. Triads a. Hepatic portal veins = groups of 3 vessels at edges of the lobules drain blood into lobule from intestinal area, bringing both nutrients & toxins b. Hepatic artery brings blood from aorta to liver via celiac branch, delivers oxygen & fats c. Bile ducts take bile formed by hepatocytes to the gall bladder & duodenum

© Radiology, Wonkwang University School of Medicine - Iksan/KR
Methods and Materials

Animal and tumor model

Twelve female BALB/c mice (6 weeks old, weighing 20 - 25g)

Fifteen BALB/C mice were induced with hepatic metastases using an injection of a murine colonic adenocarcinoma cell line (colon 26).

The CT-26 murine colon adenocarcinoma cell line was acquired from ATCC (Manassas, VA). The cells were grown as monolayer cultures in Dulbecco's Modified Eagle Medium with 10% fetal bovine serum (Invitrogen, Grand Island, NY) and supplemented with 1% glutamine, and 1% antibiotics.

The cells were maintained in a 37°C incubator with 5% CO2-humidified air. CT-26 cells (5 × 10^3 in 0.1 mL phosphate-buffered saline) were injected into the mesenteric vein using a 30-gauge needle after laparotomy.

Five mice with normal livers were used as controls.

All animal studies were carried out in accordance with the regulations set forth by the institutional review board of our university.

Intravital fluorescent microscopy

Intravital fluorescence optical microscopy using a fluorescent microscope (BX51WI, Olympus, Japan) was performed on exteriorized livers.

In brief, high-molecular-weight (HMW; 2000kDa) fluorescein isothiocyanate (FITC)-labeled dextran (Sigma-Aldrich, St. Louis, MO, USA) was injected into the tail vein (0.1 mL, 25 mg/mL). After injection (contrast enhancement by staining blood plasma), the hepatic micro-vessels were observed on a fluorescence intravital microscope.

The morphology and microvasculature of the liver tissue was observed according to reported techniques. The hepatic sinusoidal structure in the normal hepatic parenchyma and tumor microvessels were observed by the microscope.
The analysis of microvessel density was performed using MATLAB 7.1 software (The MathWorks, Inc. MA, USA).

**Micro CT system**

A compact CT scanner for non-invasive imaging with sub-micrometer resolution.

The X-ray source is an open tube type (transmission) with a tungsten target.

The minimum and maximal focal spot size of the tube is 1 um and 3 um, respectively.

The X-ray detector contains a high-resolution CCD camera with 4008 × 2672 pixels and straight fiber-optic coupling with a columnar CsI:TI scintillator with 100 um thickness.

Images were acquired at 80 kVp, 150 mA, and 5-second per frame for 360 views.

Radiation dose is approximately 6.9 mGy.

3D-rendering software (Lucion, Infinitt Ltd, Seoul, Korea) was used.

**Micro CT imaging**

The 15 hepatic metastases-burdened mice underwent micro CT scanning 15 days after cell inoculation when their tumors had grown to between 100 and 3000 um in diameter.

After surgical preparation for the micro-CT, we injected a contrast medium suspension containing barium sulfate (Solotop sol. 140 gm/mL, Taejoon Ltd, Seoul, Korea) to visualize hepatic microcirculation in the portal vein of the exteriorized livers.

We used 10 × magnifications to view images of different acini and sinusoids in the liver.

After localizing one target area, we took 360 images of the acini to generate CT images of the liver.
Image analysis

Microvascular structure and changes according to tumor size

2D plane maximum-intensity projection (MIP) and 3D volume-rendered images

Quantification of microvascular structure

- Diameter of intratumoral vessels from the portal vein supply
- Intratumoral vessel density from portal perfusion
- Volume analysis software (VG studio Max, Heidelberg, Germany).

Histopathology

The excised liver was fixed in 10% formalin after in vivo microscopy and micro CT and, embedded in paraffin; then 5-um serial sections were cut for hematoxylin-eosin (H&E) staining

The liver samples of the mice were minced to smaller than 0.2 mm3 on a glass plate on ice and prefixed with 3% glutaraldehyde, 0.2 M sucrose, 0.1 M K-phosphate buffer (pH 7.4), and 1 mM NaN3 for 2 h at 4°C. The post-fixation was performed with 1% osmium tetroxide for 2 h at 4°C.

The number of hepatic metastases were counted and their size measured.

The diameter was defined as the longest distance across the tumor.

Statistical analysis

Statistical analysis was performed using SPSS software (version 11.5, SPSS, Inc., Chicago, IL).

Comparisons of the diameter of intratumoral vessels and intratumoral vessel density according to tumor size were done using pearson correlation coefficient and paired t-tests.
For all the statistical analyses, a P value less than .05 was considered to indicate a statistically significant difference.
Fig. 2: Micro CT system

© Radiology, Wonkwang University School of Medicine - Iksan/KR

Fig. 3: Intravital fluorescence optical microscopy using a fluorescent microscope (BX51WI, Olympus, Japan) was performed on exteriorized livers.

© Radiology, Wonkwang University School of Medicine - Iksan/KR
Results

Normal sinusoid imaging on IVS and micro CT

On IVS, the morphology of hepatic sinusoids was observed.

Interconnecting network of sinusoids from portal venules to the central venule were identified. The features regarding sinusoids correlated well with micro CT findings.

On micro CT with 3-D volume rendering techniques, the hepatic microvascular unit was seen as a linear tubular vascular structure resembling a "fernbrake leaf," which was a group of sinusoids supplied by portal venules.

Histopathologically, the networks of sinusoids were corresponded to micro CT and intravital fluorescence microscopy images.

Histopathologic findings of metastatic liver tumors

All animals survived long enough to complete the full protocol. Mesenteric vein injection of $5 \times 10^3$ CT-26 cells in the BALB/C mice reproducibly generated multiple liver tumors in the 15 mice. At day 15 after cell injection, 116 tumors larger than 200 um and smaller than 3000 um were observed.

Microvasculature from portal perfusion in metastatic tumors

Diameter of hepatic sinusoids and intratumoral vessels from portal supply are shown in Fig. 6 and Table. 1.

The mean diameter of the normal hepatic sinusoid was 11.7 um. The diameter of intratumoral vessels by portal vein supply revealed an increasing tendency from 201 um to 1500 um tumor size, and a decreasing tendency from 1500 um to 3000 um tumor size ($r^2 = 0.204, p < 0.01$).

Tumoral vessel diameter of 301-500 um tumor size was significantly larger than those of 201-300 um ($P<0.001$), and those of 1500-3000 um tumor size was significantly smaller than those of 801-1500 um tumor size ($P <0.02$).
Intratumoral vessel density from portal supply according to tumor size are shown in Fig. 7 and Table. 2.

The mean intratumoral vessel density were 38.2 % from 201 um to 300 um in tumor size and 25.5 % from 801 um to 1,500 um in tumor size, and 2.5% from 1,500 um to 3,000 um in tumor size.

The mean intratumoral vessel density supply from the portal vein gradually decreased from 201 um to 1500 um tumor size ($r^2 = 0.584$, $p < 0.01$).

The intratumoral vessel density abruptly collapsed in tumors more than 1500 um in size. Tumoral vessel density of 1501-3000 um size metastases were significantly lower than those of 801-1500 um ($P <0.0001$).

**Microvasculature from portal perfusion in metastatic tumors**

Micro-CT showed a high capacity for detecting changes in the microvessels of metastatic tumors.

Compared to intravital fluorescence microscopy and H&E photomicroscopic images, micro CT images clearly revealed highly proliferating intratumoral vessels with irregular distorted and bizarrely dilated microvasculature in mouse livers.

In addition, micro CT images showed intratumoral vessels and neighboring hepatic sinusoids with communicated vessels, which provided much better informations than those of H&E microscopic images.
Images for this section:

**Fig. 4:** Intravital fluorescence microscopy of hepatic venule and sinusoids. The sinusoids course was interconnecting networks and toward the central venule.

© Radiology, Wonkwang University School of Medicine - Iksan/KR

**Fig. 5:** 3-D volume rendering techniques. The hepatic microvascular unit was seen as a linear tubular vascular structure resembling a "fernbrake leaf," which was a group of sinusoids supplied by portal venules.

© Radiology, Wonkwang University School of Medicine - Iksan/KR
Fig. 6: Diameter of hepatic sinusoids and intratumoral vessels from portal supply

The mean diameter of the normal hepatic sinusoid was 11.7 um. The A. diameter of intratumoral vessels by portal vein supply revealed an increasing tendency from 201 um to 1500 um tumor size, and a decreasing tendency from 1500 um to 3000 um tumor size ($r^2 = 0.204$, $p < 0.01$). B. Tumoral vessel diameter of 301-500 um tumor size was significantly larger than those of 201-300 um (P

© Radiology, Wonkwang University School of Medicine - Iksan/KR
Table 1: Diameter of hepatic sinusoids and intratumoral vessels from portal supply

<table>
<thead>
<tr>
<th>Tumor size (µm)</th>
<th>No. of Vessels (measured)</th>
<th>Vessel diameter (µm) (mean ± standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal sinusoid</td>
<td>15</td>
<td>11.711±4.532</td>
</tr>
<tr>
<td>201–300</td>
<td>17</td>
<td>16.046±7.224</td>
</tr>
<tr>
<td>301–500</td>
<td>70</td>
<td>23.741±12.355</td>
</tr>
<tr>
<td>501–800</td>
<td>135</td>
<td>29.130±12.344</td>
</tr>
<tr>
<td>801–1500</td>
<td>55</td>
<td>27.427±8.603</td>
</tr>
<tr>
<td>1501–3000</td>
<td>18</td>
<td>17.775±6.554</td>
</tr>
</tbody>
</table>

© Radiology, Wonkwang University School of Medicine - Iksan/KR
Fig. 7: A. The mean intratumoral vessel density were 38.2 % from 201 um to 300 um in tumor size and 25.5 % from 801 um to 1,500 um in tumor size, and 2.5% from 1,500 um to 3,000 um in tumor size. The mean intratumoral vessel density supply from the portal vein gradually decreased from 201 um to 1500 um tumor size ($r^2 = 0.584$, $p < 0.01$). B. The intratumoral vessel density abruptly collapsed in tumors more than 1500 um in size. Tumoral vessel density of 1501-3000 um size metastases were significantly lower than those of 801-1500 um size (P

© Radiology, Wonkwang University School of Medicine - Iksan/KR
<table>
<thead>
<tr>
<th>Tumor size (µm)</th>
<th>No. of tumor</th>
<th>Vessel density % (mean ± standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>201-300</td>
<td>14</td>
<td>38.217±9.453</td>
</tr>
<tr>
<td>301-500</td>
<td>25</td>
<td>35.047±9.784</td>
</tr>
<tr>
<td>501-800</td>
<td>47</td>
<td>31.940±14.794</td>
</tr>
<tr>
<td>801-1500</td>
<td>20</td>
<td>25.516±15.965</td>
</tr>
<tr>
<td>1501-3000</td>
<td>10</td>
<td>2.568±3.313</td>
</tr>
</tbody>
</table>

**Table 2:** Intratumoral vessel density from portal supply according to tumor size

© Radiology, Wonkwang University School of Medicine - Iksan/KR
Fig. 8: Intratumoral vessel images on intravital fluorescence microscopy (A), micro CT maximum intensity projection image (B) and 3D volume rendering image (C), and photomicroscopic image of H&E stain (D) of metastatic tumor (size, 402 μm) in a mouse liver. The highly proliferating intratumoral vessels with irregular distorted and bizarrely dilated microvasculature in the mouse liver are clearly visible. On 3D CT image, the intratumoral vessels were distinctly comparable to normal sinusoids.

© Radiology, Wonkwang University School of Medicine - Iksan/KR
**Fig. 9:** Photomicroscopic image of H&E stain (A) and micro CT maximum intensity projection image (B) and 3D volume rendering image (C) of metastatic tumor (size, 850 um) on mouse liver. The high vascular density and highly proliferating intratumoral vessels with irregular distorted and bizarrely dilated microvasculature in mouse liver are seen very clearly.

© Radiology, Wonkwang University School of Medicine - Iksan/KR
Fig. 10: Photograph of H &E stain and micro-CT findings of metastatic tumors 261 um (A), 316 um (B), 510 um (C), 662 um (D), and 712 um (E) in size in mice liver. This figures showed tortuous proliferated and irregularly dilated intratumoral vessels from portal supply. (Scale bar: 200 um)

© Radiology, Wonkwang University School of Medicine - Iksan/KR
Fig. 11: Photograph of H &E stain and micro-CT findings of metastatic tumors 951 um (A), 1126 um (B), 1220 um (C), 1417 um (D), and 2656 um (E) in size in mice liver. Note that intratumoral vessels from portal supply collapsed in tumors more than 1500 um in size (E). (Scale bar: 200 um)

© Radiology, Wonkwang University School of Medicine - Iksan/KR
Conclusion

The hepatic sinusoids are capillaries with open walls, allowing proteins to enter the blood from hepatocytes. These structures play a major role in metastasis in the liver. Some researchers have demonstrated the value of in vivo microscopy when applied to the study of the microcirculation of hepatic metastases. Ideally, 3D micro CT images can be defined as an imaging platform aimed at understanding cellular events by providing the possibility for collecting new qualitative and quantitative information in a large sample volume.

Colorectal cancer is one of the most common malignancies in the western world with approximately ~500,000 casualties each year. Mortality is significantly associated with a high incidence of metastasis in the liver, which is the first site of disease spread.

Hepatic metastasis is based on a multi-step process characterized by a series of structural, cellular and molecular events.

The following key events in the process of hepatic metastasis occur within the liver sinusoids.

Initially, tumor cells approach the liver tissue through the finer branches of the portal vein, where they are trapped in either the finest branch, or in the portal hepatic sinusoids.

Subsequently, specific adhesion of tumor cells within the hepatic microcirculation and active extravasation of the surviving cancer cells through the damaged hepatic endothelium occurs.

In the early stages, hepatic metastases show direct portal perfusion with hepatic sinusoid-like intratumoral vessels. The size of the tumors at this stage was approximately 201-500 mm.

With increasing metastases, microvessels began to appear in the tumor.

These intratumoral microvessels were similar to the surrounding hepatic sinusoids with respect to shape, and they anastomosed with the sinusoids. These phenomena indicate that at this stage hepatic metastases derive their blood supply directly from the portal vein.

At the second stage, hepatic metastases at least double their blood supply with lumped intratumoral microvessels. Tumors at this stage were roughly 501-1500 mm. When metastases become larger, the intratumoral microvessels increased and they were distributed in a spatially heterogeneous fashion. In addition, they appeared tortuous and developed irregular branching and abrupt diameter changes to form irregularly dilated
blood spaces. These convoluted microvessels formed irregular networks in the tumor. These findings mean that, at this stage, the hepatic metastases derive their blood supply from the portal vein.

During the third stage, hepatic metastases show slight portal blood supply with perforated intratumoral microvessels. With further growth to a massive size, the portal vein decreased in size in the tumoral parenchyma regardless of the dense inflow into the surrounding hepatic sinusoids. The size of the tumors that showed this pattern were 1501-3000 mm. During this stage, intratumoral microvessels were considered an increase in arterial supply during the reduction in portal vein supply.

These results of our serial observation were identical findings to other research studies; the hepatic sinusoids in the tumor take part in the tumor-sprouting angiogenesis process, the portal blood flow is important in small metastases, and the hepatic artery plays a predominant role in supplying large metastatic tumors.

In conclusion, the morphological characteristics of tumoral microvessels of hepatic metastases could be observed by micro CT. The images of the hepatic sinusoid and tumoral microvessels in the hepatic metastases murine model obtained using this method showed excellent histopathological correlation. This imaging study is a breakthrough in the research of liver tumors and this will facilitate novel targeted cancer therapies in the future.
References

1. McCuskey R. Morphological mechanisms for regulating blood flow through hepatic sinusoids. Liver 2000;20; 3-7


