Statistical Tracking of Murine Liver Metastases using a Hepatocyte-Selective Contrast Agent in Conjunction with MicroCT

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Introduction

Recent reports from our lab have shown that ITG, an iodinated hepatocyte-selective contrast agent, used in conjunction with microCT is an effective method for identifying and roughly quantifying hepatomas in murine tumor models. This study aims to improve upon the method of quantifying net tumor volume and also to track tumor growth (and eventually treatment) serially in living mice.

In studies of live mice without contrast, a histogram of voxel densities consistently generates four identifiable peaks (Figure 3). Analysis by selective regions of interest (ROI's) matches these peaks to air, lungs, soft tissue, and bone. A retrospective study of 12 mice has shown that in each case there is an additional peak when ITG was present (Figure 4). This study will utilize Fenestra™ LC (Alerion Biomedical Inc., San Diego, CA), the novel commercial anologue of ITG.

Preliminary studies in a single mouse have shown that liver volume can accurately be assessed by calculating the area under the ITG peak by integration, and multiplying the result by voxel size. A comparison of two such calculations (in-vivo and ex-vivo liver) shows good precision (<10% error) in this method.

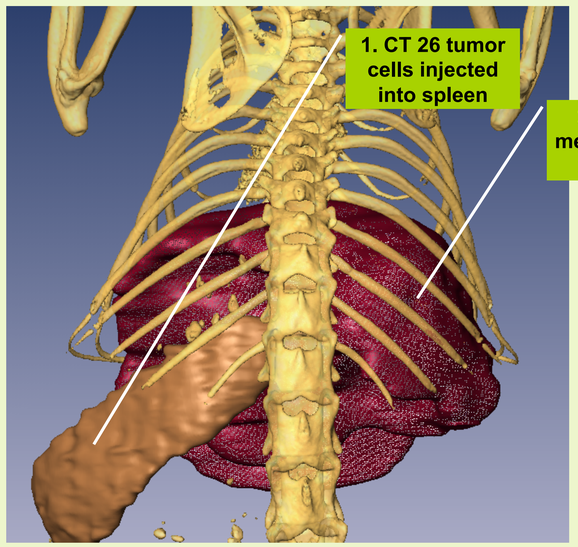

Figure 1: MicroCT of mouse abdomen with GE eXplore Locus RS at 93 micron resolution.

Procedure

Five mice were inoculated with 5e5 CT26 tumor cells (50 uL by volume) by direct splenic injection (Figure 1). The mice were scanned at days 0, 4, and 8 with microCT (GE eXplore Locus, 80 kVp, 93 um resolution). Gas anesthetic (1.5% isoflurane) was used during the inoculation and during each scan. The mice were euthanized at day 9 for gross pathological inspection. Each scan was enhanced by the contrast agent Fenestra™ LC (0.3 mL/20 g bw), injected by tail vein four hours prior to scan.

Using MicroView (GE Healthcare, Waukesha, WI), a volumetric ROI was defined to include the entire liver (Figure 2). A histogram of the ROI was rendered (Figure 3), making it possible to easily identify the peak associated with Fenestra™, and analyze the peak using the built-in Bone Mineral Density tool. Mean peak values and standard deviations were assessed for each mouse over the eight-day scanning period. To verify that the range of the peak includes the liver, MicroView has a tool that allows the user to highlight the corresponding voxel values in the image. The values recorded were averaged and plotted on a relative scale from 0 to 1.

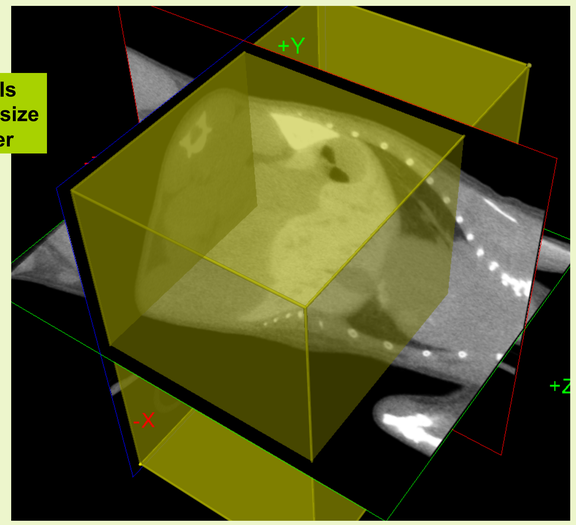

Figure 2: 3D ROI selection in MicroView. ROI includes the entire liver.

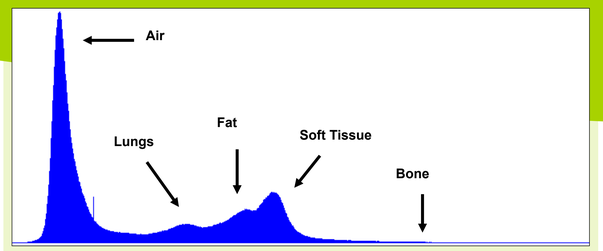

Figure 3: Histogram (frequency) of voxel densities in a mouse microCT volume. No was contrast used.

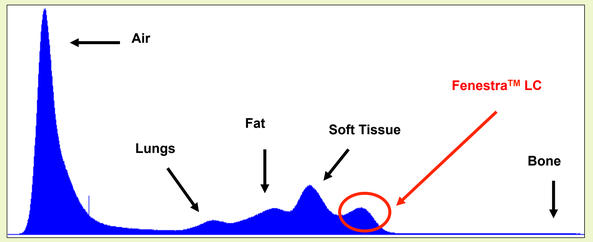

Figure 4: Histogram of volume acquired 4 hours after 0.3 mL Fenestra injected. Calculated standard deviation of peak is

Results

The standard deviation of the voxel densities in the liver showed marked increase (average ~50 HU SD) over the eight day period (Figure 5). While five mice is not enough to show statistical significance, correlation between metastatic progression and increased standard deviation in tissue density with contrast.

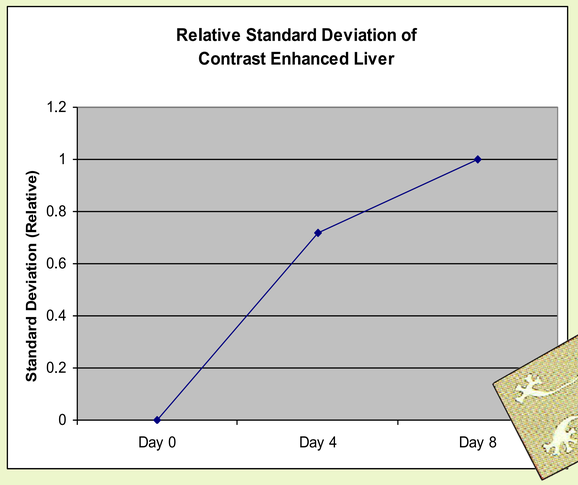

Figure 5: Standard deviation of voxel densities increased over a period of eight days.

Discussion

The results of this pilot study show the likelihood of correlation between progression of liver metastases and the standard deviation of a peak representing liver contrast agent.

The broader implication of this study is that it may be possible to track metastatic progression despite the fact that the metastases are too small to resolve, or too many to count.

Although it is impossible to establish statistical significance with only five mice, this technique of using numerical peak analysis to detect sub-resolution changes may have interesting implications in medical imaging. One notable example might be the ability to track progression and treatment of angiogenesis, despite the fact that many angiogenic vessels are beyond the resolution of the scanner.

Key References

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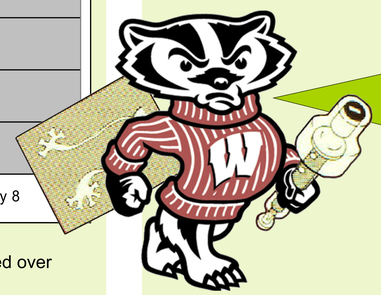

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