Histopathologic and immunohistochemical analysis of acute microwave thermal ablation zones in lung and liver

Zixuan Wang[1], Margaret A. Highland[1], Punit Prakash[2], Charan K. Ganta[3]

Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine[1], Department of Electrical and Computer Engineering[2], Kansas State University, Manhattan, KS; National Institute of Environmental Health Sciences[3], Raleigh, NC

Introduction

- Microwave thermal ablation (MTA) is an emerging therapy technique as an alternative to surgical resection for various tumor types.
- Depending on the organ, MTA can be performed percutaneously or in open surgery, and sometimes paired with laparoscopy and endoscopy[1,2].
- Energy and time are the most important parameters of MTA.
- Insufficient MTA will leave greater risk of tumor recurrence, yet excessive MTA will cause undesirable damage to the surrounding normal tissue.
- Zones of thermal ablation were previously[3] defined as: complete ablation zone, transition zone, and periphery (morphologically normal).
- Cell fate analysis is critical for evaluation of MTA safety and efficacy. Location of the margin between viable and non-viable cells is an important indicator of the destructive effect of specific microwave power-time combination.
- Regular H&E staining has limited use when cell morphology is relatively conserved after acute MTA.
- Triphenyl Tetrazolium chloride (TTC) and Nitro Blue Tetrazolium (NBT) are reduced into colored pigment by functional cellular dehydrogenase enzymes and their cofactors (NADH and NADPH). Intensity of the chromogenic reaction is a relatively reliable indicator of cell viability after MTA[3].
- Vimentin is a type of intermediate filament expressed in cells of mesenchymal origin, and its structural intactness might reflect the level of cellular damage.
- High mobility group box 1 (HMGB1) and Heat shock proteins (HSP) are damage-associated molecular patterns (DAMPs) that are released by heat-induced necrosis.

Objective

- To compare different staining methods in determining cell integrity and viability after acute MTA in normal lung and liver.

Material and Methods

Microwave Thermal Ablation (MTA)

- MTA power was supplied by a 2.45-GHz generator (SAIREM) through a water-cooled ablation applicator developed at KSU[1].
- *In vivo* MTA was performed on lung and liver of domestic female pigs (45-50kg) in previous studies.[1,2] Animals were euthanized after ablation and the tissue was fixed in 10% neutral buffered formalin and embedded in paraffin blocks.
- For *ex vivo* MTA, fresh bovine liver from a local slaughter house was kept in a cooler with ice over night and cut into 8 × 8 × 8 cm^3 blocks. 60W MTA was performed for 5 minutes. The block was cut in half along the applicator. Two 3mm-thick axial sections from each side of the central cut were submitted for viability assays (Figure 1a).

Histology and Immunohistochemistry (IHC)

- Sections of 4-5 µm thickness were obtained from formalin-fixed, paraffin-embedded tissue blocks and stained with hematoxylin and eosin (H&E) and IHC antibodies.
- All IHC staining was performed on Leica BOND Rx^m automatic stainer. The following monoclonal antibodies were used for detection of corresponding markers in tissue sections: Vimentin (Leica BOND RTU PA0640), HMGB1 (Abcam Ab11354, 1:25) and HSP70 (Abcam Ab2787, 1:200).
- BOND Polymer Refine Red Detection system (Alkaline Phosphatase/Fast Red) was used for Vimentin, while BOND Polymer Refine Detection system (Horseradish Peroxidase/DAB) was used for HMGB1 and HSP70.

Triphenyl Tetrazolium chloride (TTC) staining

- *Ex vivo* bovine liver slices were submerged in freshly made TTC bath for 1 hour.
- The sections were then fixed in 10% neutral buffered formalin for color enhancement as well as future H&E and IHC staining.

Nitro Blue Tetrazolium (NBT) staining

- Non-ablated liver was heated at 60°C for 15 minutes as negative control.
- Ablated *ex vivo* bovine liver slices were trimmed into quarters, snap-frozen in ethanol-dry ice slurry, recovered to -20°C in cryostat and then cryosectioned at 6µm.
- The sections were then air-dried, incubated in 1mg/ml NBT (Sigma-Aldrich N6876) and 1mg/ml NADH (Sigma-Aldrich N8129) TBS solution (pH=7.35), fixed in formalin, dehydrated in 95% to 100% ethanol, cleared in xylene and coverslipped.

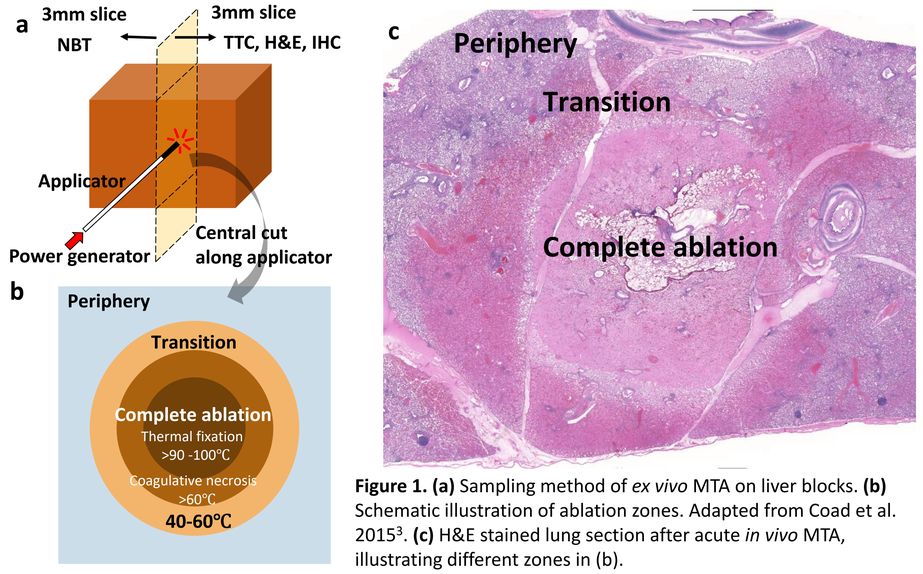

Figure 1. (a) Sampling method of *ex vivo* MTA on liver blocks. **(b)** Schematic illustration of ablation zones. Adapted from Coad et al. 2015[3]. **(c)** H&E stained lung section after acute *in vivo* MTA, illustrating different zones in (b).

H&E and IHC results

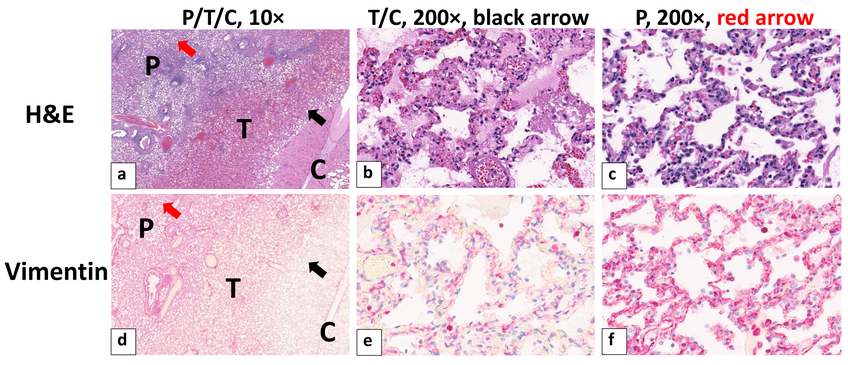

Figure 2. (a) H&E and **(d)** Vimentin staining of lung after acute *in vivo* MTA, showing three ablation zones: Periphery (P), Transition (T) and Complete ablation (C). **(b)(e)** and **(c)(f)** are 200× snapshots taken at the T/C boundary (black arrow) and the periphery (red arrow), respectively.

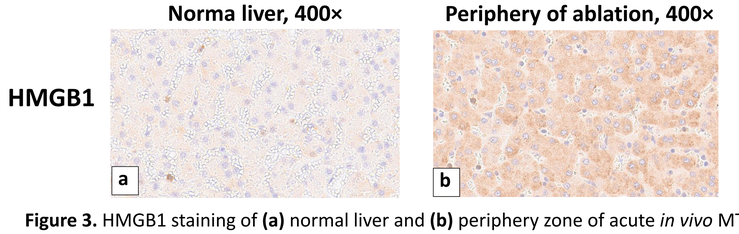

Figure 3. HMGB1 staining of **(a)** normal liver and **(b)** periphery zone of acute *in vivo* MTA

Viability Staining results

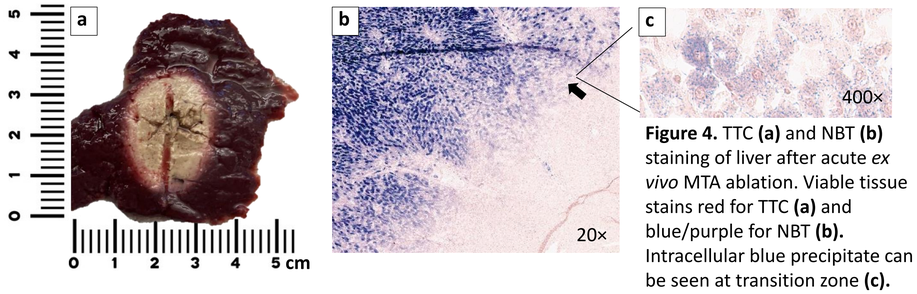

Figure 4. TTC **(a)** and NBT **(b)** staining of liver after acute *ex vivo* MTA ablation. Viable tissue stains red for TTC **(a)** and blue/purple for NBT **(b).** Intracellular blue precipitate can be seen at transition zone **(c).**

Results

- H&E and vimentin staining of ablated lung and liver both yielded well-defined zones of complete ablation, transition and periphery (Figure 1c, Figure 2).
- Various degree of cell shrinkage and pyknosis was noticed in the complete ablation zone and transition zone; prominent hemorrhage was observed around transition zone.
- Cells of mesenchymal origin, including endothelial cells, fibroblasts, smooth muscle cells, lymphocytes and macrophages were stained positive with vimentin in the periphery (Figure 2f).
- HMGB1 staining was not observed in hepatocytes of normal pig liver (positive control), while abundant staining was seen in cytoplasm of peripheral hepatocytes of MTA liver (Figure 3).
- HSP70 staining was reduced in the ablation center, and there was a gradient change towards normal staining intensity through the transition or hemorrhagic zone. HSP70 was also detected in normal pig liver.
- TTC and NBT staining both yielded positive (dark red and blue/purple, respectively) result for non-ablated liver as well as the periphery of ablated liver.
- For TTC staining, there was a red-pink-pale color transition towards the ablation center. The diameter of the pale center is about 2cm. (Figure 4a)
- For NBT staining, blue precipitate was absent in ablation center and transitioned to dark blue/purple in periphery (Figure 4b). Precipitate dots could be observed inside individual cells at higher magnification in the transition zone (Figure 4c).

Discussion/Conclusion

- The staining pattern change of selected proteins suggests direct cellular alteration and injury caused by extreme heat.
- Vimentin as a intracellular structural protein could provide better information than regular H&E in regard to cellular integrity (Figure 2). However, the relationship between vimentin staining intensity and cell viability is unknown.
- The presence of HMGB1 in cytoplasm of peripheral hepatocytes (Figure 3b) might be caused by its translocation from nucleus to cytoplasm, indicating its structural alteration as well as the ongoing degenerative and inflammatory process in the periphery. However, an adequate positive control is needed to confirm the true staining in ablated liver.
- HSPs can prevent cellular proteins from denaturation under stressful conditions including heat. It is not clear whether HSP70 expression was upregulated after acute MTA in the periphery zone.
- TTC and NBT provides the most reliable information of cell viability. However, the destiny of weakly stained cells in the transition zone is still not clear.
- Combining H&E, IHC, TTC and NBT staining together is a promising tool for optimal setup of MTA parameters in order to achieve highest safety and efficacy of tumor treatment.

Future Study

- HMGB1 and HSP staining will be performed on further periphery as well as other types of tissue to fortify the explanation of staining results.
- Samples from different ablation zones of *ex vivo* ablated liver has been submitted for Transmission Electron Microscopy (TEM) imaging.
- *Ex vivo* MTA experiment will be repeated and optimized for statistical analysis and future acute/survival *in vivo* MTA studies.
- Acute and survival *in vivo* MTA will be performed for comparison of ablation size, morphology, viability and IHC properties.

References

1. Sebek, J. et al. (2020), Bronchoscopically delivered microwave ablation in an *in vivo* porcine lung model, ERJ Open Research, In press -- accepted for publication.
2. Pfannenstiel, A., Sebek, J., Fallahi, H., Beard, W. L., Ganta, C. K., Dupuy, D. E., & Prakash, P. (2020). Directional Microwave Ablation: Experimental Evaluation of a 2.45-GHz Applicator in Ex Vivo and In Vivo Liver. *Journal of Vascular and Interventional Radiology*.
3. Beatty, C. J., Thomsen, S. L., Vos, J., & Coad, J. (2015, March). Practical pathology for thermal tissue applications. In *Energy-based Treatment of Tissue and Assessment VIII* (Vol. 9326, p. 932602). International Society for Optics and Photonics.

Acknowledgements

- This study was generously supported by Boehringer Ingelheim Veterinary Scholars Program and KSU Veterinary Research Scholars Program.
- Research funding was provided by NIH grant R01 CA218357.
- Special thanks to Shawna Fitzwater and Jennifer Phinney at Kansas State Veterinary Diagnostic Lab for their expertise in immunohistochemistry and frozen section technique.
- Special thanks to staff at VDL Histopathology Lab for their assistance with my experiment and feedback for my poster design.