Autophagy Contributing to Tumor Recurrence after Radiofrequency Ablation in a Clinically Relevant Murine Model of HCC

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Hepatocellular carcinoma (HCC) is the second leading cause of cancer death worldwide and continues to increase in the west, particularly in the United States [1]. HCCs lead to approximately 700,000 deaths annually worldwide [2, 3]. The clinically available therapeutic options are limited and prognosis is extremely poor. Radiofrequency ablation (RFA) [4] is a minimally invasive treatment and emerging as the first therapeutic option for primary and metastatic liver cancer when the patients are not suitable candidates for surgical resection. Unfortunately, local recurrence and distant metastasis after RFA has been an issue, especially with larger tumors. The underlying mechanism of tumor recurrence after RFA is not entirely clear. An increasing number of studies have demonstrated that heat stress-induced the generation of heat shock proteins (HSPs), facilitates recovery of tumor cells from heat damage [5] by acting autophagy signaling. Hsp90 is a member of HSPs superfamily. Hsp90 has been reported to play a critical role in cellular autophagy via regulating the stability and activity of autophagy-related protein [6, 7]. Further studies to explore how RFA affects autophagy and impact HCC tumor relapse might provide significant targets which could be used to improve RFA in the treatment of HCC.

Recently, we developed a clinically-relevant murine model of HCC in which histologically-normal hepatocytes from transgenic mice are transplanted into liver of the recipient immunocompetent mice with progressive hepatic fibrosis and develop into discrete foci of HCC. Using this successful strategy, half million hepatocytes isolated from SV40 T antigen transgenic mice were orthotopic inoculated into wild type C57BL/6 mice by intra-spleen injection (ISPL). Tumor initiation and progression in the mice were monitored by magnetic resonance imaging (MRI) after two months. The mice with similar tumor size were selected and divided into two groups: one without treatment, one receiving RFA. With optimized parameters of 75°C for 60s, RFA were performed in the mice [8]. Seven days after RFA, all mice were terminated, tumor tissue were collected and used for IHC and electron microscope imaging following the instruction of EMC in University of Missouri-Columbia. Imaging with JEOL JEM-1400 Transmission Electron Microscope was used to detect the alteration and autophagy of different cell types in tumors. The IHC was performed following standard protocol.

On day 7 post RFA, macroscopic detection observed obvious tumor necrosis within tumor induced by RFA (Figure 1a), which were further validated by H & E staining (Figure 1b). Antibodies-mediated IHC staining (Figure 2a) indicated RFA treatment induced dramatic upregulation of HSP90 in tumors. Electro-microscopy is able to detect typical autophagy structures which present in mice from control and experiment groups. But more autophagy cells were observed in in RFA-treated mice (Figure 2b). These preliminary results suggest that effective RFA drives abundance of Hsp90 in tumors which is companioned with the occurrence of more autophagy tumor cells. Hsp90 or autophagy might be a useful targets which could be modulated to improve RFA in the treatment of HCC [9].
References:

[9] This work was supported by a funding “EXCELLENCE in ELECTRON MICROSCOPY AWARD” from Electron Microscopy Core, University of Missouri-Columbia,

Figure 1. RFA induced tumor necrosis in HCC orthotopic murine model. a. 7 days after RFA performance, tumor necrosis is shown on the tumor mass (black arrow). b. H&E staining show RFA induced tumor cell necrosis (black arrow), scale on the images: 100 µM.

Figure 2. RFA upregulates autophagy through Hsp90 in HCC tumor microenvironment. a. dramatically increased Hsp90 expression is shown by IHC staining with Hsp90 antibody (CST. #4877). b. autophagy structure (black arrow) under electron-microscopy, scale on the images: 2 µM.