Cerenkov Radiation Energy Transfer Imaging Combined with probe-based confocal laser endomicroscopy for precise image-guide tumor resection

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Background: Cerenkov luminescence imaging (CLI) has been extensively studied in the area of image-guided tumor resection surgery. However, the extending of CLI application remains challenging due to the CLI signal intensity is weak and the penetration ability is insufficient. Here, we used the clinical contrast agent fluorescein sodium to enhance the CLI signals through the Cerenkov Radiation Energy Transfer (CRET). Moreover, probe-based confocal laser endomicroscopy (pCLE) imaging was combined with the proposed CRET imaging to provide tumor information of cellular level and show the precise tumor margins for resection during the surgery.

Methods: A series EP-tube samples were firstly prepared for *ex vivo* CRET imaging studies. ^{18}F-FDG of same activity (50 μCi) and same volume (50 μl) was putted in each tube, and 200 μl fluorescein sodium in different concentration (0%, 0.005%, 0.01%, 0.05%, 0.1%, 0.5%, 1%, 5%) were added into different tubes respectively in order to determine the optimal dose of fluorescein sodium for *in vivo* CRET imaging. Signal intensity and emission spectrum were both measured during *ex vivo* experiments. To investigate the penetration ability of CRET imaging, we used polyethylene phantoms and nude mouse pseudo tumor models. Next, we used subcutaneous 4T1 tumor mice models to verify the *in vivo* CRET performances of tumor detection. Then pCLE was combined to detect the tumor margins intraoperatively on these mice models.

Resultes: *Ex vivo* experiments showed the CRET signal intensity reached its peak when the fluorescein sodium concentration was 0.05%. Compared with CLI, the signal intensity of CRET was over 5 times higher. The observed emission peak wavelength of CRET (~540nm) was longer than CLI (~380nm), which theoretically leads to better penetration ability. Phantom pseudo tumor models imaging results also clearly verified that CRET owns better penetration comparing with CLI. By combining CRET and pCLE, we succeeded to identify the tumor margins and determine the resection site on subcutaneous 4T1 tumor mice models. Contrast between preoperative and postoperative imaging results showed that precise tumor resection was able to achieve based on this proposed dual-modality imaging.

Conclusion: This study proposed a novel combined imaging for tumor detection and guided resection. We firstly extended the conventional CLI to CRET imaging by

using fluorescein sodium, in order to enhance the signal intensity and the penetration ability. After *ex vivo* feasibility study, CRET imaging showed improvements in signal intensity and penetration during the *in vivo* tumor detection on subcutaneous 4T1 nude mice models (n=6). Then CRET imaging was combined with pCLE and achieved the research goal of precise tumor resection *in vivo*. All the experiments were accomplished by using clinical proved pharmaceuticals. We believe this proposed CRET/pCLE dual-modality imaging is promising in clinical translation.

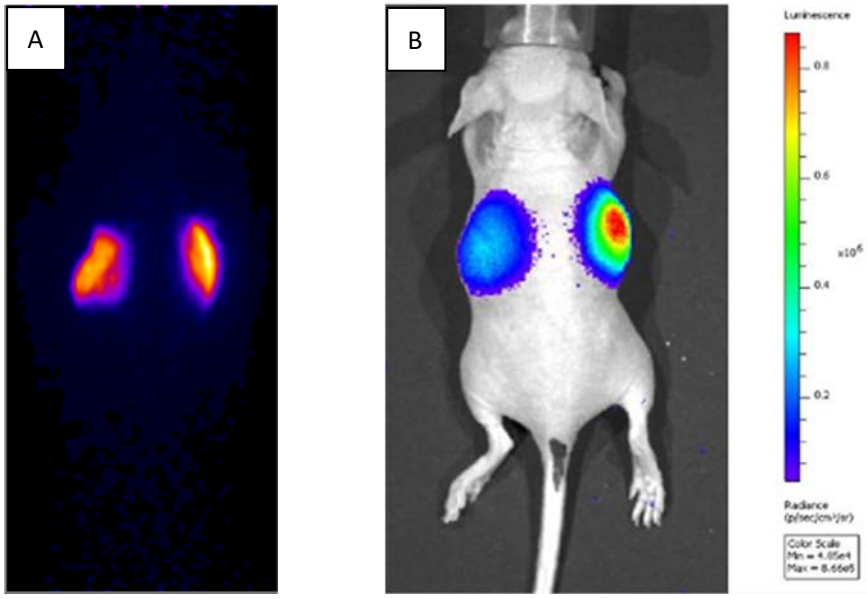

Figure 1. *In vivo* imaging of the subcutaneous pseudo tumor nude mouse model. (A) PET results of 50 μCi (50 μl) radiopharmaceuticals (left flank: ^{18}F-FDG mixed with physiological saline; right flank: ^{18}F-FDG mixed with fluorescein sodium); (B) CLI results of the same pseudo tumor mouse model.