Image-guided cancer surgery using a novel nanoparticle-mediated radiopharmaceutical-excited fluorescence molecular imaging

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Objectives

Fluorescent molecular imaging (FMI) technique has been widely used for image-guided cancer therapy. However, the signal-to-noise ratio (SNR) of FMI is generally not very high because FMI requires external excitation, resulting in background reflection and autofluorescence. Therefore the efficacy of the fluorescent image-guided cancer surgery is not ideal. This paper presents a novel image-guided cancer surgery strategy using nanoparticle-mediated radiopharmaceutical-excited fluorescence molecular imaging (REFI), which employs the dual internal excitation of europium oxide (EO) nanoparticles by both gamma rays and Cerenkov luminescence of radiopharmaceuticals.

Methods

Firstly, *in vitro* fluorescence imaging of EO nanoparticles was performed. Bcap37 cell was incubated in 6-well plate with/without EO nanoparticles. Bright field and fluorescence images were acquired at 24 h and 72 h.

In order to perform the REF image-guided cancer surgery, the subcutaneous breast cancer 4T1 tumor model, orthotropic hepatocellular carcinoma (HCC) tumor-bearing mice, and orthotropic and ectopic HCC tumor-bearing mice were established by injecting 1×10^6 4T1 cells in the mice right upper flank, 5×10^6 HCC cells in the mice liver lobes, and 5×10^6 HCC cells in the mice liver lobes and into the peritoneum, respectively. The mice bearing subcutaneous 4T1 tumor (n=4 per group) was injected with ^{18}F-FDG (100 μCi, 0.1ml) and then EO nanoparticles (1mg/ml, 0.1ml) through the tail vein for REF image-guided surgery. The control group was injected fluorescent molecular probe (IntegriSense^{TM} 750, 20mM, 100μL) for fluorescent image-guided surgery. The orthotropic HCC tumor-bearing mice (n=4 per group) were injected with ^{11}C-CHO (100 μCi, 0.1ml) through the tail vein and then EO nanoparticles (1mg/ml, 0.1ml) for REF image-guided surgery. The mice were injected with indocyanine green (ICG; 100mg/L, 100μL) as the control group. The orthotropic and ectopic HCC tumor-bearing mice (n=4 per group) injected with ^{18}F-FDG (400 μCi, 0.1ml) and then the EO nanoparticles (1mg/ml, 0.1ml) through the tail vein were used for REF image-guided surgery. The other mice were injected with ICG (100mg/L, 100μL) as the control group. Hematoxylin and eosin (H&E) staining was conducted on the cryosection of resected tissue.

Results

In vitro imaging results showed there were no significant cell morphologic changes and no obvious cell aggregation, when they were treated with EO nanoparticles (Figure 1(a)). EO was found to accumulate in the cells. Fluorescent image-guided cancer surgery of the mice bearing subcutaneous 4T1 tumor showed that the tumor was successfully resected (Figure1b). The tumor could also be successfully removed by REF image-guided surgery (Figure1c). Histological analysis validated that the resected samples by REF image-guided surgery were malignant tissues (Figure 1d). The tumor signal-to-normal tissue ratio of REFI was much better than that of FMI (p=0.0158, Figure1e). The REF image-guided cancer surgery of the orthotropic HCC-bearing mice showed that residual cancer tissue was detected after the surgical resection of the orthotropic HCC, whereas fluorescent image-guided cancer surgery failed to detect residual tumor tissues. Furthermore, REF image-guided cancer surgery of the orthotropic and ectopic HCC tumor-bearing mice successfully detected an invisible tiny tumor (less than 1 mm), which fluorescent image-guided cancer surgery failed also.

Conclusions

Our study demonstrates the high potential use of the novel image-guided cancer surgery using REFI technique which employed dual internal excitation of EO nanoparticles by both gamma rays and Cerenkov luminescence of radiopharmaceuticals. The REF image-guided cancer

surgery technique exhibits the excellent performance of detecting invisible tiny tumor (less than 1 mm) and residual cancer tissue. REFI is a promising technique for precise tumor resection.

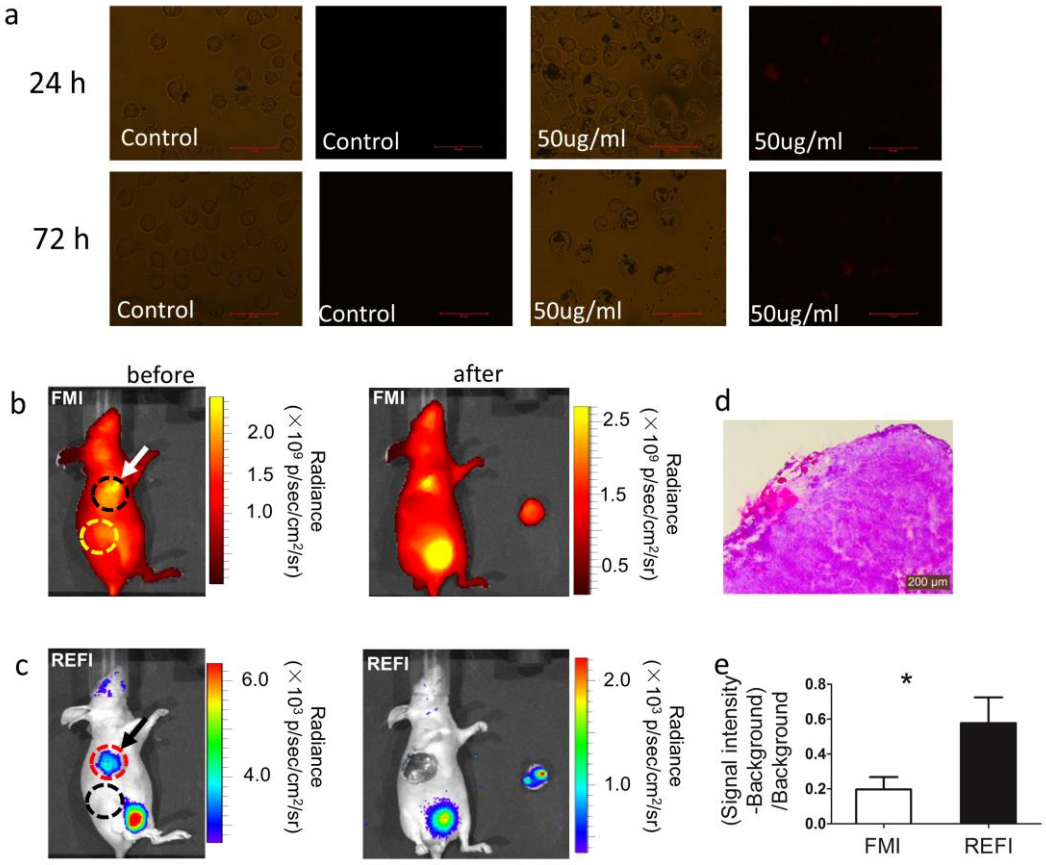

Figure 1. Fluorescent image-guided cancer surgery and REF image-guided cancer surgery. (a) *In vitro* cytotoxicity assay which indicates no significant cell morphologic changes and no obvious cell aggregation treated with EO nanoparticles. (b) Fluorescent image-guided cancer surgery. (c) REF image-guided cancer surgery. (d) The H&E staining result of the resected 4T1 tumor tissues. (e) The comparison of SNR of fluorescent image-guided cancer surgery and REF image-guided cancer surgery.