EGF targeted fluorescence molecular tomography as a predictor of PDT outcomes in pancreas cancer models

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ABSTRACT

Verteporfin photodynamic therapy (PDT) is a promising adjuvant therapy for pancreas cancer and investigations for its use are currently underway in both orthotopic xenograft mouse models and in human clinical trials. The mouse models have been studied extensively using magnetic resonance (MR) imaging as a measure of surrogate response to verteporfin PDT and it was found that tumor lines with different levels of aggression respond with varying levels to PDT. MR imaging was successful in determining the necrotic volume caused by PDT but there was difficulty in distinguishing inflamed tissues and regions of surviving tumor. In order to understand the molecular changes within the tumor immediately post-PDT we propose the implementation of MR-guided fluorescence molecular tomography (FMT) in conjunction with an exogenously administered fluorescently labeled epidermal growth factor (EGF-IRDye800CW, LI-COR Biosciences). We have previously shown that MR-guided FMT is feasible in the mouse abdomen when multiple regions of fluorescence are considered from contributing internal organs. In this case the highly aggressive AsPC-1 (+EGFR) orthotopic tumor was implanted in SCID mice, interstitial verteporfin PDT (1mg/kg, 20J/cm) was performed when the tumor reached ~60mm³ and both tumor volume and EGF binding were followed with MR-guided FMT.

Keywords: photodynamic therapy, verteporfin, magnetic resonance imaging guided diffuse optical tomography, orthotopic pancreas cancer

1. INTRODUCTION

The therapeutic outcome for patients with pancreatic cancer is dismal. The only curative treatment is complete surgical resection of the both the tumor and the pancreas, and even then only 15-20% of patients survive to 5 years.¹ The many advances in the detection and treatment of various cancers in the last several decades have made virtually no impact on the outcome of patients diagnosed with pancreas cancer. There have been several investigations into using photodynamic therapy (PDT) as a single treatment or in combination with gemcitabine or antibody therapy in certain populations.² ³ ⁴ Currently, Phase I/II clinical trials for verteporfin PDT are underway at University College London Hospital (London, UK)⁵ and it appears that PDT could be a viable option for pancreas tumor therapy. It was previously reported in a Phase I clinical trial from the same institute that using mTHPC PDT that regrowth of the tumor at the edges of the treatment volume occurred in all cases.⁶ We have recently demonstrated that changes in tumor volume monitored by magnetic resonance imaging (MRI) as early as 48 hours post-interstitial verteporfin PDT could be indicative of a positive response to PDT.⁷ ⁸ However, MR imaging alone provides information only on structural details and vascular perfusion, which can often be misinterpreted due to the rapid inflammation observed for several days post-PDT. Additionally, MRI lacks the ability to provide molecular information that reports on the metabolic function of the tumor or the expression of key growth factors, such as epidermal growth factor (EGF) and its receptor (EGFR). This information could assist in individualized therapies based on the structural and molecular status of the tumor. We propose that performing MRI-guided fluorescence molecular tomography (MRI-FMT) could improve patient outcome by monitoring and aggressively*

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treated early changes in the structure and molecular function of the tumor. MRI-FMT has been thoroughly developed and tested in our laboratory using an EGF conjugated near infrared fluorescing dye in both murine orthotopic brain and pancreas tumors. Here we demonstrate that monitoring the growth and metabolic function of pancreatic tumors is possible using MRI-FMT.

2. MATERIALS AND METHODS

2.1 AsPC-1 Cell Culture

The procedure for AsPC-1 human pancreatic acinar cell adenocarcinoma culture and preparation for surgical implantation has been described in depth previously. Briefly, cells are cultured in RPMI with 10% (v/v) fetal bovine serum (FBS), 1% (v/v) penicillin-streptomycin, and 1 mg/mL sodium pyruvate. For implantation, the cells are prepared in a 1:1 mixture of cell culture medium and Matrigel® (BD Biosciences, San Jose, CA). Sterile insulin syringes (½ cc U-100 Lo-Dose Insulin Syringe 28G½, Becton Dickinson & Co., Franklin Lakes, NJ) were loaded with the cell-Matrigel® mixture.

2.2 Murine Orthotopic Pancreas Model

All animals were used in accordance with an approved protocol from the Institutional Animal Care and Use Committee (IACUC) at Dartmouth College. The techniques and procedures for the implantation of the Matrigel® orthotopic pancreas model in SCID mice has been described in detail elsewhere. Briefly, 50 μL of the 1:1 cell-Matrigel® mixture containing 1×10⁶ cells was injected directly into the tail of the pancreas and allowed to solidify prior to the removal of the needle. The surgical incision was closed with 3-4 sutures (Ethilon 5-0 PS-3, Ethicon, Piscataway, NJ) and the animals were administered an analgesic (0.1 mg/kg of buprenorphine hydrochloride, Hospira Inc.) immediately after surgery and every 12 hours as needed by sign of physical pain or stress.

2.3 Verteporfin Photodynamic Therapy

Photodynamic therapy was performed two weeks after implantation when the tumors reached ~60 mm³, determined by MRI growth curves of the identical tumor model. The procedure for interstitial verteporfin PDT has been described previously. The procedure is described here briefly, including all modifications. Any fur that had regrown post-surgery was removed with a combination of shaving and a depilatory cream. Verteporfin for injection was administered intravenously via the tail vein (1 mg/kg, 75 μL total injection volume) one hour prior to interstitial PDT. Prior to PDT, the surgical incision was re-opened and the tumor exposed. A diffusing fiber (320 μm diameter) protected by a 20-guage needle was implanted into the center of the tumor along the long axis. PDT was performed with a 690 nm laser (Applied Optics, South Plainfield, NJ) at a linear irradiance of 74 mW/cm for 4.5 minutes for a total dose of 20 J/cm. The control mouse was not administered verteporfin but underwent ‘sham’ PDT with fiber implantation and light delivery identical to the treated mouse. After PDT or sham PDT, the fiber was removed and the incision site closed with 4-5 sutures. The mice were administered 0.1 mg/kg buprenorphine hydrochloride immediately after treatment and every 12 hours after if pain symptoms persisted.

2.4 Magnetic Resonance Imaging Guided Fluorescence Molecular Tomography (MRI-FMT)

The MRI-FMT system images both MRI and near-infrared diffuse molecular fluorescence simultaneously on a system built in-house and described previously. Imaging took place one day prior to PDT (13 days post-tumor implantation) and every seven days after for 21 days (PDT control group) and 28 days (PDT treated group).

2.4.1 Magnetic Resonance Imaging

The tumor location, volume and the fiber plane for FMT imaging were determined using a modified rodent coil (Philips Research Europe, Hamburg, Germany) inside of a human Philips Achieva 3T X-series MRI scanner. The mice were
anesthetized with isofluorane (2.5% for the induction period and 1-1.5% for the imaging session with 1 L/min oxygen). Gadolinium contrast agent (Gd-DTPA, Magnivist™) was loaded in a tail vein catheter (MTV-01, Braintree Scientific Inc., Braintree, MA) and placed in the intraperitoneal cavity of the mouse for administration during the MRI procedure. The anesthetized mouse was placed supine in a specially designed resin holder that accommodates placement of the FMT optical fibers 360° on the surface of the mouse abdomen. The holder contains fiducial markers to determine both the orientation of the holder in the MRI and the plane of optical fibers for FMT reconstruction. Motion artifacts that can distort abdominal imaging were minimized by fasting the mice for 12 hours prior to imaging to reduce the peristaltic motions of the digestive system, placing the mouse supine in the holder to reduce breathing motions, and lightly restraining the mouse with loose surgical tape restraints to reduce involuntary motions while under anesthesia.8,12

The MR imaging parameters for the sequences performed have been described previously7-9 and are summarized in Table 1. Briefly, the mouse was imaged with a survey scan to determine the center of the mouse and to align the imaging with the plane of FMT optical fibers. A pre-contrast T1-weighted turbo spin echo (T1W) scan was followed by injection of the Gd-DTPA contrast agent, then a T2-weighted turbo spin echo (T2W) scan was performed and 10 minutes after the injection of the contrast agent, a second T1W scan was performed. A T1W contrast difference (T1WCD) image sequence was produced by subtracting the T1W pre-scan from the T1W-post scan using the Philips software. Subsequently, tumor and organ segmentation as well as the location of the optical fibers was performed using Mimics Medical Software (Materialise, Version 13.0).

Table 1. The axial MR image sequence parameters used during MRI-FMT imaging sessions

<table>
<thead>
<tr>
<th>Sequence Parameter</th>
<th>MR Image Sequence Protocol</th>
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2.4.2 Near-Infrared Fluorescence Molecular Tomography

The MRI-couple FMT system has been described elsewhere in great detail.11 Briefly, the parallel 16-channel spectroscopy FMT system is coupled directly into the MRI scanner by long bifurcated spectroscopy fibers that enter the MRI scanner room via conduits in the wall. The contrast agent, EGF-IRDye800CW administration is described in the next section. Due to the spatial limitations of the mouse abdomen only 8 of the 16 fiber optic bundles and spectrometers are used for imaging and are inserted through the specially designed mouse holder9 and pressed directly against the skin of the mouse, ensuring sufficient contact to reduce reflectance at the fiber-skin interface. Both fluorescence and transmission data were collected by sequentially illuminating one fiber and detecting in the other seven, providing 56 measurements in total for each mouse. A 690 nm laser was used for fluorescence excitation, and fluorescence emission was collected with a 720 nm long pass filter (300 lines/mm grating). The 690 nm laser transmission was collected with a 2 OD filter (1200 lines/mm grating). MRI-guided finite element method (FEM) image reconstruction was performed using NIRFAST v2, a software package developed at Dartmouth College and the reconstruction techniques previously described in detail.13
2.4.3 EGF IRDye 800CW Administration

The contrast agent used in this study was the commercially available near-infrared dye IRDye 800 CW conjugated to EGF (EGF-IRDye800CW) from LI-COR Biosciences (Nebraska, USA). The mice were injected with 1nmol (75 μL of the prepared dye as per manufacturer’s instructions) of the EGF-IRDye800CW via the tail vein 48 hours prior to MRI-FMT imaging. This procedure was repeated for each imaging session as the dye completely clears within a week of administration.14

3. RESULTS AND DISCUSSION

EGFR was selected as a molecular target because the majority of pancreas tumors are found to over-express EGFR and high levels of EGFR are correlated with aggressive, advanced disease and a shorter patient survival time.15 The pancreas adenocarcinoma cell line AsPC-1 used here is known to over-express EGFR,15 and thus monitoring the EGFR levels pre- and post-PDT will provide insight into the molecular activity and therapeutic response of the tumor to verteporfin PDT.

Figure 1. MRI-FMT uses structural information from the MRI to guide the fluorescence reconstruction. A) The axial T1W post-Gd-DTPA image sequence was used to determine tissue regions. B) The MRI fiducials are placed perpendicularly in the plane of fibers (blue lines) to denote the location of the fiber plane in the MR image and to guide the placement of the fibers post-imaging. C) The position of each of the eight fiber bundles is determined (red lines) and each is marked at the edge of the axial MR image. D) The MR image is segmented into four regions (tumor – green, bowel – blue, kidney – yellow, and abdomen – pink), which are assigned individual optical properties for the hard and soft prior reconstruction. The location of each of the fibers (red bulls-eyes) and the iso-center of the fiber place is determined from the information in B & C. E) An example of a soft-prior reconstruction performed in NIRFAST v2 demonstrates that each of the four regions assigned in the MR images show distinct fluorescence intensities. F) The reconstructed fluorescence image in E can be overlayed on the MR image from A to demonstrate the fluorescence of each region corresponds to the tissue in the MRI.

MRI and FMT were performed simultaneously and the MRI tumor volume and vascular perfusion volumes were analyzed separately from the MRI-FMT data. For the MRI-FMT data collection and analysis (Figure 1) the T1W post-Gd MRI sequence was used such that the tumor, fiducials and surrounding organs could all be visualized.
simultaneously (Figure 1A). The fiducial markers sit in the same plane as the FMT optical fiber bundles and are perpendicular to each other (Figure 1B). From these perpendicular fiducials, the location of each of the 8 fiber locations can be determined as shown in Figure 1C. The fiber marker is placed along the red line at the extreme edge of the mouse abdomen (at the skin-air interface). These fiber positions can be seen in Figure 1D as the red bulls-eye markers. The red marker near the center of the mouse abdomen denotes the iso-center of the optical fibers. Additionally, the axial image of the mouse abdomen can be segmented into the tissues that display high levels of fluorescence (tumor, bowel, kidney), as shown previously. The tissue regions are assigned individual absorption and scattering optical properties based on the excitation and emission wavelengths determined from Alexandrakis et al 2005. The fiber positions and the segmented regions are provided to the NIRFAST reconstruction software to produce the soft-prior image guided reconstruction. The soft-prior reconstruction uses L-matrix regularization works to relax the ‘smoothness’ constraints at the boundaries of the segmented tissues in order to achieve non-homogeneous optical properties within the tissues.

Figure 2. MRI and MRI-FMT provides information on both the physical structure and the EGFR expression levels of the treated and control tumors. The MRI data of the control and PDT treated (A & C, respectively) provides information on the physical nature of the tumor. The total tumor volume (T2W, black line) shows the overall size of the tumor, while the vascular perfusion volume (T1WCD, red line) shows the regions of the tumor with active blood vessel activity. The data reveals that although the overall tumor volume is similar between the PDT treated and control mice, that there is less tissue with active vasculature. This suggests that there is a large necrotic region in the PDT treated, even though the treatment was sub-curative. The MRI-FMT determined fluorescence within the tumor region (as determined from soft priors) for the control and PDT treated mice (B & D, respectively) indicates that while the control tumors has a slowly decreasing level of EGFR as the tumor increases in size, the PDT treated tumor fluctuates at a much high fluorescence intensity level. Additionally, EGF-targeted fluorescence is highest 7 days post-treatment indicating the highest levels of EGFR at this time point.
Both MRI and fluorescence determined from the tumor region of the MRI-FMT soft-prior reconstruction were analyzed for both the PDT treated mouse and the PDT control (Figure 2). The total tumor volumes are similar in both the control and treated mouse (Figures 2A & C, respectively; black line). This is not unexpected as a sub-curative PDT treatment was given in order to monitor the tumor re-growth. It is most likely that the edges of the tumor were not completely treated with PDT, as seen previously at 20 J/cm for AsPC-18, and that the tumor continued to grow from these untreated edges. The vascular perfusion volume does show a difference between the control and treated tumors (Figures 2A & C, respectively; red line). The untreated tumor has a large vascular perfusion volume; only slightly smaller than that of the tumor indicating that it is likely there is a small necrotic region characteristic of these tumors. The vascular perfusion volume of the treated tumor is much lower than the total tumor volume after 7 days post-PDT. This indicates that the treatment was successful in creating a necrotic core larger than that found in the control animal, and that the molecular response of these tumors may be different due to the large change in actively viable tumor tissue.

The MRI-FMT data demonstrates that there is a difference between the control and PDT treated tumors (Figures 2B & D). The control tumor shows that the EGFR levels (proportional to the fluorescence from bound EGF-IRDye800CW complex) are somewhat constant through the three weeks post-surgery, with a slight decrease in EGFR levels over these three weeks. The PDT treated tumor on the other hand shows high fluctuations in the EGFR expression levels. At 7 days post-PDT, the fluorescence is at the highest intensity but decreases again at 14 days post-PDT before leveling out. This indicates that the PDT treated tumor is over-expressing EGFR post-PDT in order to promote regrowth of the tumor. It is possible that 7 days after PDT may be the best time point in order to treat the tumor with an adjuvant therapy such as Erbitux, a monoclonal anti-body against EGFR. It is also possible that the fluorescence difference in the PDT treated mouse is indicative of vascular changes in the tumor (i.e. increased enhanced permeability and retention (EPR) effect); however, more investigations are required in order to determine the reason for theses fluorescence changes.

4. SUMMARY AND FUTURE DIRECTION

MRI is successful at providing structural and vascular perfusion information regarding the treated and control tumors. We found that although the total tumor volumes of the treated and control tumors were comparable, the vascular perfusion volumes showed a large difference. This indicated that the PDT treated mouse had a larger necrotic core than the control mouse even though the treatment was sub-curative. Additionally the MRI data alone showed larger differences at longer time points from the PDT treatment, whereas the MRI-FMT data provided information on the molecular changes of the tumor at much shorter time periods. MRI-FMT provides information regarding the EGFR expression levels of a tumor, and we have demonstrated that changes as early as 7 days post-PDT in the treated tumor are indicative of rapid and large changes in the EGFR expression. It is difficult to make any broad conclusions as a small number of mice were used in this study, but the addition of more mice in the future will clarify the experimental results. Ex vivo analysis of the fluorescence levels of the tissues compared to the MRI-FMT results, in addition to the vascular structure and distribution in the tumor will provide insight into the nature of the treatment and the tumor re-growth. Additionally, the actual binding levels of EGF in the treated and control tumors can be analyzed to determine whether we are truly measuring EGF binding or simply changes in the vascular perfusion.

5. ACKNOWLEDGEMENTS

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6. REFERENCES


