Towards Early Detection of Pancreatic Cancers by CA19-9 Conjugated Magnetic Nanoparticles and Active Feedback MR

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Target audience Physicians and physicists interested in the research of gastrointestinal cancer, pancreatic cancer, cancer biomarkers, MR molecular imaging, preclinical animal studies, and early tumor detection.

Purpose Pancreatic cancer (PC), called the silent killer, is the fourth leading cause of cancer-related death in both men and women in US. Due to difficulties in diagnosis and therapy, PC's five-year survival rate is only about 1% in US. Nonetheless, hope for mitigating PC arises from the early detection of small, resectable tumors through imaging modalities like magnetic resonance imaging (MRI). Since late-stage PC exhibits chemo- and radiotherapy resistance, early detection of this malignancy using enhanced MRI imaging techniques increases not only the treatment options available, but also the patients' survival rate. This can be realized with antibody-conjugated superparamagnetic iron oxide (SPIO) nanoparticles capable of binding to early stage pancreatic cancer cells to improve imaging specificity and innovation methods that can sensitively detect SPIO to improve imaging sensitivity. The enhanced contrast from SPIO can then be used to visually assess the distribution and magnitude of SPIO-targeted tumor cells. Therefore, the purposes of this work are: (i) to enhance detection specificity through effective targeting of PC biomarkers, (ii) to enhance detection sensitivity through contrast-enhanced imaging of magnetic nanoparticles.

Methods (i) To enhance detection specificity, anti-CA 19-9 antibodies were conjugated to NH2-PEG-coated SPIO nanoparticles utilizing reductive amination chemistry. Conjugation was verified using dynamic light scattering (DLS) for particle size determination, and the Bradford protein assay. The antigen binding capacity to CA 19-9 over-expressing cell lines (BxPC3) was witnessed with in vitro MR cellular images. A control experiment using mouse models bearing both CA19-9(+) and CA19-9(-) PCs was used to additionally confirm specific, reliable binding, as shown in Fig. 1. (ii) To enhance detection sensitivity, we have developed a new approach, called "Active Feedback Magnetic Resonance". The general principle of the "Active-Feedback MR" is based on the feedback-induced nonlinear spin dynamics that we discovered, for examples [1-4]. Here, its specific applications to sensitively image SPIO/aggregates was developed [5,6]. Our theoretical, numerical, and in vitro cellular imaging studies show that "Active Feedback MR" is sensitive to magnetic field fluctuations arising from diffusion motion within strong magnetic field gradients and thus can be applied to sensitively imaging magnetic nanoparticles.

Results The binding capacity of the bio-conjugated SPIO-CA19-9 "molecular beacon" to CA 19-9 over-expressing PC cell lines (BxPC3) was first confirmed by a control experiment using mouse models bearing both CA19-9(+) and CA19-9(-) PCs through i.v. injection (**Fig. 1**). *In vivo* images of human PC from both subcutaneous (Fig. 2) and orthotopic (Fig. 3) xenograft mouse models were carried out, where the PC were targetd by the SPIO-CA19-9 "molecular beacon" and imaged by the Active Feedback MR method.

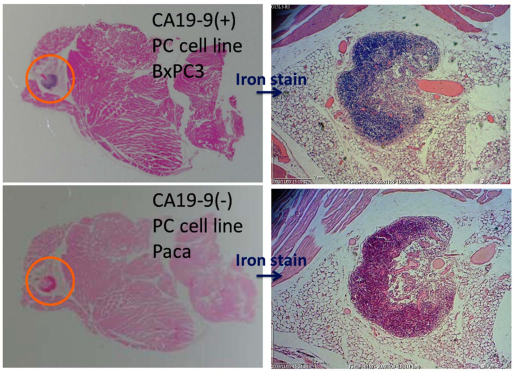

Fig. 1: The binding capacity of the bio-conjugated SPIO-CA19-9 "molecular beacon" to CA 19-9 over-expressing PC cell lines (BxPC3) was first confirmed by an in vivo control experiment.

Discussion Subcutaneous xenografts PC mouse models (Fig. 2A) show that, while T2-weighted image cannot clearly locate the magnetic nanoparticles (Fig. 2B), the active-feedback images (Fig. 2C) successfully highlight the magnetic nanoparticles distribution with a close correlation with iron-stained histopathology (Fig. 2D). In addition, for the orthotopic xenografts PC mouse models (Fig. 3), while CPMG-T2 weighted image cannot clearly locate the SPIOs (Fig. 3A), the "Active Feedback MR" images shown in Fig. 3B and 3C (acquired with two different active feedback pulse sequences and with imaging processing) successfully highlight the SPIO distribution (red circles) with positive contrast and a close correlation with iron-stained histopathology (Fig. 3D).

Conclusion *In vivo* subcutaneous and orthotopic xenografts PC mouse models validated the superior contrast/sensitivity and robustness of this approach towards early PC detection. Statistical results (N>10) for PC mouse models at various cancer stages, alternative active feedback pulse sequences with further improved performance will also be presented.

Reference [1] Science 290, 118 (2001) [2] Magn. Reson. Med. 56, 776 (2006) [3] Magn. Reson. Med. 61, 925 (2009) [4] J. Phys. Chem. B 110, 22071 (2006) [5] Magn. Reson. Med. (in press) [6] Magn. Reson. Med. (in presss)

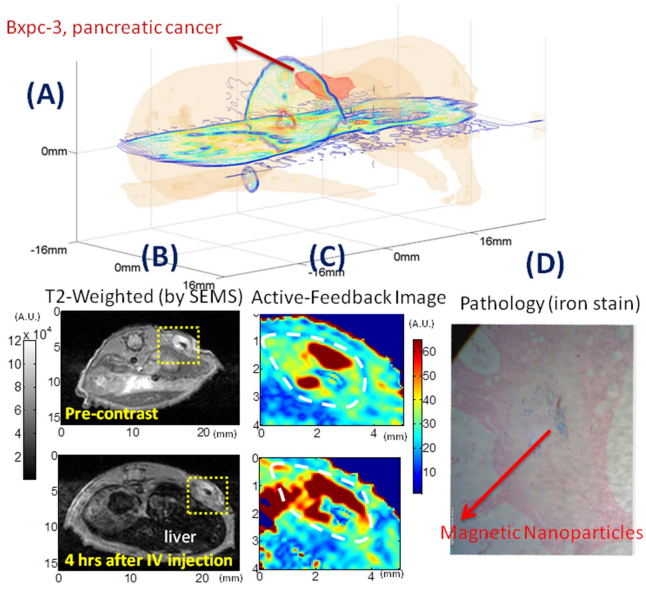

Fig. 2: Detection of pancreatic cancers in subcutaneous xenograft PC mouse models (**A**) by sensitive imaging of the dipolar fields induced by magnetic nanoparticles (**D**). The active feedback images show much improved post-injection contrast (**C**), compared with spin echo T2W images (**B**).

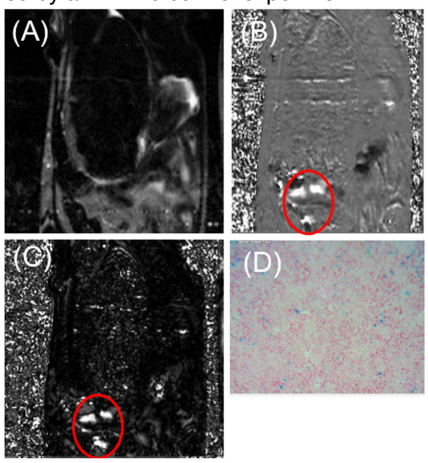

Fig. 3: In vivo images of orthotopic mouse model bearing human PC (from CA 19-9 positive BxPC3 cell lines) labeled by SPIO were acquired. While CPMG-T2 weighted image cannot clearly locate the SPIOs (**A**), the "Active Feedback MR" images shown in (**B**) and (**C**) (acquired with two different active feedback pulse sequences) successfully highlight the SPIO distribution with positive contrast and a close correlation with iron-stained histopathology (**D**).