Creation of an Multimeric anti-p53 scTCR-SPIO Conjugate For The Detection of Cancer in MR

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Background: The concept of targeted contrast agents can be readily adapted for use in applications such the specific and selective detection of cancer using MRI. Common implementations of this targeted approach have used targeting proteins specific to biomarkers overexpressed in certain cancers, conjugated to common contrast agents, such as superparamagnetic iron oxide (SPIOs) nanoparticles or Gd-based constructions.1 A biomarker ideal for this application is p53, an intracellular tumor suppressor protein that is overexpressed in a wide range of human malignancies. Previously, we have isolated a T-cell receptor from a p53 (aa 264-272)-specific high avidity CTL clone derived from HLA-A*0201 transgenic mice (sc264TCR). Thus, by coating SPIO nanoparticles with 264scTCR, we can selectively deliver SPIO nanoparticles to p53-expressing cells and probe their presence using MRI. A positive outcome could lead us to develop 264scTCR-based SPIO nanoparticles as a targeted generalized cancer contrast agent.

Method: Creating Multimeric scTCRs: The previously isolated sc264TCR was multimerized into dimeric and tetrameric forms. Dimeric forms were created via interaction from IL-15/IL15R sites cloned into two corresponding 264scTCRs. Tetrameric forms were created by further fusing two dimeric sc264TCRs onto each of two heavy chains of human IgG1.

scTCR Specificity Assay: scTCR specificity against p53-expressing cells was observed using flow cytometry. Human melanoma tumor cells, A375 (ATCC, Manassas, VA), and human lymphoblast cells, T2 (ATCC, Manassas, VA) pulsed with p53 peptide (aa 264-272), were stained with Phycoerythrin-labeled monomeric, dimeric, or tetremeric scTCRs at varying concentrations. Staining curves were obtained at FL-2, 575nm.

scTCR-Conjugate Formation and Validation: scTCR-SPIO conjugates were constructed through interaction between biotinylzed monomeric and dimeric scTCRs, and streptavidin-coated SPIOs. Conjugates were purified using a magnetic cell separation column and conjugate formation was validated through ELISA. scTC-SPIO conjugates were captured using BF1 mAb coated plates (BF1 binds to the β1 constant region of the scTCR). Streptavidin-HRP dye was linked using WF1-biotin mAb linker (WF-1 binds to a different region of the β1 constant region of the scTCR), and developed with ABTS.

Quantitative MR Imaging: A375 cells and T2 cells loaded with p53 peptide were incubated with scTCR-SPIO conjugates constructed from monomeric or dimeric scTCRs. After two hours of incubation, cells were washed and centrifuged into a pellet in a standard centrifuge tube. The cell apparatus was scanned using a 3T Signa GE scanner (General Electric, Milwauke, WI) with a homemade birdcage coil and using an multi-TE EFGRE 3D sequence at an isotropic resolution of 500um^3. Images were reconstructed and iron content was determined using the COSMOS methodology, as described in Liu 2008. Traditional T2 images were also obtained for qualitative observation.

Results and Discussion: scTCR Specificity Assay: Staining curves of the Phycoerythrin-labeled 264scTCR monomers, dimers, and tetramers against A375 cells and p53-pulsed T2 cells are shown in Figure 1. Tetramers show a greater degree of staining compared to the dimers and monomers, and exhibit a low degree of staining against unpulsed cells. A nonspecific scTCR (anti-Mart1) exhibits a similarly low degree of staining.

These results demonstrate the specificity and selectivity of the scTCR against cells expressing p53. Phycoerythrin-labeled monomeric scTCRs show a significant degree of staining compared to p53 negative controls. Likewise, dimeric and tetremeric 264scTCRs show a progressively greater degree of staining compared to the monomer, suggesting that the increased avidity contributes to increased affinity against p53 peptide.

scTCR-Conjugate Formation and Validation: ELISA results are depicted in Figure 2. The relative concentration of each scTCR-SPIO preparation is plotted against the optical density at 405nm after final HRP development with ABTS. Results indicate a nonlinear dose-dependent response for the scTCR-SPIO conjugates, and a flat background with preparations containing over SPIOs or scTCRs.

These results demonstrate the successful formation of the scTCR-conjugates. As BF1 and WF4 antibodies bind only to the scTCR, and the magnetic filtration process captures magnetic SPIOs while eliminating all unbound scTCRs, only scTCR-SPIO conjugates should be detected by this assay. The dose-dependent nature of the results indicate that the magnetic purification process effectively removes unbound scTCRs from the preparation while preserving the scTCR-SPIO conjugates

Quantitative MR Imaging: Traditional T2 weighted images, relative field maps, and results of the COSMOS reconstruction for select samples are shown in Figure 3. Results of iron quantification analysis from COSMOS is depicted in Figure 4. Conjugates created from the dimeric form of the scTCR and incubated with p53-pulsed T2 cells show greater iron accumulation compared to that of the monomeric form, with unpulsed T2 cells and nonspecific scTCR-SPIO conjugates (anti-Mart1) exhibit a small degree of nonspecific binding. When incubated with A375 cells, all conjugates show a lesser degree of iron accumulation compared to that of p53-loaded T2 cells. The resulting SPIO accumulation is readily observable using both traditional and MR-QSM based techniques, allowing for a qualitative and quantitative measure of overall contrast agent delivery. The effectiveness of the tetremeric form over the monomeric and dimeric forms for p53-specific staining under flow cytometry, and the effectiveness of the dimeric form over the monomeric form of the scTCR-SPIO conjugates for MR labeling under QSM, demonstrates that an increase in avidity corresponds to an increase in affinity, resulting in a greater degree of staining and a greater degree of contrast agent accumulation among p53-expressing cells in both fluorescent and MR applications.

These results strongly support an expansion into *in vivo* studies, whereby scTCR-SPIO will be used to target xenographed tumors in cancer mouse models for detection using MRI.

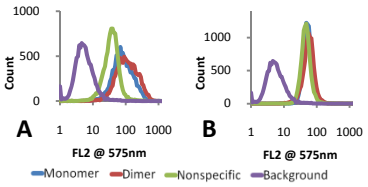

Figure 1. Flow Cytometry – Staining profiles for **(A)** T2 cells loaded with p53 peptide, and **(B)** A37545N exhibit positive staining for monomeric and dimeric forms of the scTCR compared to that of a nonspecific TCR and the unstained background.

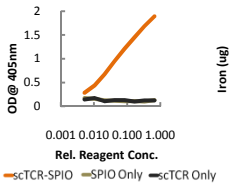

Figure 3. ELISA – Concentration dependent response is shown for purified scTCR-SPIO conjugates, indicating successful conjugation.

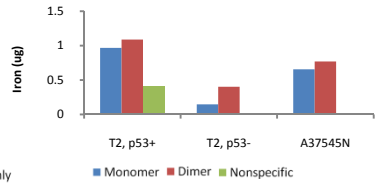

Figure 4. Quantitative MRI – scTCR-SPIO conjugates incubated with p53-expressing cells demonstrate SPIO accumulation when measured using MRI-QSM. The dimeric form of the scTCR contributes to greater SPIO accumulation over that of the monomeric form; nonspecific binding and binding against p53 negative T2 cells is minimal.

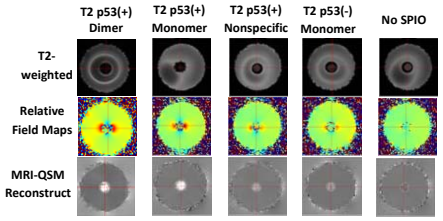

Figure 5. MRI Example Images – Representative images are shown from varying cell and scTCR-SPIO conditions. Traditional T2 weighted images **(TOP)** depict negative contrast with increasing SPIO accumulation. Relative field maps **(MIDDLE)** exhibit an increasingly large "dipole" pattern with increasing SPIO accumulation. MRI-QSM reconstructions **(BOTTOM)** show positive contrast; larger, more intense pixels represent more concentrated SPIO accumulation.