Assessment of Mucin 13 (MUC13) as an Imaging Target for Guiding Colorectal Cancer Treatment: A Pathway Towards Theranostic Development

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Background: A theranostic strategy combining diagnostic imaging and targeted therapy in a single regimen is proposed for improved management and treatment of colorectal cancer (CRC). Increased specificity in detection by the noninvasive imaging technique positron emission tomography (PET) can be achieved by radiolabeling antibodies (Abs) designed to target tumor-associated antigens with increased expression post-translational modifications present in cancer cells. In this study, an Ab designed to target the transmembrane glycoprotein mucin 13 (MUC13) was radiolabeled with the positron-emitting radionuclide zirconium-89 (^{89}Zr) for PET imaging of a xenograft mouse model of CRC. Specified uptake of this radioimmunoconjugate was observed in the presence of increased MUC13 expression was observed through imaging along with *in vitro* and *ex vivo* analyses.

Methods:

Radiochemistry: The MUC13-targeting Ab C14 conjugated with desferrioxamine (DFO) was radiolabeled with ^{89}Zr alongside isotype control Ab MOPC-21 (IgG) at a 59 kBq/μg (1.6 μCi/μg) ratio, producing [^{89}Zr]Zr-DFO-C14 and [^{89}Zr]Zr-DFO-IgG. Radiochemical purity (RCP) was determined using radio-iTLC and radio-SEC. Radiochemical yield (RCY) was determined with a well-type dose calibrator.

Cellular Binding and Internalization: Cultured human CRC cell lines T84 (MUC13+) and SW480 (MUC13-) were incubated with either [^{89}Zr]Zr-DFO-C14 or [^{89}Zr]Zr-DFO-IgG. At 2 and 24h, cell membranes were separated and radioactivity measured to compare membrane-bound and cell-internalized activity. To determine binding specificity of radiolabeled C14, cells were co-incubated with excess unmodified Ab.

μPET/CT Imaging: T84 and SW480 cells were introduced subcutaneously in athymic nude mice. Once palpable tumors were detected, mice were placed in the following treatment groups for 1.9 MBq (50 μCi) injection: T84+[^{89}Zr]Zr-DFO-C14 (n=5), T84+[^{89}Zr]Zr-DFO-C14 with 350 μg C14 (n=2), SW480+[^{89}Zr]Zr-DFO-C14 (n=5), and T84+[^{89}Zr]Zr-DFO-IgG (n=4). PET imaging was performed 24, 48, and 120h post-injection (p.i.) alongside computational tomography (CT) imaging to provide anatomical context. After 120h, mice were euthanized and blood, organs, and tissues were collected to measure radioactivity biodistribution and radioimmunoconjugate distribution in tumor tissue.

Results: Radiolabeled C14 and IgG were successfully produced with RCY>83% (n.d.c.) and RCP>95%. Reflecting rapid internalization observed *in vitro* (57.9±13% [^{89}Zr]Zr-DFO-C14 uptake in T84 at 2h compared to 6.57±0.6% uptake in SW480 ($p<0.0001$) and 0.39±0.1% [^{89}Zr]Zr-DFO-IgG uptake ($p<0.0001$)), mice bearing T84 xenografts displayed greater signal intensity from [^{89}Zr]Zr-DFO-C14 at 24h p.i. through 120h p.i. compared to that measured in SW480 xenografts (5.5±0.7% ID/cc vs. 2.8±0.5% ID/cc at 24h p.i., $p<0.0001$) as well as that in T84-bearing mice injected with [^{89}Zr]Zr-DFO-IgG (1.9±0.2% ID/cc at 24h p.i., $p<0.0001$). Autoradiography revealed high, homogeneous distribution of [^{89}Zr]Zr-DFO-C14 within the tumor. Furthermore, co-injection with excess C14 resulted in reduced PET signal (2.7±0.1% ID/cc, $p=0.0002$), supporting the targeting specificity of [^{89}Zr]Zr-DFO-C14. *Ex vivo* biodistribution comparison confirmed high, persistent [^{89}Zr]Zr-DFO-C14 uptake in T84-derived tumor (18.5% ID/g at 120h p.i.).

Conclusion: MUC13 expression was clearly represented by PET/CT imaging in a xenograft mouse model of CRC using a ^{89}Zr-labeled MUC13-targeting Ab, which also demonstrated target specificity both *in vitro* and *ex vivo*. These promising results justify further exploration into developing a theranostic platform for CRC treatment. Future work will test the therapeutic efficacy of the MUC13-targeting Ab radiolabeled with a beta particle-emitting radionuclide.