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New biarsenical imaging probes for radiolabelling of biologicals

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Aim

The radiolabeling of biologicals for PET imaging has so far been hampered by a lack of generalised radiolabeling methodology. We have developed a novel approach for this purpose, RadTags, which enables rapid, general, and simple radiolabeling of biologicals based on the biarsenical-tetracystein system (Fig 1). One unique characteristic of the methodology is that RadTags may be labeled with any isotope of choice, using established radiolabeling technology, allowing imaging using the same constitutionally distinct molecular probe in a variety of settings, from the test tube to the clinic. The aim of the present study was to show proof-of-principle in a variety of studies, including in vitro radioligand binding studies and in vivo microPET nuclear imaging of melanoma tumors in xenograft mice.

Materials and methods

The MC1 receptor ligand, melanocyte stimulating hormone (MSH) was modified to enable site-specific labeling using the biarsenical-tetracysteine methodology. Radiolabeled RadTag (3H or 11C) was efficiently prepared by O-methylation of the desmethyl phenolic precursor using either or [3H]methyl nosylate or [11C]methyl triflate under mild alkaline conditions. The modified MSH analog (NDP-MSH) was reacted with Radtag (3H or 11C) in an aqueous buffer according to a published procedure (Fig 2). The radiochemical purity of [11C]RadTag-NDP-MSH was >95%. In vitro radioligand binding characterization of [3H]-RadTag-NDP-MSH was performed using B16F10 melanoma cells or transfected cells Fig 3). In vivo [11C]RadTag-NDP-MSH microPET imaging of melanoma tumors (A375 and B16F10), with different levels of MC1 receptor expression was performed in xenograft mice. A375 tumor cells show low MC1 receptor expression, whereas B16F10 show high MC1 receptor expression.

Results

The reaction of radiolabeled RadTag with NDP-MSH proceeded with near quantitative conversion after a ten minute incubation at room temperature. Both NDP-MSH and RadTag-NDP-MSH bind with low nM affinity to MC1 receptors, showing that modification of NDP-MSH does not influence the primary pharmacology of the peptide analogue (Fig 3). In the in vivo microPET experiment, [11C]RadTag-NDP-MSH successfully visualized the B16F10 melanoma tumor with a tumor/muscle ratio of about 1.5 at 60 minutes and 2 at 90 min (Fig 4-6). The tumor/muscle ratio for the low MC1 receptor expressing tumor was around 1.

Conclusion

- RadTag can be [11C]- or [3H]-labelled with high efficiency
- RadTag radiolabeled NDP-MSH retain pharmacological profile
- [11C]RadTag-NDP-MSH successfully visualized melanoma tumor (B16F10) with a tumor/muscle ratio of about 2 at 90 minutes
- Biarsenical tetracystein system may be successfully used for rapid multimodal radiolabeling of biologicals for in vivo molecular imaging using RadTag

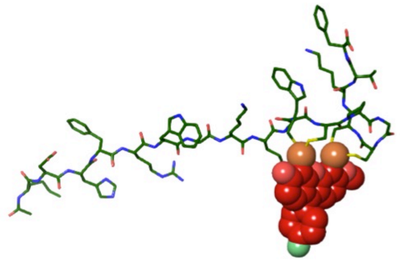

Figure 1. Illustrates RadTag (space filled) covalently bound the NDP-MSH-CCPGCC. CCPGCC is the consensus sequence for biarsenical-tetracysteines.

Experimental design & image analysis

- C57Bl/6J mice were transplanted with B16F10 melanoma cells, non-SCID-gamma mice were transplanted with A375 melanoma cells subcutaneously
- PET measurement was perfomed 10 days after injection of the cells.
- Immediately upon injection of [11C]RadTag-NDP-MSH a 93 minute dynamic PET measurement was initiated (37GBq/µmol)
- Manual Region of Interest (ROIs) were drawn on the CT image
- Time activity curves (TAC) were generated in the different ROIs–values expressed as % SUV according to the following equation:

$$\%SUV = \frac{Radioactivity}{Injected\ RA/weight} * 100$$

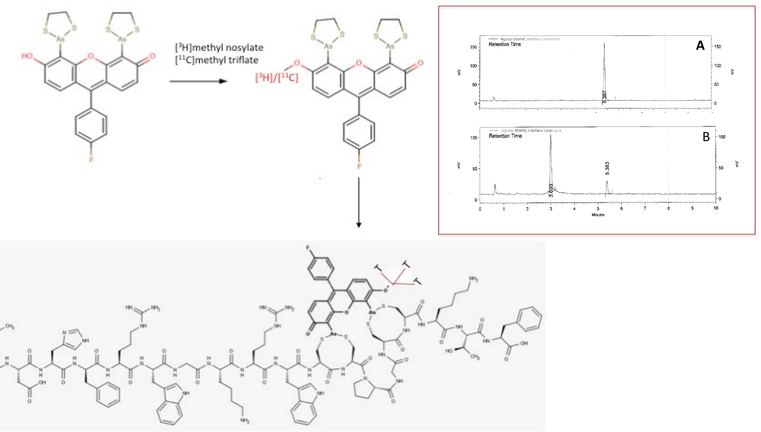

Figure 2. Schematic scheme of [3H] or [11C] radiolabeling of RadTag and subsequent binding of RadTag to NDP-MSH-CCPGCC. Insert show HPLC radiochromatogram of [11C]RadTag (A) and [11C]RadTag-NDP-MSH (B)

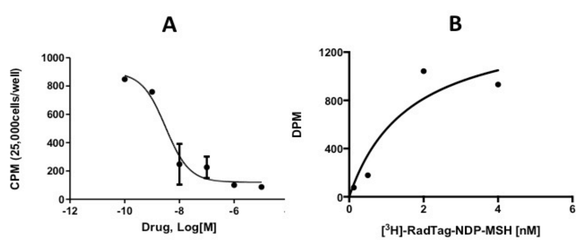

Figure 3. In vitro binding profile of RadTag-NDP-MSH (A) displacement of [125I]NDP-MSH at hMCR1 expressed in HEK 293, (IC50 3nM) (B) saturation binding of [3H]-RadTag-NDP-MSH at B16/F10 melanoma tumor cells (Kd 2nM)

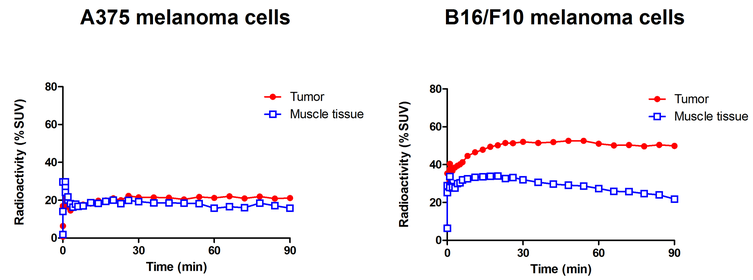

Figure 4. Average time activity curves of [11C]RadTag-NDP-MSH uptake in the tumor and muscle tissue in A375 primary melanoma-bearing non-SCID-gamma mice and in B16F10 primary melanoma-bearing C57BL/6J mice

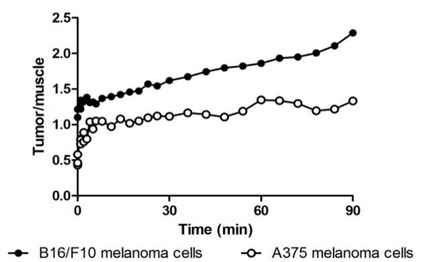

Figure 5. Tumor/muscle ratio of [11C]Rad-Tag-NDP-MSH in B16F10 primary melanoma-bearing C57BL/6J mice (high MC1 receptor expression) and A375 primary melanoma-bearing non-SCID-gamma (low MC1 receptor expression).

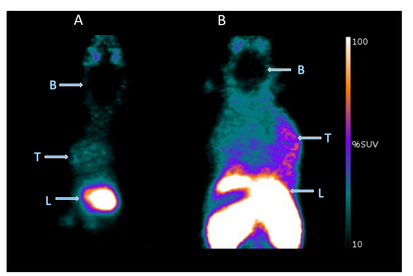

Figure 6. Horizontal %SUV images averaged from 1 – 93 min. (A) shows [11C]RadTag-NDP-MSH biodistribution in A375 primary melanoma-bearing non-SCID-gamma mice. (B) shows [11C]RadTag biodistribution in B16F0 primary melanoma-bearing C57BL/6J mice. Arrows indicate the brain (B), tumor (T) and liver (L).