Development of selective peptide-based radiopharmaceuticals for targeted therapy and diagnosis of high malignant cancers

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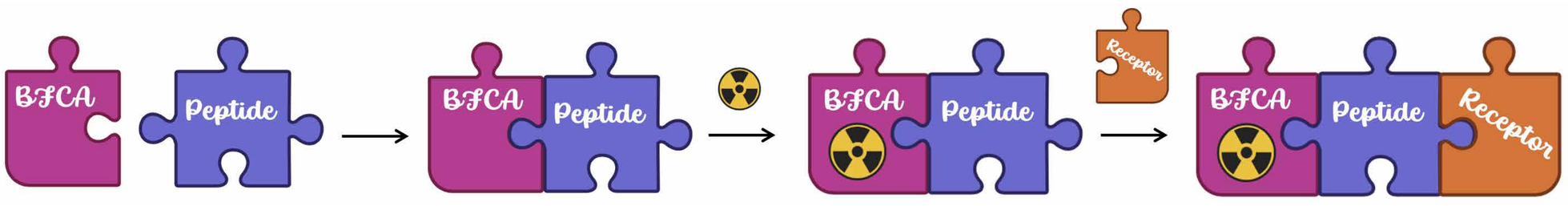
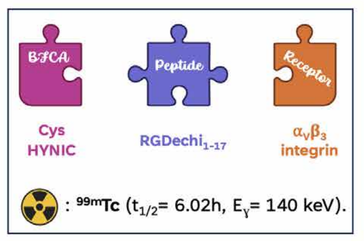
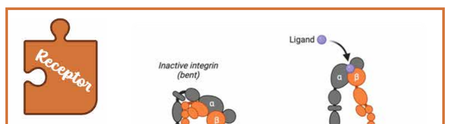
https://doi.org/10.17952/37EPS.2024.P1227

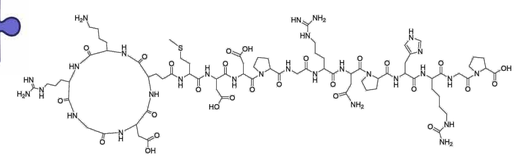
RGDechi peptide derivatives can be considered interesting candidates as non-invasive diagnostic tracers in tumor imaging.







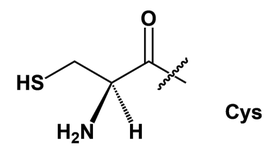
Integrins are heterodimeric trans-membrane glycoproteins composed of noncovalently associated α and β subunits.



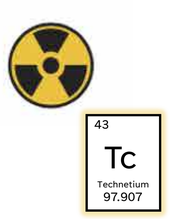
Properties[1,2,3,4]

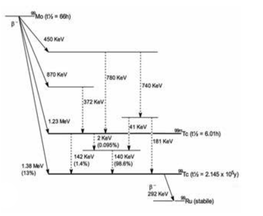
- antiangiogenic activity;
- antiadhesive effect;
- antiproliferative effect;
- pro-apoptotic effect on human malignant melanoma cells.

$RGDechi_{1-17}$ is a bifunctional chimeric peptide composed of a cyclic RGD containing pentapeptide covalently linked by a spacer to an echistatin domain. This peptide can selectively modulate $\alpha_v\beta_3$ function, which is highly expressed in activated endothelial cells and in many solid tumors (e.g. melanomas).



To prepare the $[^{99m}Tc(N)(PNPn)]^{2+}$-system conjugate to $RGDechi_{1-17}$ peptide, the labeling approach exploited involved the use of a Cys as a bifunctional chelating agent (BFCA), conjugated on Lys1 side chain.

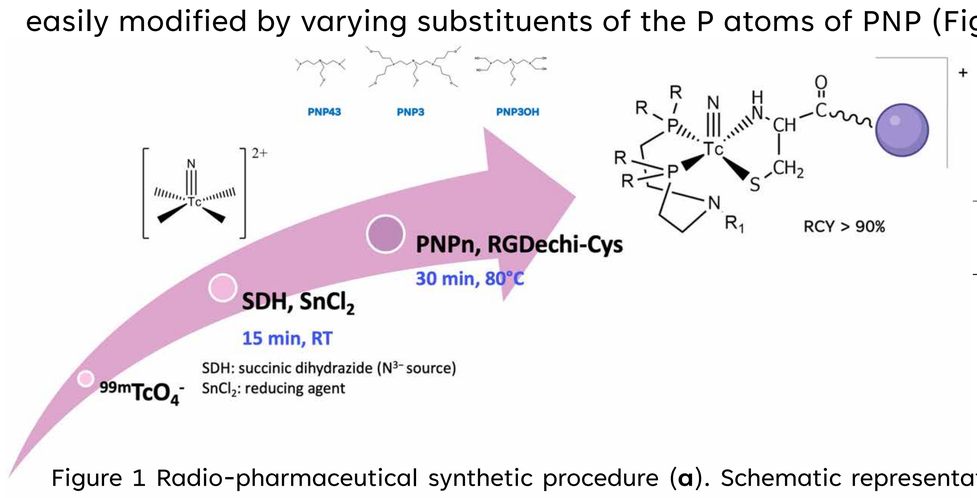




Technetium-99m ($t_{1/2}$=6.02 h, E_γ=140 keV) is the workhorse radionuclide in Nuclear Medicine for SPECT applications.

Radiosynthesis

$RGDechi_{1-17}$ peptide was prepared via solid-phase peptide synthesis. Exploiting the $[^{99m}Tc(N)(PNPn)]^{2+}$-technology,[5,6,7] a Cys residue was coupled on the side chain of Lys1, obtaining $RGDechi_{1-17}$-Cys, affording the final monocationic complex.[8]
Moreover, both the molecular weight and lipophilicity of the radiolabeled peptides were easily modified by varying substituents of the P atoms of PNP (Figure 1).

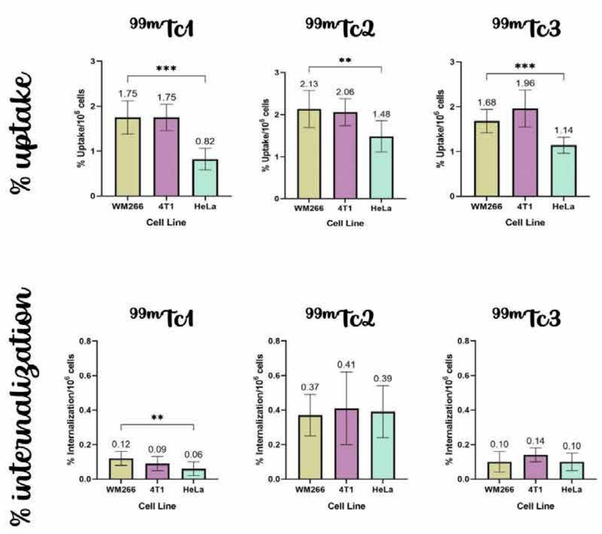


Complex	
$[^{99m}Tc(N)(RGDechi_{1-17}\text{-}Cys)PNP43]^+$	^{99m}**Tc1**
$[^{99m}Tc(N)(RGDechi_{1-17}\text{-}Cys)PNP3]^+$	^{99m}**Tc2**
$[^{99m}Tc(N)(RGDechi_{1-17}\text{-}Cys)PNP3OH]^+$	^{99m}**Tc3**

Table 1 – Radiolabeled peptides synthesized.

Figure 1 Radio-pharmaceutical synthetic procedure (**a**). Schematic representation of the PNP (PNP: bis-phosphino-amine) selected as ligands of Tc (**b**).

In vitro studies



Preliminary studies were carried out incubating ^{99m}**Tc1-3** with $\alpha_v\beta_3$ overexpressing cell lines WM-266-4 (metastatic human melanoma) and 4T1 (breast cancer), and HeLa cells as control (Figure 5).
The radiolabeled peptides possess an almost similar accumulation profile showing a specific uptake in $\alpha_v\beta_3$-positive cells, which was dependent on the nature of $[^{99m}Tc(N)(PNPn)]$-synthon. ^{99m}**Tc1** seems to give the best results.

Figure 5 – Cells studies of ^{99m}**Tc1-3**. The cell uptakes were assessed in suspensions at 37 and 4°C. Data are expressed as percentage cell uptake of the total activity on 10^6 cells. Experiments were performed in triplicate.

Stability studies and Metabolite identification

Stability studies on radiolabeled peptides showed that they are stable in cell culture media, human and murine sera and after incubation in murine liver homogenate. Different behavior was detected after the exposure of ^{99m}**Tc1** to Mouse Kidney Homogenate (MKH): HPLC chromatogram collected after only 15 min incubation clearly shows a significant peak shifting.

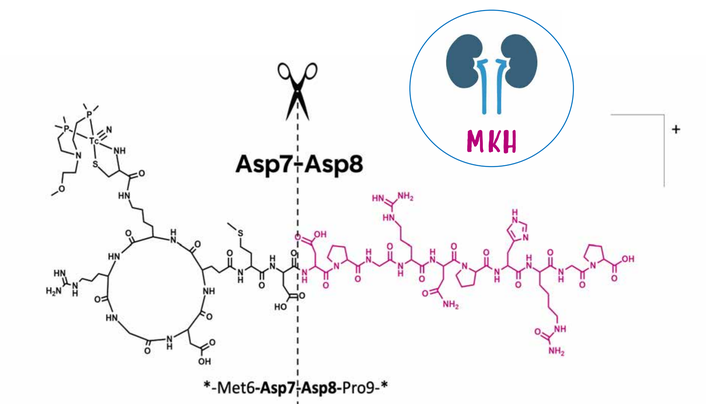


Figure 2 – Schematic representation of the enzymatic cleavage site observed after MKH exposure.

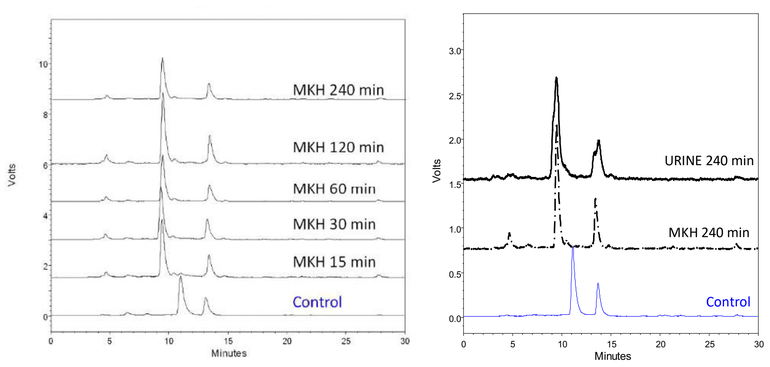


Figure 3 – Radio-HPLC of ^{99m}**Tc1** before and after incubation in MKH (left) and in murine urine sample (right).

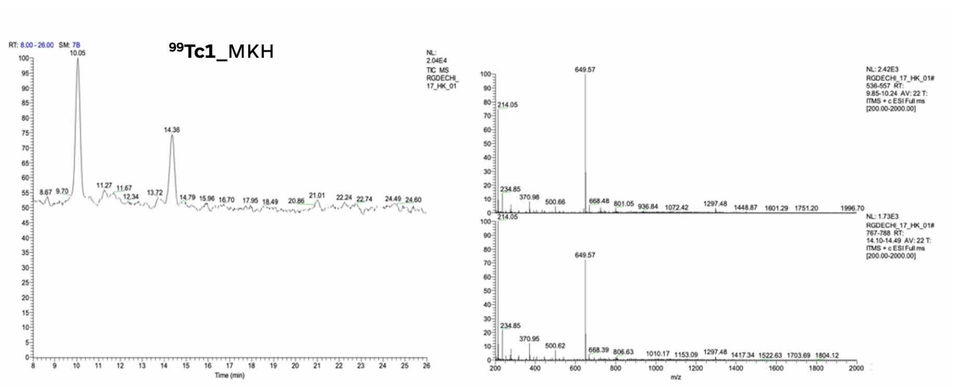


Figure 4 –LC-MS profiles of ^{99}**Tc1** after 1h of incubation, at 37°C, in MKH.

LC-ESI-MS analyses revealed for the two peaks (syn and anti isomers) a similar pattern consisting in a double-charged ion at m/z 649.96 (Figure 4), corresponding to a fragment that lacks the portion starting from Asp8 (Figure 2).

Stabilization of radio-peptide

Given this result, Asp7 residue was replaced by α-methyl-L-Aspartic acid. $RGDechi_{1-17}$(αMeAsp7)-Cys peptide (^{99m}**Tc4**) was synthesized and characterized. Also, this peptide undergo biotransformation after incubation with MKH, but after 1h and not after only 15 minutes.

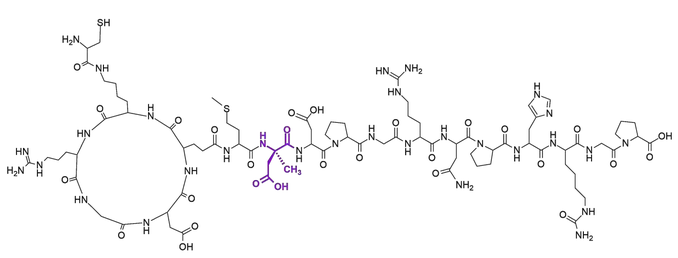


Figure 5 – Schematic representation of ^{99m}**Tc4**.

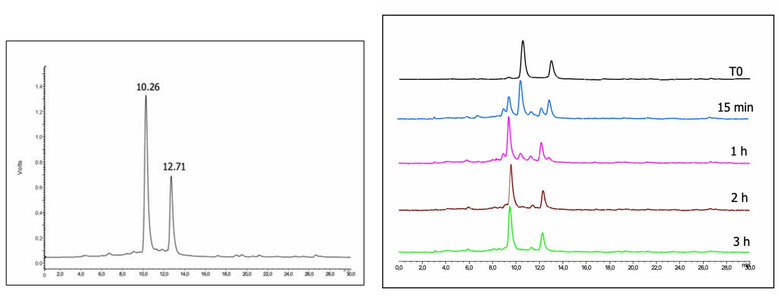


Figure 6 – Radio-HPLC of ^{99m}**Tc4** after purification (left) and before and after incubation in MKH (right).

In conclusion …

^{99m}**Tc1–3** can selectively bind $\alpha_v\beta_3$ integrin and not to cross-react with $\alpha_v\beta_5$.
A cleavage site, common for different peptides, was identified between the Asp7 and Asp8 as a site of enzymes present in circulation and highly expressed in kidneys. The information collected from metabolism studies should be utilized to design, synthesized and characterized a more stable derivative, ^{99m}**Tc4,** in order to improve bioavailability and accumulation at tumor site.

The authors acknowledge AIRC for financial support (AIRC, IG2020 ID 24528)

References:

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