Mitochondrion-Tropic Radioconjugates Carrying TPP and PSMA Derivatives: Radiobiological and Imaging Studies in Prostate Cancer Models

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In the past few years, there has been a significant interest in the design of new theranostic radiopharmaceuticals targeted at the Prostate-Specific Membrane Antigen (PSMA), which is overexpressed in the majority of prostate cancer (PCa) and its metastases [1]. A variety of PSMA ligands were labelled with different imaging and therapeutic radionuclides, namely with the soft beta minus emitter ^{177}Lu. This intense research work led to the development of Lu-177-PSMA-617, undergoing a Phase III clinical trial to treat metastatic castration-resistant PCa (mCRPC) [1,2]. However, the use of beta minus emitters in targeted radionuclide therapy (TRT) of cancer has some limitations, such as the nephrotoxicity and beta radiation resistance encountered in a non-negligible number of patients. Auger electron (AE) emitters can be an attractive alternative to circumvent these difficulties, namely those that are already commonly used in nuclear medicine imaging (e.g. ^{67}Ga, ^{99m}Tc or ^{111}In) [4].

The radiobiological effects induced by AE emitters might include hardly repairable and lethal DNA damage in the targeted tumor cells, if the AEs are emitted in close proximity to a radiosensitive cellular target, such as the nuclear DNA or the mitochondria. In particular, mitochondria-targeted radioconjugates may be an attractive alternative because mitochondrial DNA is damaged by exposure to ionizing radiation, which is also able to elicit other deleterious effects such as ROS production or apoptosis. For this reason, recently, the energized mitochondria of tumor cells started to be studied as a subcellular target for therapeutic AE-emitting radionuclides [3]. Having this in mind, we have designed dual-targeted ^{111}In-DOTA complexes carrying a PSMA inhibitor (PSMA617 derivative) and a triphenyl phosphonium (TPP) group to promote a selective uptake by prostate cancer (PCa) cells and their accumulation in the mitochondria, respectively. Conjugates bearing a cathepsin B cleavable linker between the PSMA617 moiety and the DOTA chelator were also synthesized, aiming at a further enhanced accumulation in the mitochondria upon enzymatic cleavage of the linker. In this way, we expected to obtain AE emitting radioconjugates suitable for a more selective TRT of mCRPC (see Figure 1).

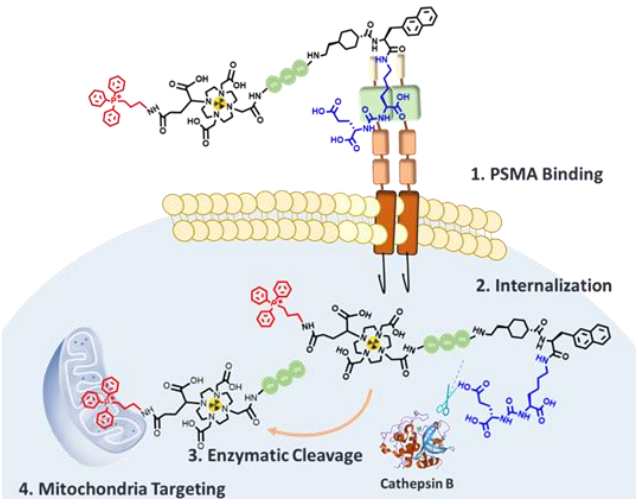

Figure 1. *Schematic drawing of the devised strategy for a cell-specific targeting of tumor mitochondria.*

In this communication, we describe novel DOTA-based chelators functionalized with PSMA617 and/or TPP derivatives and their respective ^{nat}In and ^{111}In complexes. The "cold" compounds (ligands and ^{nat}In complexes) were fully characterized by HPLC, ESI-MS and multinuclear NMR analysis. The ^{111}In complexes were obtained by reaction of the different ligands with $^{111}InCl_3$, in high radiochemical yield and purity at high specific activity; their chemical identity was ascertained by HPLC comparison with the cold congeners. All the radiocomplexes show high *in vitro* stability in physiologic conditions and in the presence of cell culture medium. The biological evaluation included cellular uptake and internalization and PSMA-blocking studies in different cell lines (LNCaP, PC3 PIP and PC3 Flu), subcellular localization experiments and the assessment of radiobiological effects based on the clonogenic survival assay. The PSMA-targeted ^{111}In-radiocomplexes display high cellular uptake and internalization in the PSMA-positive PC3 PIP cells while presenting a negligible internalization in the PSMA-negative PC3 Flu cells. In some cases, the radiobiological studies indicated that the complexes compromise the cellular viability in a dose-dependent manner. In summary, the high and specific cellular uptake and internalization in prostate cancer cells displayed by the PSMA-targeted ^{111}In-complexes, as well as their significant radiotoxicity in the same cell lines, are indicative of their potential for Auger therapy of cancer. MicroSPECT imaging studies in PSMA-positive PCa xenografts are underway to assess how the different components (TPP and PSMA617 pharmacophores, cleavable linker) influence the *in vivo* behavior of the radioconjugates.

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