Mitochondria-targeted Radiocomplexes for Cancer Theranostics

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Objective

Prostate cancer (PCa) is the most common cancer and the second highest cancer-related cause of death in men in the Western world. In particular, metastatic castration-resistant prostate cancer (mCRPC) still remains incurable. The radiobiological effects induced by Auger electron (AE) emitters might result in severe DNA damage, which may be unrepairable, 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. Nuclear DNA has been considered the most relevant target of Auger electrons to have augmented radiotoxic effects and significant cell death. However, mitochondria-targeting radiocompounds 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 reactive oxygen species (ROS) production or induced-apoptosis. Currently, the energized mitochondria of tumor cells are also being studied as a subcellular target for therapeutic AE-emitting radionuclides[1].

Towards this goal, we have designed dual-targeted ^{111}In-DOTA complexes carrying a Prostate Specific Membrane Antigen (PSMA) inhibitor (PSMA617 derivative) and a triphenyl phosphonium (TPP) group to promote selective uptake by 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 to further enhance 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 targeted radionuclide therapy of mCRPC.

Materials and Methods

Novel DOTA-based chelators, functionalized with PSMA617 and/or TPP derivatives, and their respective ^{nat}In complexes were synthesized and fully characterized by HPLC and ESI-MS. The synthesized ligands were radiolabeled with ^{111}In by reaction with $^{111}InCl_3$. The radiochemical purity and *in vitro* stability of the ^{111}In complexes were evaluated by HPLC. The biological evaluation included cellular uptake studies, 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.

Results

The designed dual-targeted conjugates were successfully synthesized and radiolabeled with ^{111}In in high radiochemical yield and purity at high specific activity. The chemical identity of the resulting radiocomplexes was ascertained by comparing its HPLC profile with that of the non-radioactive indium congeners previously prepared. All the radiocomplexes show high *in vitro* stability under physiological conditions. The PSMA-targeted ^{111}In-radiocomplexes display high cellular uptake and internalization in the PSMA-positive PC3 PIP cells while presenting negligible internalization in the PSMA-negative PC3 Flu cells. In some cases, preliminary radiobiological studies indicated that the complexes compromise the cellular viability in a dose-dependent manner. MicroSPECT imaging studies are underway.

Conclusions

The high and specific cellular uptake, 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.

References

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