Theranostic targeting of fibroblast activation protein (FAP) with gold nanoparticles decorated with FAPI fragments.

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1. Authors

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2. Context of the project (800 characters max. including spaces)

Fibroblast activation protein (FAP) is a serine protease expressed by cancer-associated fibroblasts (CAFs) and many tumor cells, but minimally in normal tissues. Its roles in invasion, immune modulation, and matrix remodeling make it a key biomarker and therapeutic target. UAMC1110 is the most potent and selective FAP inhibitor and forms the basis of FAP inhibitors (FAPIs), which link tumor targeting with diagnostic or therapeutic payloads. While FAPIs excel in PET imaging, therapy is limited by short tumor retention. To overcome this, we attach multiple FAPI units to gold nanoparticles (AuNPs). These AuNP–FAPI constructs, featuring UAMC1110 and a Cu-67-NOTA complex, aim for enhanced tumor targeting and radionuclide therapy efficacy.

3. Results and discussion (1000 characters max. including spaces)

AuNPs for in vivo theranostic use (10.6 ± 2.35 nm) were synthesized and characterized in-house. Two variants differing in linker length to the FAP-targeting UAMC1110 were made. FAPI-modified AuNPs showed 19.4 nm size (PDI 0.340) by DLS, 519 nm UV–Vis peak, and high FAP affinity (IC_{50} = 50 pM). Initial Cu-67 radiolabeling of NOTA-PEG-FAPI AuNPs failed. A successful route used pre-labeling of NOTA-lipoic acid with Cu-67, then conjugation to AuNPs followed by PEG and FAPI attachment (RCY = 60%, RCP = 98%), yielding stable, water-dispersible particles. *In vivo* biodistribution of Cu-64-NOTA-PEG-FAPI AuNPs showed predominant liver (30 % ID/g) and spleen (25 % ID/g) uptake for both FAPI-decorated and control AuNPs. Tumor uptake was 2.6 % ID/g vs. 2.8 % ID/g, respectively, indicating minimal enhancement by FAPI functionalization. Nonetheless, the system establishes a basis for further nanoparticle-based theranostic development.

4. Conclusions (800 characters max. including spaces)

Cu-64/-67-NOTA-PEG-FAPI AuNPs were successfully prepared and radiolabelled, but the tumour targeting was not substantially enhanced compared to control nanoparticles. Both in vivo and in vitro data indicate that interactions were non-specific, regardless of FAP presence in the tumor/CAFs. However, the study provides important insights into nanoparticle behaviour and supports future optimization of FAP-targeted nanocarriers, including strategies to improve specificity, uptake, and overall theranostic performance.

5. Involvement of the PRISMAP services (600 characters max. including spaces)

PRISMAP services allowed us to practically execute our research proposal, which would otherwise be impossible since there is limited access to ^{67}Cu in Belgium. The ^{67}Cu delivery from DTU allowed us to optimize the radiolabeling conditions, leading to the development of Cu-67-NOTA-PEG-FAPI AuNPs as a potential therapeutic candidate.

6. Feedback to PRISMAP (600 characters max. including spaces)

PRISMAP has provided us full support for the project application. Additionally, the timely deliveries of ^{67}Cu were ensured so that our experiments could be carried out. The collaboration with DUT not only for the delivery of the radionuclide but also the troubleshooting on the radiolabeling of the AuNPs, contributed to the success this project.

7. Publications and other dissemination activities (conferences etc.)

A manuscript is “in preparation” phase.

Appendix 1. Dissemination guidelines for user projects as agreed in the signed User Agreement

Dissemination rules

Only user groups that are allowed to disseminate the results which they have generated under the project may benefit from the access, unless they are working for SMEs.

For each user group project, a publishable project summary and a publishable summary of the results will be published on the European Union Horizon 2020 PRISMAP project website www.prismap.eu. The publication of results in journals or at conferences is strongly encouraged.

To ensure the long-term sustainability of the PRISMAP initiative, proper recognition of the contributing facilities, their services and the involved persons is necessary. All participating PRISMAP facilities shall be acknowledged in the publication. Acknowledgement and co-authorship of PRISMAP staff members who participated in the experiment shall be considered according to the research field best practices and verified with the PRISMAP Technical Manager before any publication.

The user group shall contact the PRISMAP Technical Manager 30 days prior to submission of publications or other communications of results that were obtained by making use of services provided by PRISMAP (radionuclides delivered or medical services provided). The Technical Manager will communicate to the user group the list of PRISMAP facilities and persons that have contributed to each specific project and the way this contribution must be acknowledged in the publication/communication or where co-authorship is required to reflect specific scientific contributions.

Users must comply with Horizon 2020 dissemination rules (i.e. acknowledge that their work was financially supported by the European Union's Horizon 2020 Research and Innovation Programme by including the following acknowledgement: "This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 101008571 (PRISMAP)"), and grant open access to resulting publications and related data.

Dissemination shall take place only once legitimate interests regarding intellectual property have been safeguarded. A maximum publication delay of 90 days may be granted for this purpose.

Acknowledgements

The list of name(s) to be mentioned in the acknowledgment section is sent to the technical manager by the main contact of the involved facilities.

A general sentence will be added by the corresponding author of the article (user side):

"The authors would like to thank the members of the PRISMAP consortium and of the PRISMAP user selection panel, coordination and management team for their advice and support."

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