Evaluation of aptamers for targeted radiotherapy: Binding specificity and labelling with natural lutetium

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Introduction

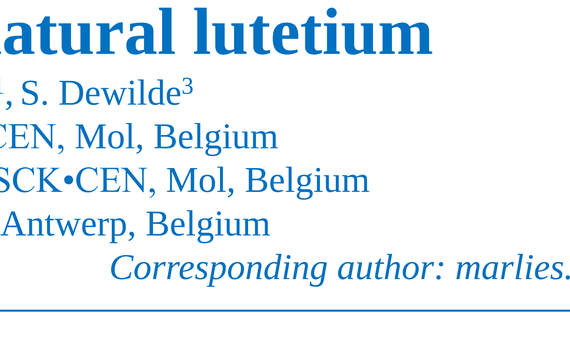
Many cancer patients suffer from serious side effects when treated with external beam radiotherapy or chemotherapy because of damage to healthy tissues by lack of selectivity. In this regard, specific targeting of tumours through radiopharmaceuticals is increasingly considered as a promising strategy in oncology. Radiopharmaceuticals consist of a radionuclide coupled to a vector that specifically targets cancer-related molecules.
Aptamers are small (5-15 kDa) synthetic oligonucleotides (DNA or RNA) that possess several advantages compared to other vectors, such as an relatively easy and cheap chemical synthesis allowing the introduction of different chemical modifications, a selection possible against almost every target, a non-immunogenicity and a good tumour penetration. That is why aptamers are regarded as promising molecules for the development of radiopharmaceuticals.
An aptamer targeting the Human Epidermal growth factor Receptor 3 (HER3), which plays an important role in cancer development and progression, was chosen for the development of aptamer-based radiopharmaceuticals.

Materials and methods

An RNA aptamer sequence (53-mer, ΔG = -24,77 kcal.mole^{-1}) targeting the HER3 receptor (Chen et al, PNAS (2003) 100, 9223-9231) was synthesized by Integrated DNA Technologies (Haasrode, Belgium).

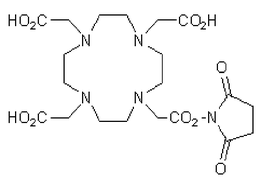
The binding properties of this aptamer were tested on HER3-expressing cells (LS174T cells, ATCC) by flow cytometry (using the RiboGreen fluorescent dye, Invitrogen) and on the recombinant human HER3 protein (R&D systems) by two electrophoretic mobility shift assays based on the detection of proteins (High Sensitivity Protein 250 kit, Bioanalyzer 2100, Agilent Technologies) or nucleic acids (1% agarose gel electrophoresis).

Labelling of the aptamer with natural lutetium after coupling to a bifunctional chelator (1,4,7,10 tetra-aza-cyclo-dodecane-1,4,7,10-tetraacetic acid N-hydroxysuccinimide ester or DOTA-NHS ester), was evaluated using electrospray ionization mass spectrometry (ESI-Q-TOF MS, Mass Spectrometry Laboratory, University of Liège, Belgium).

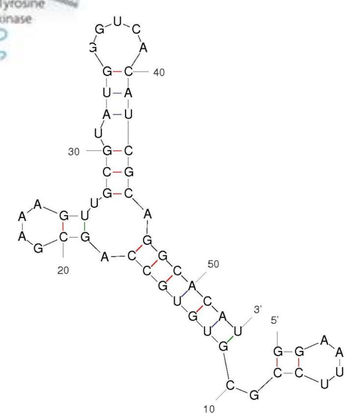


Baselga et al (2009)
Nat. Rev. Cancer 9,463-475

HER receptor family



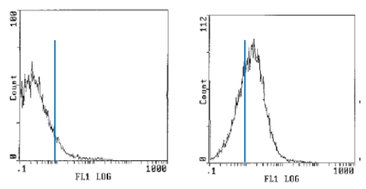
DOTA-NHS ester



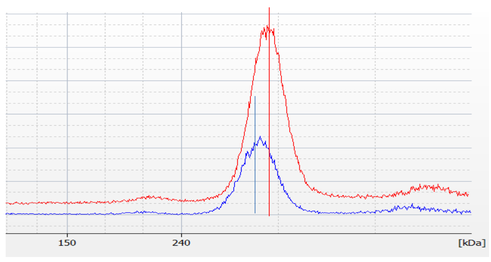
Anti-HER3 aptamer

Results

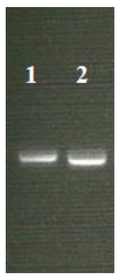
Evaluation of binding properties



Flow cytometry using the LS174T cells incubated without (negative control, left) and with the anti-HER3 aptamer (right) resulted in two different spectra. The cells incubated with the aptamers have a higher fluorescence (shift of the peak to the right) which suggests binding of the aptamer to the LS174T cells.



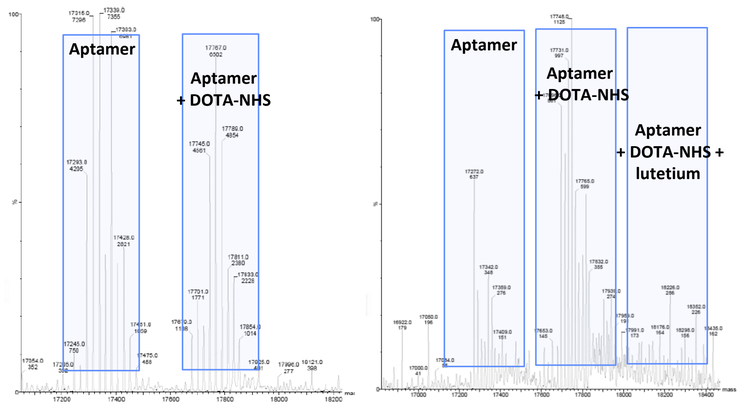
Protein electrophoresis showed a shift of the peak (aptamer-HER3 protein complex, red, compared to the HER3 protein, blue) which corresponds to a difference in migration time. This difference suggests binding of the aptamer to the HER3 protein.



Nucleic acid electrophoresis revealed a difference in migration distance between the aptamer-HER3 protein complex (lane 1) and the aptamer (lane 2) which also suggests binding of the aptamer to the HER3 protein.

Labelling

The ESI-Q-TOF MS spectrum of the DOTA-NHS ester-coupled aptamer (left spectrum) revealed a moderate yield of the coupling reaction. Two populations of peaks (sodium adducts) can be recognised as aptamers with and without the DOTA-NHS ester.
After labelling three populations of ammonium adducts are visible (right spectrum); aptamer with and without the DOTA-NHS ester and aptamers with the DOTA-NHS ester including an extra mass of 175 Da corresponding to the lutetium ion.



Conclusions

In this study, different techniques were evaluated to test the binding properties of an anti-HER3 aptamer to its target on cells or as pure protein. Furthermore, the aptamer was successfully coupled to DOTA-NHS ester and labelled with natural occurring lutetium. However, for a better yield, the coupling and labelling reactions need further optimization. To this end, it is foreseen to radiolabel the anti-HER3 aptamer with radioactive lutetium-177 and to perform preclinical evaluation of this potential therapeutic radiopharmaceutical *in vitro* and *in vivo*.