Development of aptamer-based radiopharmaceuticals for targeted cancer imaging and therapy

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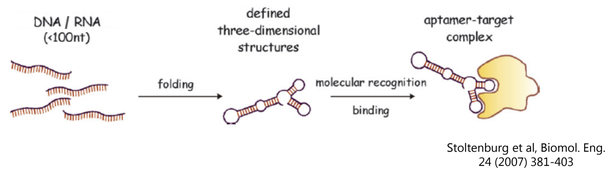
Introduction

Aptamers are small (5-15 kDa, 15–60 mer), non-coding, single-stranded nucleic acids (DNA or RNA) that can fold into unique three-dimensional structures which allow the interaction with a target with high affinity and specificity. Aptamers exhibit significant advantages relative to protein therapeutics in terms of size, synthesis, modifications, possible targets and immunogenicity. Therefore, aptamers are regarded as promising vector molecules for the development of radiopharmaceuticals for molecular cancer imaging or targeted cancer therapy.



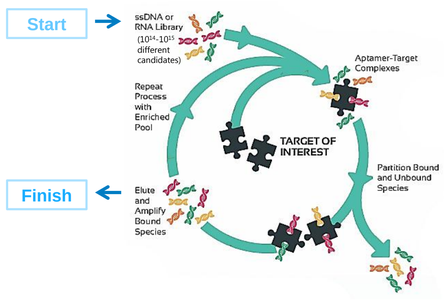
Stoltenburg et al, Biomol. Eng. 24 (2007) 381-403

The Human Epidermal growth factor Receptor 2 (HER2) is a transmembrane receptor tyrosine kinase and a member of the HER family. Overexpression of HER2 is observed in 20 to 30% of breast cancers. Since its role in cancer development and progression, HER2 is attractive for targeted imaging and therapies.
In this study, we aim to select aptamers targeting HER2 by the use of an *in vitro* selection process called SELEX (Systematic Evolution of Ligands by EXponential enrichment) in collaboration with the Astbury Centre for Structural Molecular Biology of the University of Leeds (United Kingdom). The resulting aptamers are evaluated for their binding properties, i.e. affinity and specificity, to their target on cancer cells. In regard to the specificity, a HER2 negative cancer cell line was created by removing specific HER2 expression through gene silencing.

Results

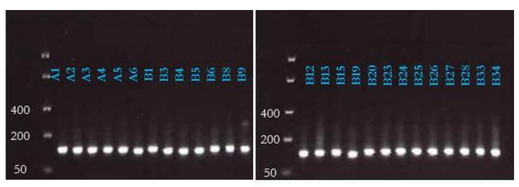
The SELEX process

A pool of 10^{14} different aptamer candidates (the library), containing a 50-mer randomised region flanked by two fixed primer ends, was subjected to iterative rounds of selection, separation and amplification to find candidates with the best binding properties. As a target, both the purified HER2 protein and HER2 overexpressing breast cancer cells (SK-BR-3 cells) were used. In order to avoid non-specific binding, extra steps using the purified HER3 protein and HER2 low expressing breast cancer cells were included.



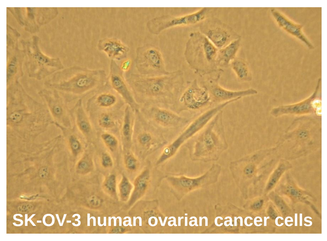
Adapted from AptaSol, 2012

After 15 rounds of selection, the resulted aptamer pool was cloned in *E. coli*. Colony PCR revealed 26 selected RNA aptamers with the correct insert length (i.e. 121 bp). These aptamers will be evaluated further *in vitro*.

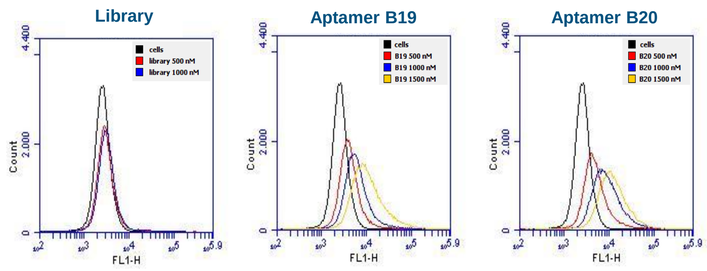


Evaluation of binding of selected aptamers

Binding of the individual selected RNA aptamers was tested on SK-OV-3 cells, a human ovarian cancer cell line with overexpression of HER2 by flow cytometry. To this end, the aptamers are fluorescently labelled via T7 *in vitro* transcription using fluorescein-12-UTP (Roche Applied Science).

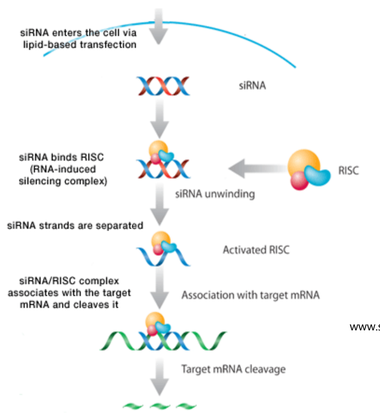


The unselected library serves as a negative control for non-specific binding. We observed a dose-dependent fluorescent signal for two tested aptamers, B19 and B20, compared to no signal for the unselected library which suggests binding to the SK-OV-3 cells.



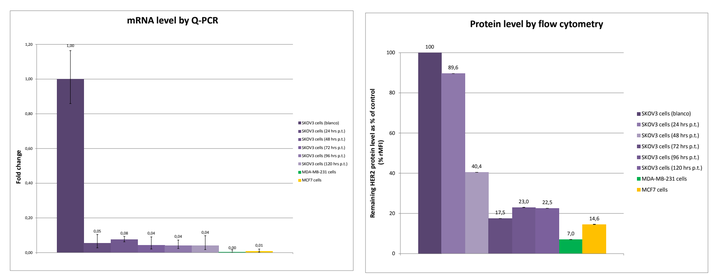
Generation of a HER2 negative cancer cell line through gene silencing

50 nM small interfering RNA (siRNA) targeting HER2 mRNA (Dharmacon) was introduced into SK-OV-3 cells through lipid-based transfection using Lipofectamine RNAiMAX (Invitrogen). The HER2 mRNA level was measured by Q-PCR and the HER2 protein level was tested by flow cytometry.



www.scbt.com/gene_silencers 2012

Both levels (mRNA and protein) were compared to cells which were not transfected (represented as 0 hours after transfection). We observed a knock-down of 96 % of the mRNA level and 82.5 % of the protein level at 72 hrs and 96 hrs after transfection, respectively.



Discussion

This SELEX experiment, for the selection of HER2 targeting aptamers, resulted in 26 selected RNA aptamers for further individual evaluation. Up till now, we were able to identify two aptamers that show binding to HER2 overexpressing SK-OV-3 cancer cells. In addition, we were able to generate silenced SK-OV-3 cells with minimal remaining HER2 levels, which will be used to obtain more information regarding the binding specificity of the selected aptamers

Future

Based on several binding properties (affinity, specificity, kinetics), internalization potential, and primary and secondary structures, we will select a small set of aptamers which will be conjugated to a chelator and radiolabelled with lutetium-177. These potential therapeutic aptamer-based radiopharmaceuticals will be further evaluated *in vitro* and *in vivo*.

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