AAMC Theranostics: A Step Toward Personalized Medicine

Next Generation X-Aptamers in Cancer Therapeutics and Companion Diagnostics

David G. Gorenstein, Ph.D.
Brown Foundation Institute of Molecular Medicine for the Prevention of Disease
Department of NanoMedicine & Biomedical Engineering
UT HSC Houston

Disclosure:

AMBiotech
A Frustrating Decline in the Development of New Therapeutics

Overall trend in productivity (adjusted for inflation)

“Eroom’s Law?”


F. Collins, AAMC Grand/Great 2012
Drug & Companion Diagnostics Development

Problem:
Still takes 10-14 years and now Billions $

Organize:
UTH Institute for Molecular Medicine

UTH / MDACC Center for Clinical and Translational Sciences

NCI Nanomedicine Center
Different Approach to Personalized Therapeutics and Companion Biomarkers?

- **Aptamers**: Nucleic acids that bind a specific molecular target
- **DNA or RNA**
- **Synthetic or naturally occurring**
- Affinity & specificity comparable to antibodies *yet stable at room temperature*
- **Susceptible to nucleases**
- Targets include proteins, carbohydrates, small molecules, intact cells, virions
- Applications in research, diagnostics, imaging, therapeutics
- **PMSA targeted aptamer nanoparticle in prostate cancer clinical trial** (Langer)
Thioaptamers: Thiophosphate Backbone Modifications

- Enhanced nuclease stability
- Phosphorodithioates are achiral at phosphorus
- Incorporated at selected positions during chemical synthesis
- Enhance aptamer binding affinity without sacrificing specificity
- Incompatible with conventional SELEX
Personalized Companion Diagnostics and Therapeutics: Thioaptamer Selection against Human Ovarian Tumor Endothelial cells

The normal and tumor endothelial cells were isolated from the ovarian tissue samples from the patients.

In vivo validation
Orthotopic Ovarian Cancer Mouse Model

Fresh normal ovaries

Counter selection

library

n iterations

Heat denaturation

Next-gen Sequencing

Selected Sequences after n cycles

Normal endothelial cells

Tumor endothelial cells

Endothelial cell (EC) isolation (CD31⁺; CD146⁺)

Human tumor

Selected Sequences

PCR

ID biomarker target of thioaptamer

By pulldown LC MS/MS proteomics

i.v. inj of identified promising thioaptamers

i.p. injection of ovarian tumor cells (tumor establishment)

The normal and tumor endothelial cells were isolated from the ovarian tissue samples from the patients.

Simultaneously identify a personalized companion diagnostic biomarker and reagent as well as potential therapeutic and targeting agent for imaging and nanomedicine.
One thioaptamer identified via cell-based screen can target tumor vasculature following i.v. administration (HeyA8ip2, Nude mice, 4hr).

Thioaptamer targets a membrane protein involved in angiogenesis and metastasis
Delivery of TA28-conjugated chitosan nanoparticles in vivo
NIR Images E-selectin targeting thioaptamer gold nanoparticles

Gold nanoshell Nanoparticles

IRdye 800-labeled ESTA-1-NPs. Pancreatic tumor xenograft model
Next-Gen X-Aptamers: Base Modifications with Novel Chemical Functionalities

- Virtually unlimited chemical functionality
  - Positive charges
  - Hydrophobic groups
  - Amino acids
  - Drugs
- Easily incorporated at selected positions
  - Directly during synthesis or post-synthetically
  - Use amide coupling or click-chemistry
- Modifications that are incompatible with SELEX
Our Bead-Based Process

random sequence library on beads: one bead, one compound (DNA or RNA)

labeled target (in solution)

isolated individual beads with high target binding

Advantages vs. SELEX:
- Very high selective enrichment
- Single cycle required
- No PCR amplification bias
- No chemistry limitations
- Modifications at any positions
- Freedom to operate (patents issued)
- Nanobead libraries as well
Bead-based X-aptamer Library: Split-pool Chemical Synthesis

Combinatorial Bead Library Synthesis: One bead one sequence \((10^8)\)

“One Bead One Library” \(10^8 \times 10^6 = 10^{14}\) Library of Random Libraries

Fully Automated DNA split pool synthesizer

Chemical Modifications can be incorporated randomly at any positions, not just dNTP
IgE Model System

- DNA aptamer\(^1\)
- Binds human IgE with \(K_D \sim 10\) nM
- Requires MgCl\(_2\) (1 mM)
- Very well characterized\(^2\)
  - all single & double base mutants

Library Selection by Fluorescence Microscopy

- Incubate beads with biotinylated protein
- Incubate with streptavidin-coated QDot-605
- Visualize by fluorescence microscopy
Library Selection by Fluorescence Microscopy

- 13 of 15 positive beads (87%) recovered of correct sequence (IgE aptamer)
- 100% of negatives (>100,000) have mutated sequence that do not bind
**X-aptamer Selection**

1. **Starting library** ~10^6 compounds
2. **In silico screening**
3. **Focused library** ~10^3 compounds
4. **Best hits**
   - Hit 1
   - Hit 2
5. **X-aptamer Library on beads** (Binding to target protein validated by NMR)
6. **Bead-based Combinatorial selection**
7. **PCR on beads and sequencing**
8. **X-aptamer with highest binding affinity**

**X-aptamers:** Amino acid-like sidechains, backbone phosphate modifications, and drug-like moieties inserted at multiple positions
Results: X-aptamer to CD44 (stem cell biomarker)

In silico screen

Docking

Prediction

Validation

HSQC NMR

Rendered in 3D Model

DANA drug binding

$K_d \approx 2 \text{ mM}$

X-aptamer Library

Selection

Optimization

Selected: $K_d \approx 200 \text{ nM}$

$K_d \approx 2-15 \text{ nM}$

~100-fold better binding than thioaptamer but with full thiophosphate backbone and DANA drug ~1,000,000-fold better drug binding!

He et al., Biochemistry, October, 2012
Molecular Dynamics Simulation
CD44-HABD + ligand

5 ns MD simulation at 300K
MD Simulation

CD44-HABD + X-aptamer (hairpin)

38 ns unrestrained MD, AMBER 11
explicit water (~50,000 atoms), 300K
Proteomics and POC Clinical Diagnostics

Traditional: Imaging, ELISA, IHC
Biomarkers
Microarrays/nanochips/
biosensors / SRM MS
Ligands (antibodies, aptamers)
Goal?
X-aptamer Sandia Microfluidic Chips for POC Diagnostics

Incubation

Antigen-containing sample + Receptor (Ab*) \rightarrow Immune complex

Fluorescent Receptor

Microchannel

100 µm

Immune complex

Detection

Fluorescence

Elapsed time

Complex

Antigen Concentration

Herr et al., PNAS, 2007
Herr et al, Anal Chem, 2005
Conclusions: Next-gen Aptamer Opportunities for Theranostics?

- Can we short-cut long and expensive theranostics pipeline by simultaneously identifying imaging agent, therapeutic and biomarker to accelerate drug and companion diagnostics development?
- Cell-based screening and Next-gen X-aptamers are excellent alternatives to antibodies in diagnostics, imaging, therapeutics and nanomedicine
- Very high affinity ligands – nM to pM
- Very high specificity – single protein target binding
- Thioaptamers greater stability towards nucleases
- Bead-based X-aptamer libraries
  - Click or amide coupling chemistry allows us to introduce virtually any base modification in the starting library
  - Full chemical diversity of different amino acid-like sidechains or even small molecule drugs is achievable at every site in a molecule
  - Modifications can be easily identified by simple PCR and sequencing
- Applicable as a substitute for antibodies in micro- and nanotechnologies for POC diagnostics (eg, Atactic, Nanosphere, Bioscale, NanoInk, Sandia microfluidics chips)
- Personalized theranostics – personalized thioaptamers and X-aptamers for drug delivery and companion diagnostics imaging in nanomedicine
# Acknowledgments

## PROTEOMICS / TA

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## X-APTAMERS

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## Cancer

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