

Versatility of Nucleic Acid for Cancer Theragnosis

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Requirements for development of cancer gene therapeutics

Targeting
Efficiency
Efficacy
Safety

Delivery

-Systemic
administration

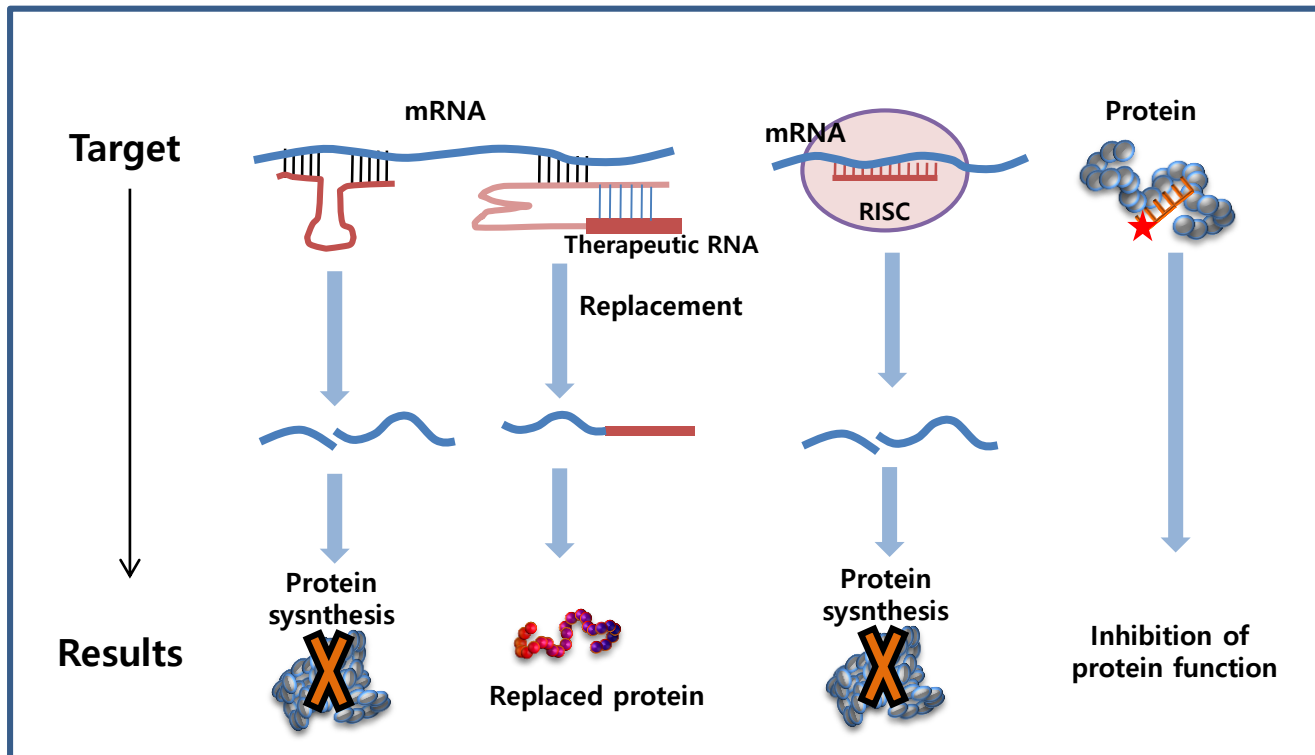
Monitoring

-Non-invasive
-Reflection of
individual
differences

Nucleic acid-based therapy

in vivo Imaging

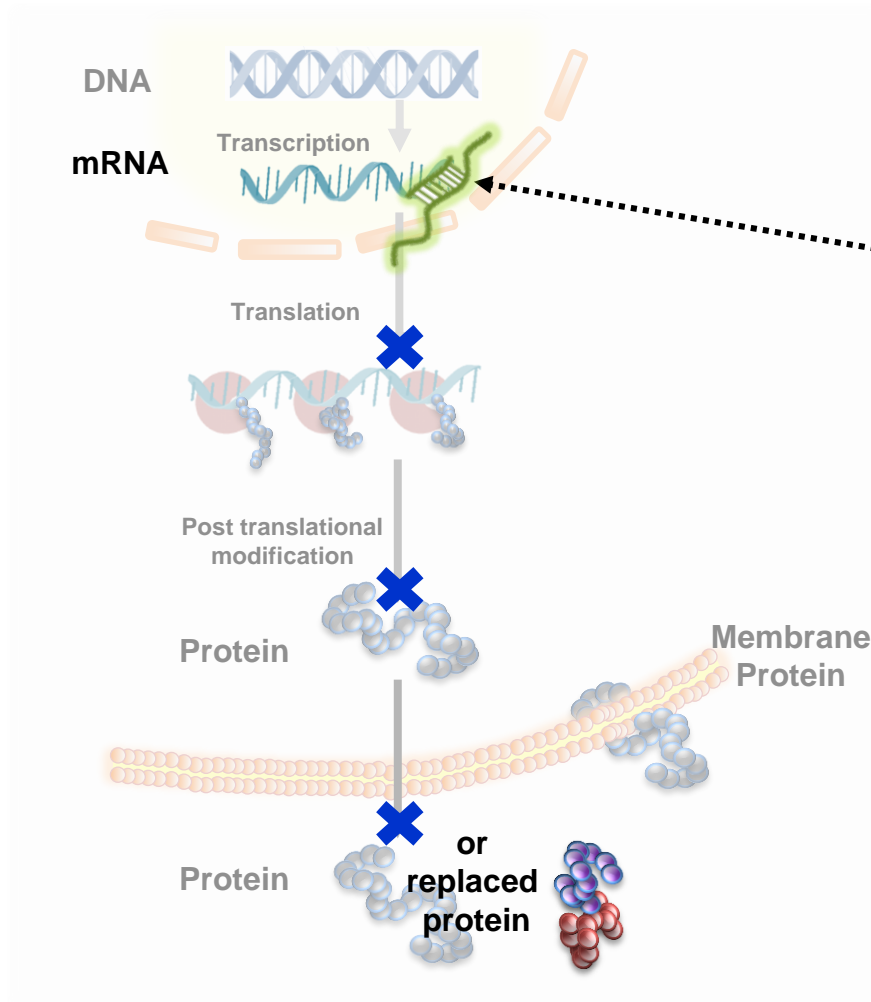
Nucleic acid-based therapeutic strategies



- ❖ **Gene Silencing:** antisense RNA , ribozyme, RNAi, miRNA
- ❖ **RNA replacement:** trans-splicing ribozyme, spliceosome
- ❖ **Modulating protein function:** aptamer

General strategy of Nucleic acid-therapeutics

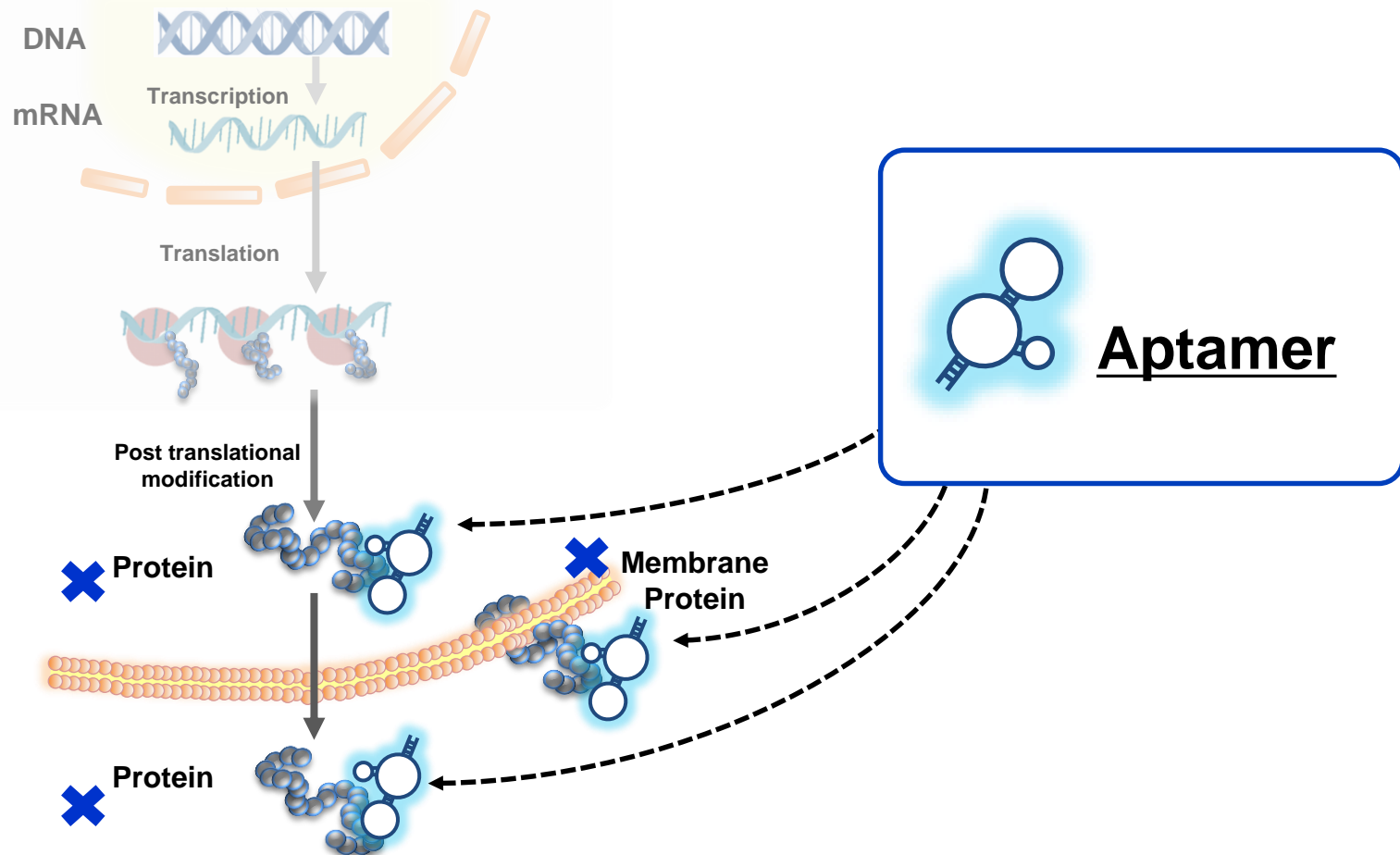
1) Inhibition of protein biosynthesis



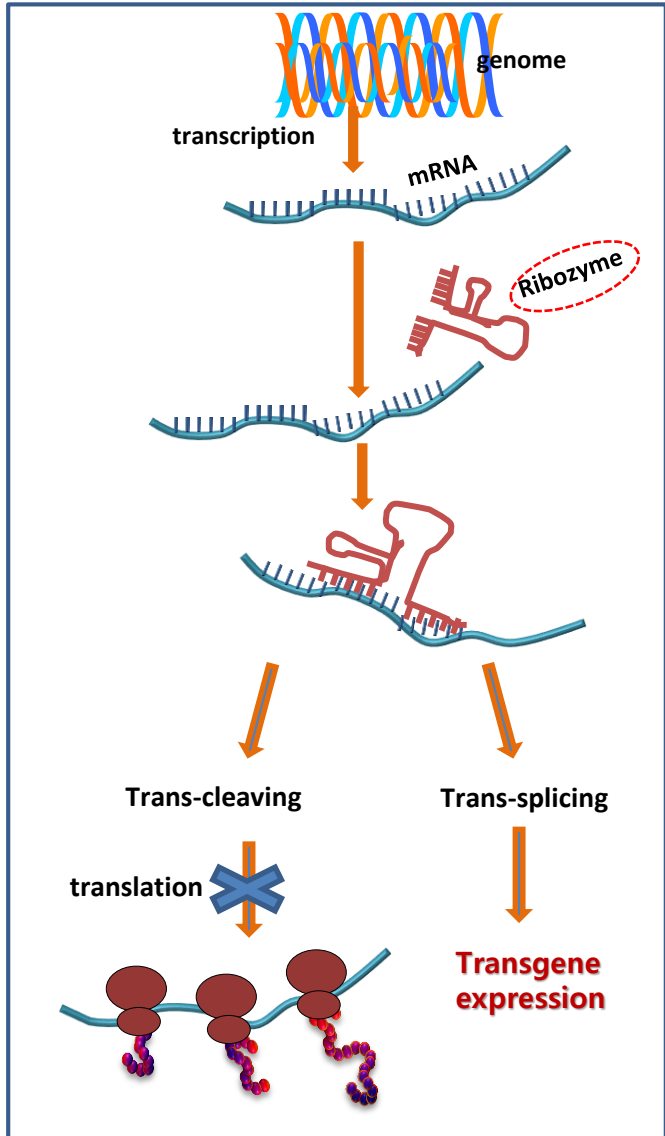
- Antisense oligonucleotides
- Ribozymes
- Small interfering RNAs (siRNA)
- Short hairpin RNAs (shRNA)
- Micro RNA (miRNA)

Strategy of Nucleic acid-therapeutics

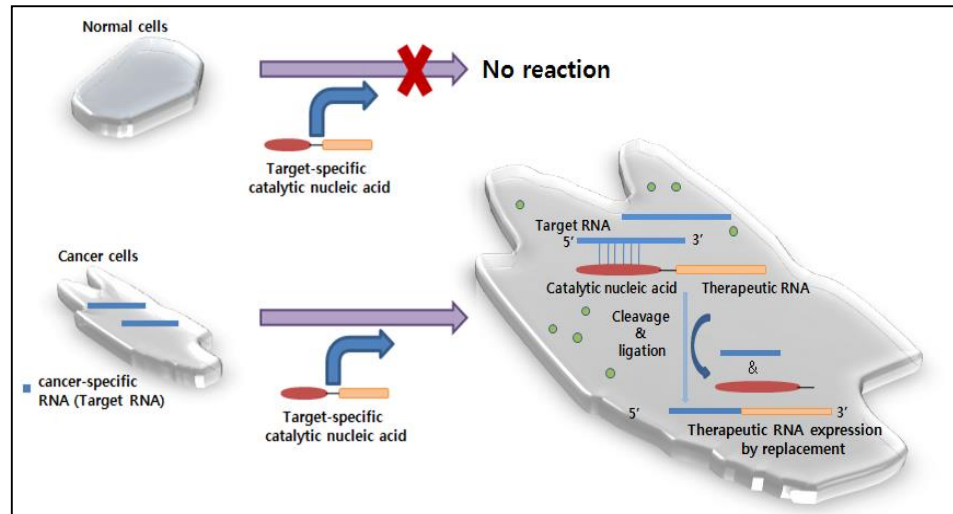
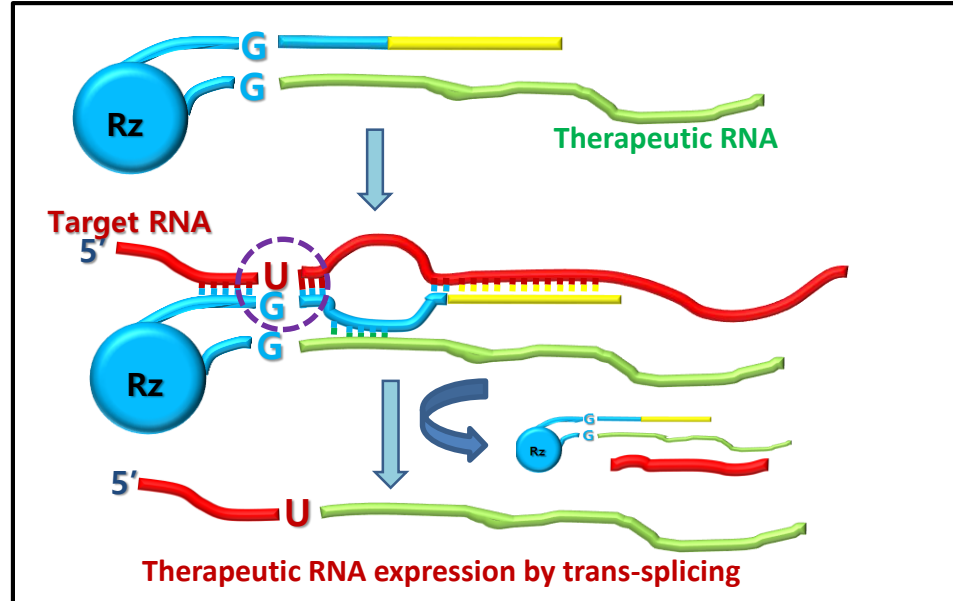
2) Capturing agents to bind protein



RNA replacement : trans-splicing Ribozyme

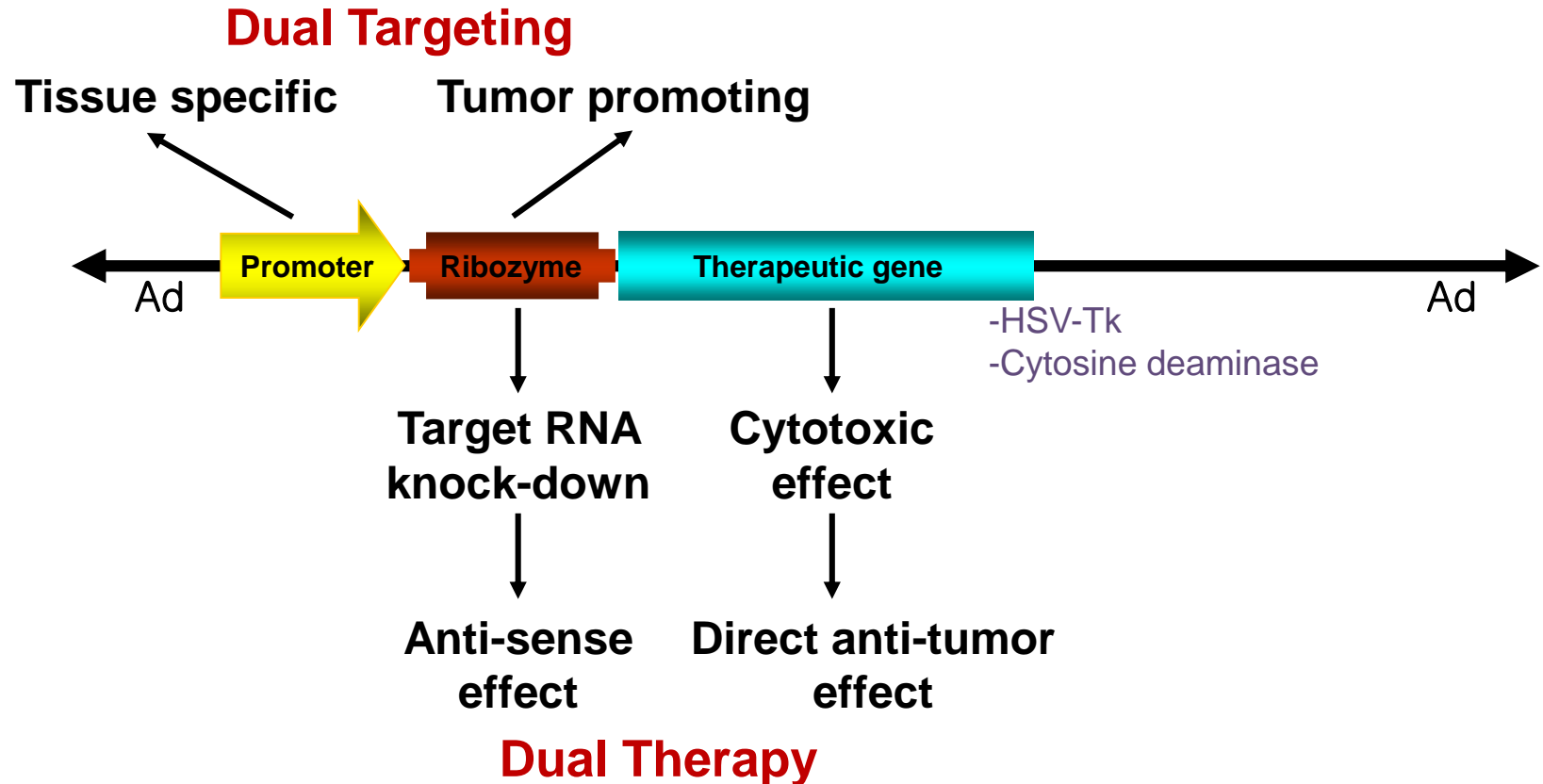


Trans-splicing ribozyme



Multifunctional Tumor Targeting Device

by *trans*-splicing ribozyme

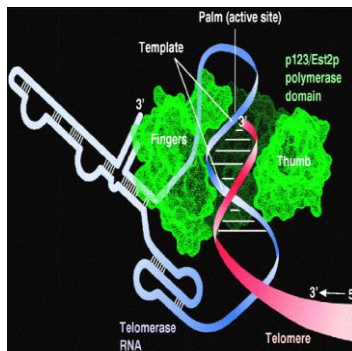


Target & therapeutic gene for replacement in trans-splicing ribozyme

Target

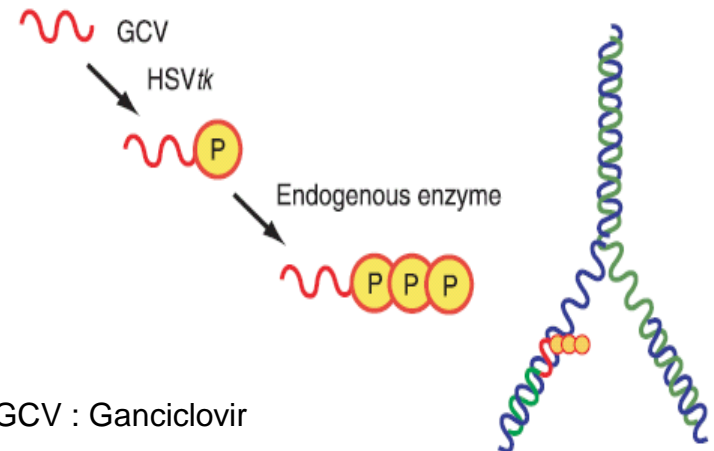
hTERT : component of telomerase gene
- potential anti-cancer therapeutic target -

1. Ribonucleoprotein enzyme that maintains the protective telomere structures (tandemly repeated (TTAGGG)_n seq.) in eukaryotic chromosome
2. Expression level of hTERT relates with telomerase activity
3. Selective expression in highly proliferative cells (bone marrow stem cells, germ cells...) and **~90% of cancer cells**



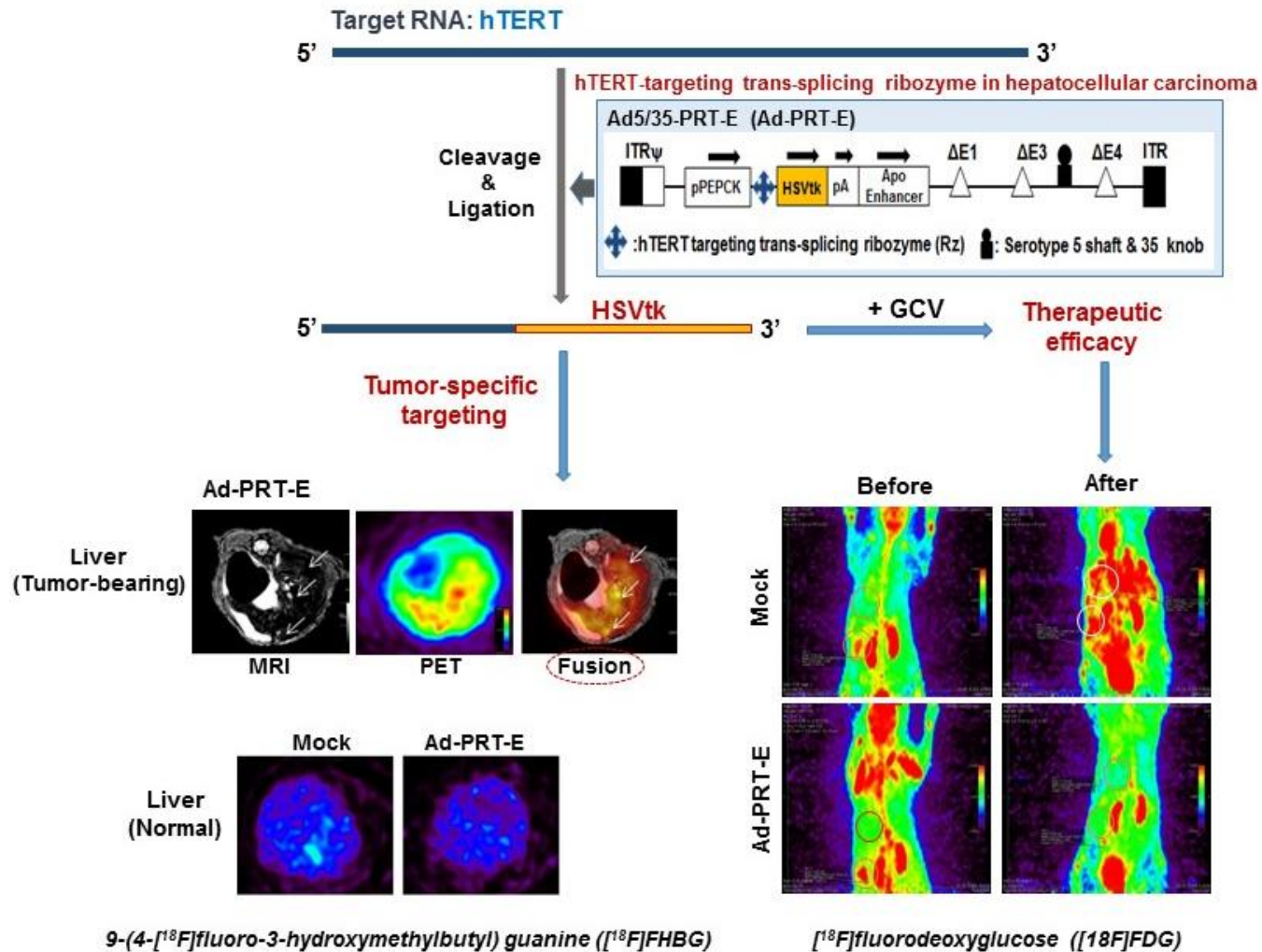
Therapeutic gene

Herpes simplex thymidine kinase (HSVtk)
- Cell death by bystander effect



- Well-known mechanism of action
- Strong cell killing potential due to neighboring effect
- No toxic effect without GCV
- Expression can be monitored by PET *in vivo*

Multifunctional Devices(trans-splicing ribozyme) for Hepatocellular Carcinoma Therapy

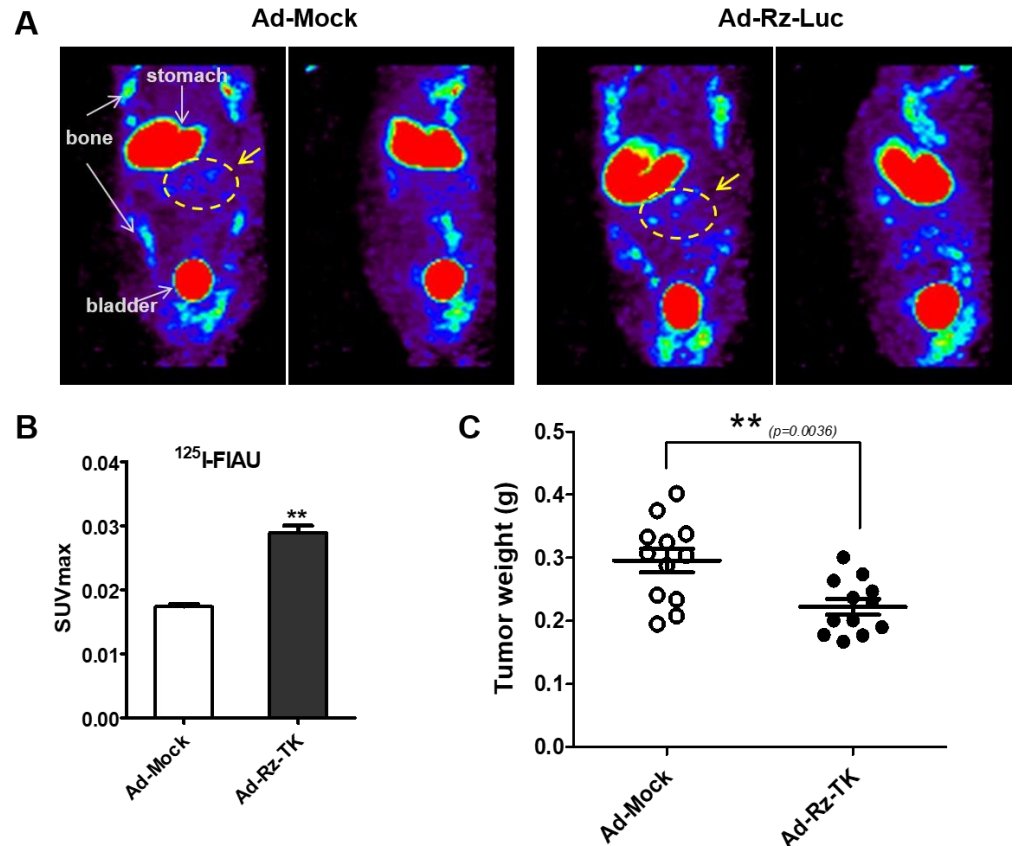
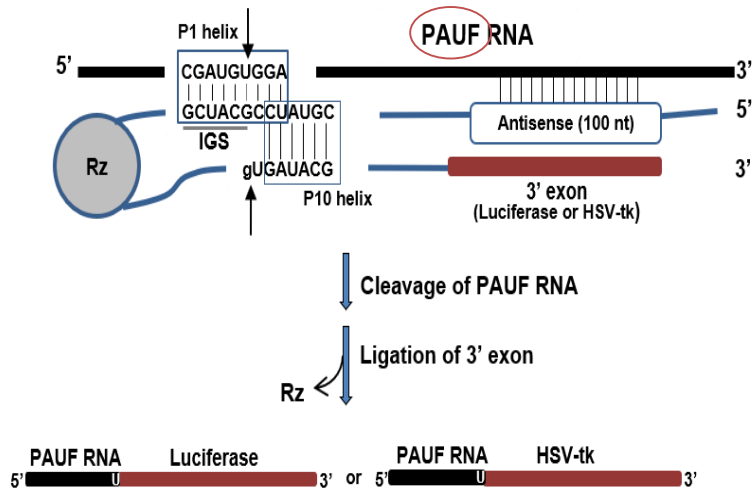


* Image-aided *in vivo* Evaluation

Multifunctional Devices(trans-splicing ribozyme) for Pancreatic Cancer Therapy

Target RNA:

PAUF (pancreatic adenocarcinoma up-regulated factor)



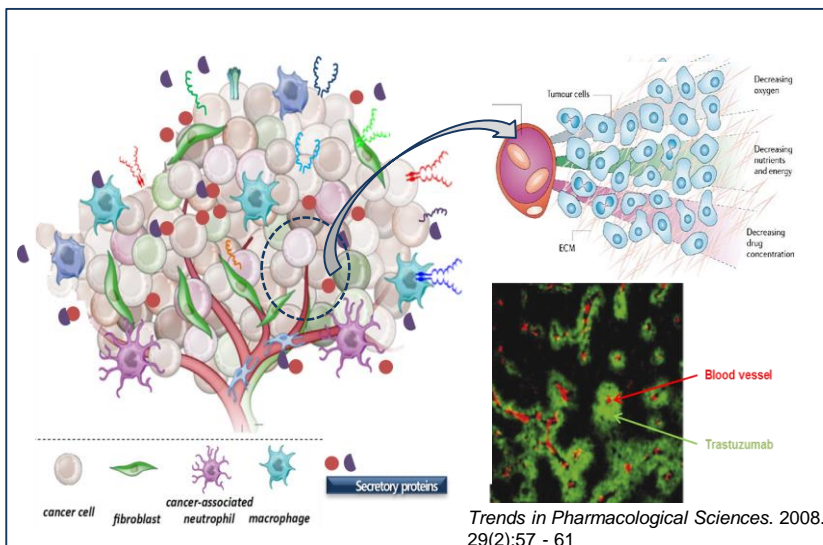
* Image-aided *in vivo* Evaluation

Additional Requirements for Cancer Therapy

1. Target (tumor specificity, natural conformation,...)
2. Specific-Targeting efficiency
3. Penetration into tumor tissue
4. Easy manipulation for optimization
5. Speed & Cost for development
6. Multiple targeting (simultaneously)
 - Complexity of tumor microenvironment



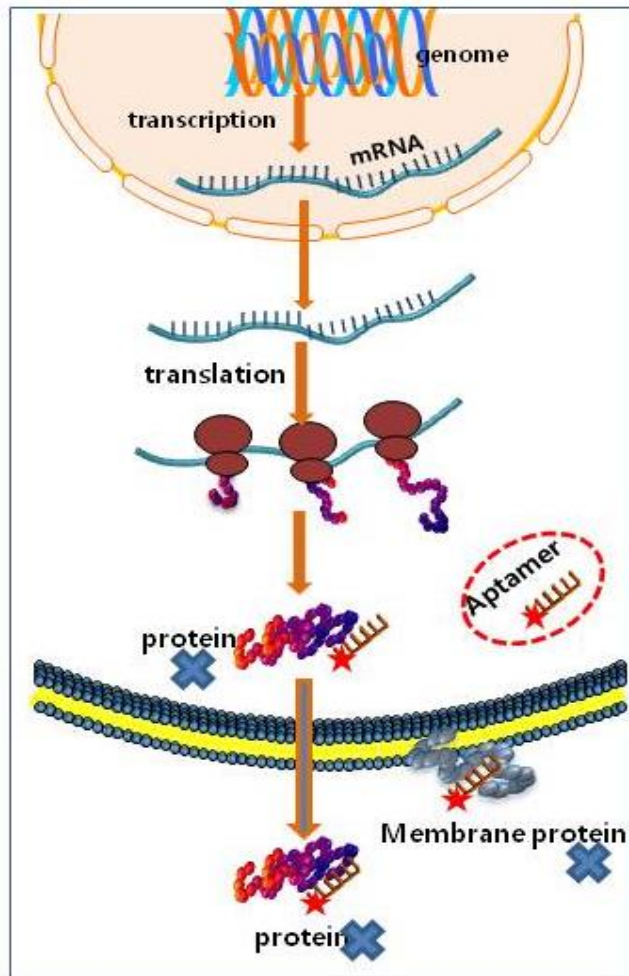
Aptamer



<Advantages of aptamers>

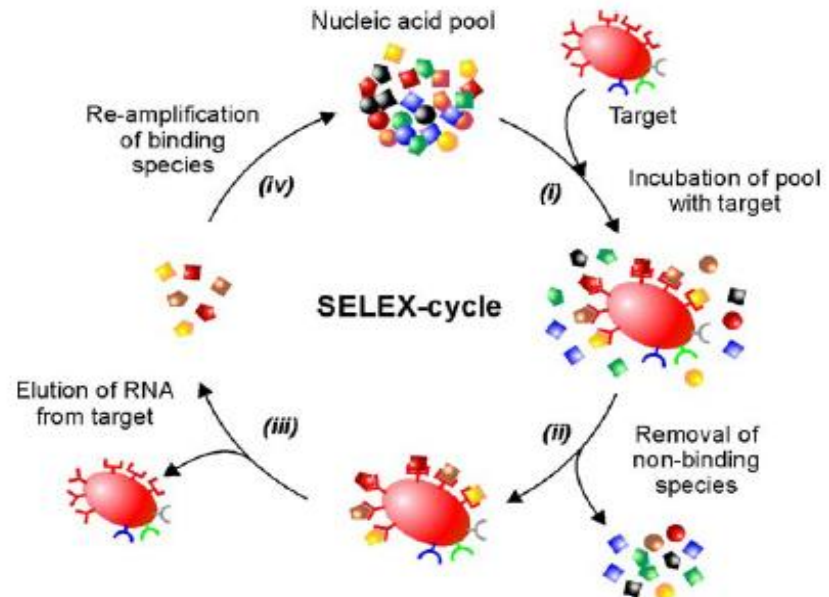
- **Specifically bind** a target of interest
($K_D = \text{pM} \sim \text{nM}$)
- Produced by **chemically process** (*in vitro*)
- **Conjugation chemistries** are easy
- **Smaller size** allows tumor tissue penetration
- Able to select for **cell-surface targets**

Modulating protein function : Aptamer

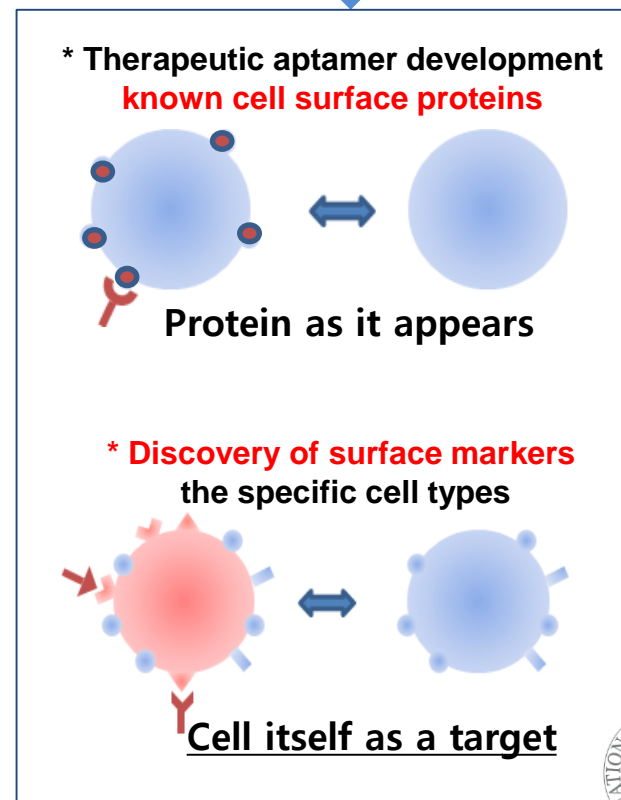
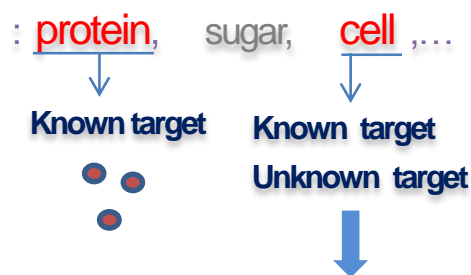
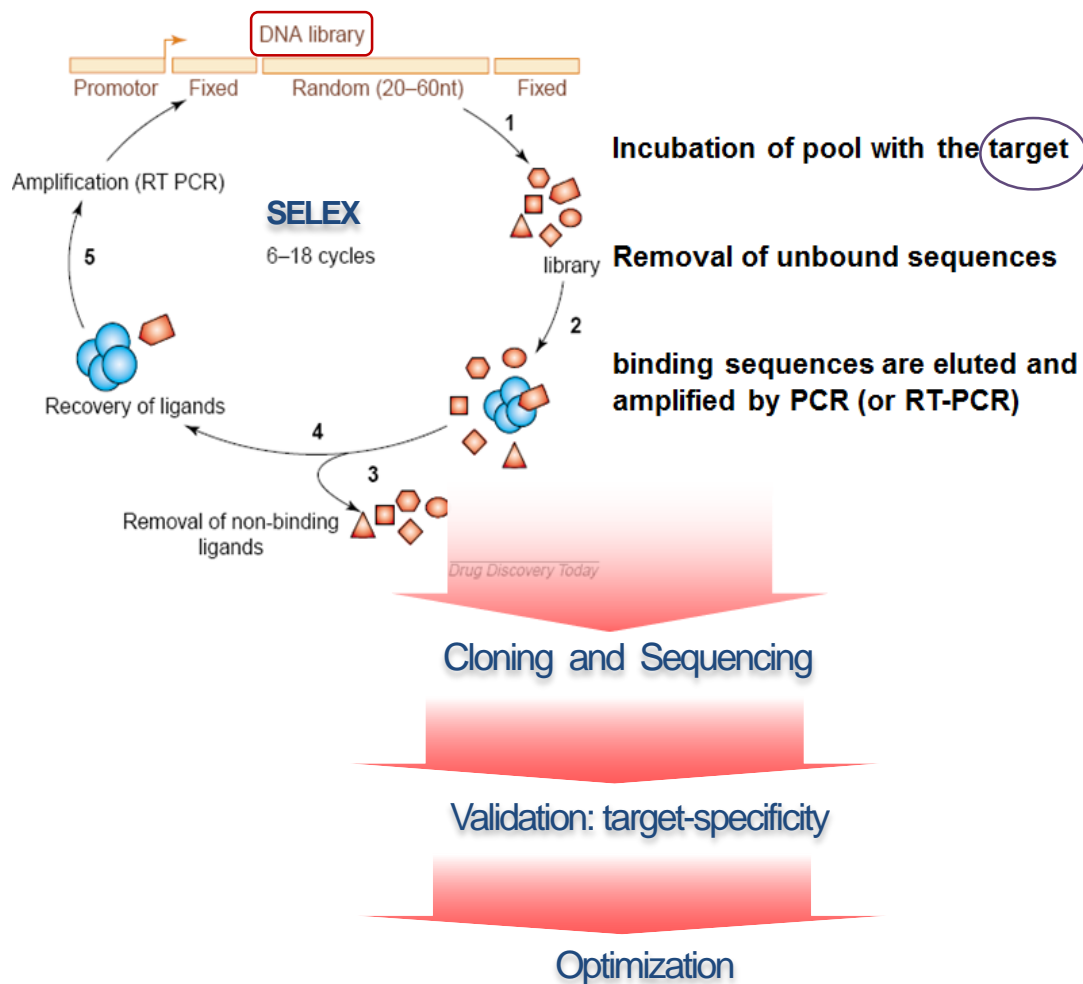


- ❖ Latin: “aptus” – to fit
- ❖ Oligonucleic acid (DNA or RNA) or peptide molecules that bind a specific target molecule
- ❖ Aptamer can be thought of as the nucleic acid antibodies
- ❖ Sequences are selected from very large pools (10^{15} or greater)
- ❖ Discovered through *in vitro* selection (**SELEX**) to recognize and specifically bind a target of interest

SELEX : systematic evolution of ligands by exponential enrichment



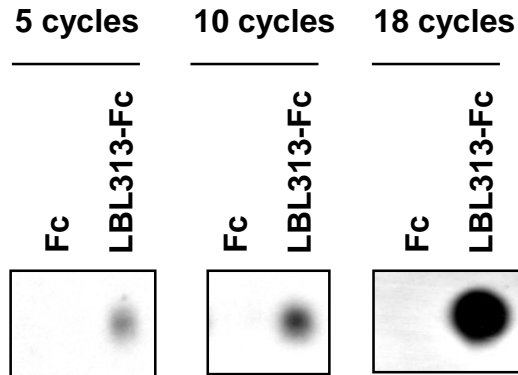
SELEX (systemic evolution of ligands by exponential enrichment)



PAUF-targeting Aptamer

for known secretory target

Protein-based SELEX → Aptamer



L1, L2, L4, L6, L7, L8, L9, L10, L11, L14, L17, L18, L19
CCTGTAAATACACGCATCGTATCTCGATTCTCGATCCTTGACC

L3
CCTGTAAATACACGCATCGTATCTCGATTCTCATATCCTTGACC

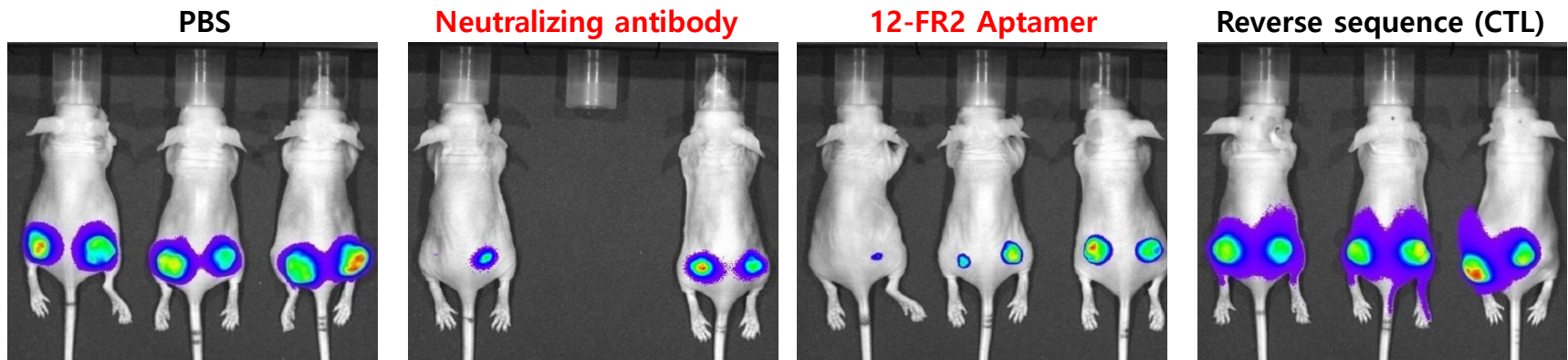
L12
CCTGTAAATACACGCATCGTATCTCGATTCTCGATCCCTGACT

L15, L20
CCTGTAAATACATTCTTCCGCGTGTGATACCGTCCGTGACTA

L5
CCTGTAAATACACTGATCTTCCGATATGTTCTCGCCATCATAC

PAUF targeting aptamer – *in vivo* function

Tumor: CFPAC-1-Luc (pancreatic tumor cell)
Aptamer: 10 day (every 2day injection)



Pancreatic cancer -targeting Aptamer

for unknown cell surface marker

Why Cell-SELEX ?

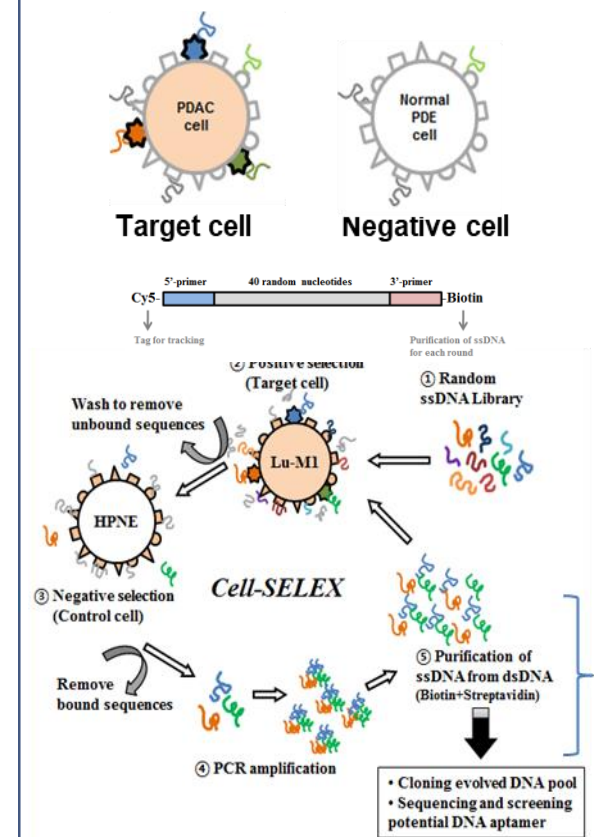
1. Target identification (in case of unknown target)
 - binding to target & pulldown & purification
2. Specific recognition for target protein
3. Easy optimization for therapeutic utilization
4. Small size benefits-penetration, imaging probe,...

&

1. Cell surface target without destruction of 3-dimensional structure (SELEX in live cells)
2. Recognition ability to small sized epitope
3. Recognition to tumor status-dependent modification of target

Cell SELEX (Target vs Counter)

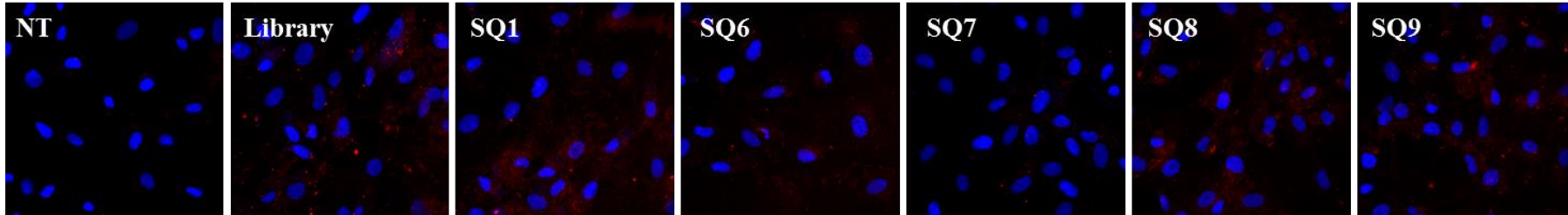
PDAC: pancreatic ductal adenocarcinoma
PDE: pancreatic ductal epithelial cells



Cell surface binding of aptamer candidates

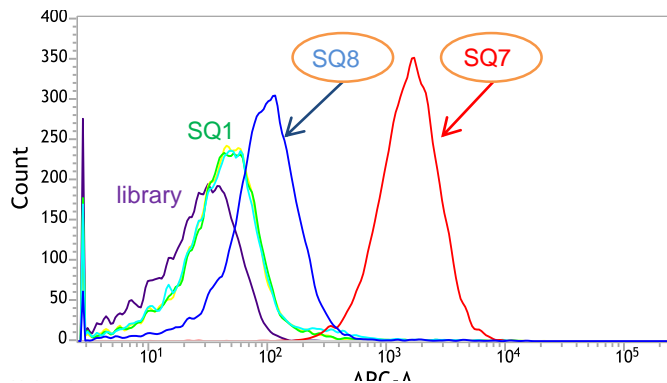
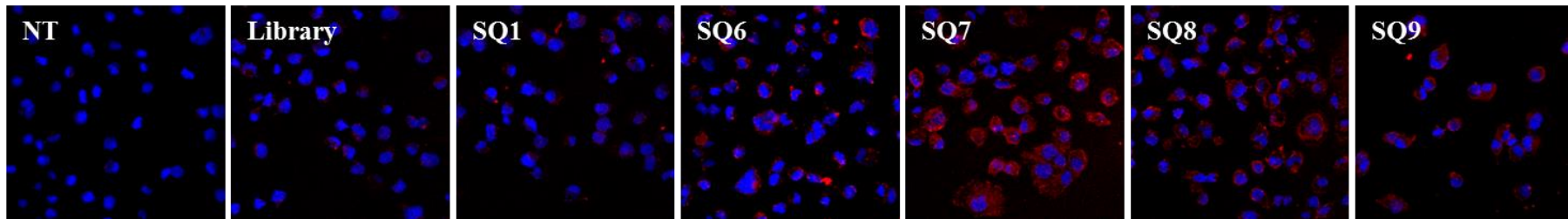
■ nucleus
■ Aptamer-Cy5

A Control cell (HPNE)



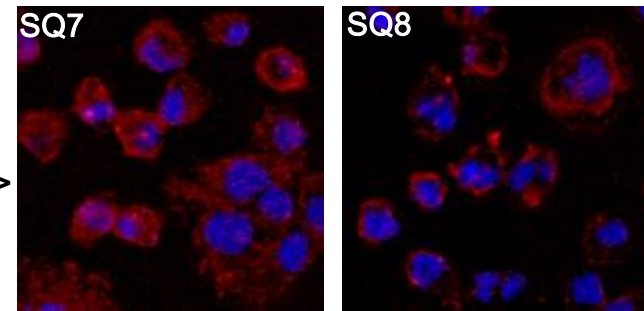
B Target cell (Lu-M1)

Negative
control
aptamer



Aptamer candidates

<Internalization>

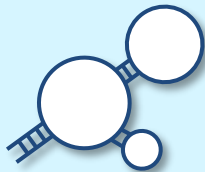


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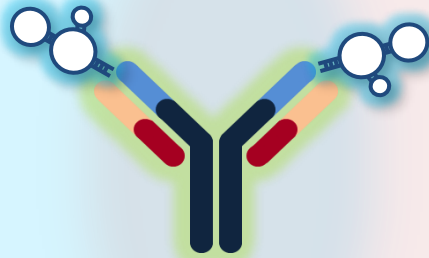
Win-Win : Antibody-based delivery of Aptamer

**Aptamer
(Oligomer)**



High penetration

Oligobody



High stability in blood

High penetration

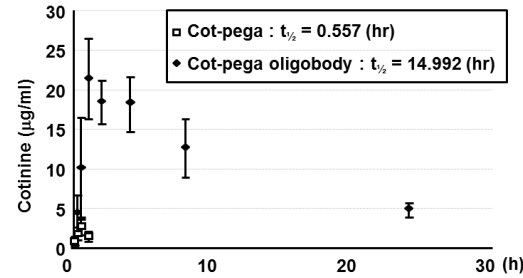
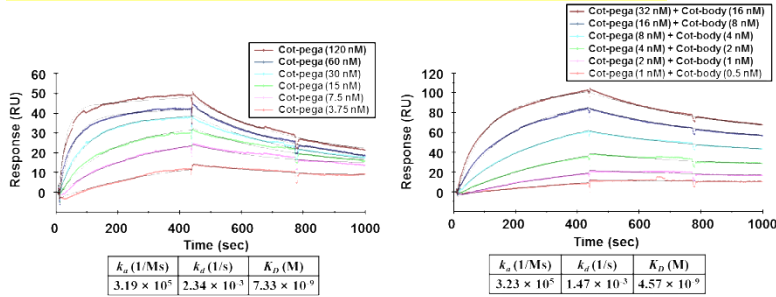
Antibody



High stability in blood

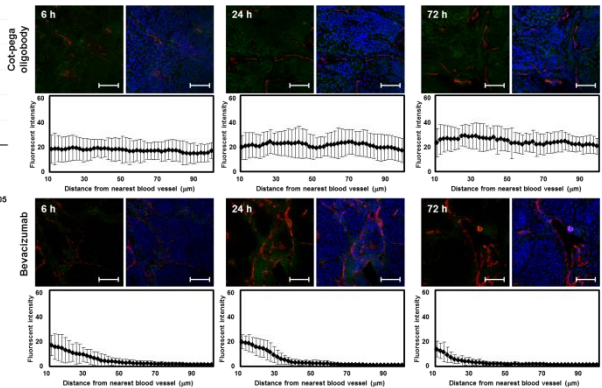
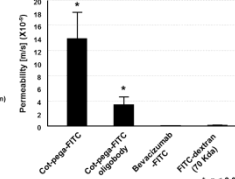
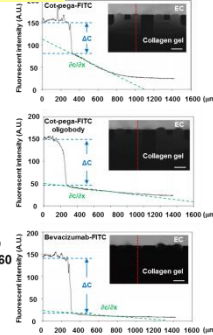
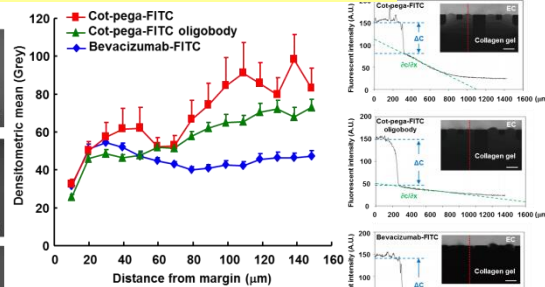
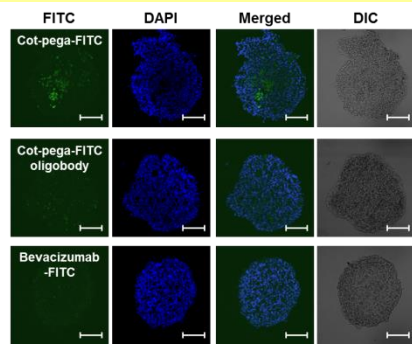
Proof-of-Concept of Oligobody

Increase pharmacokinetics

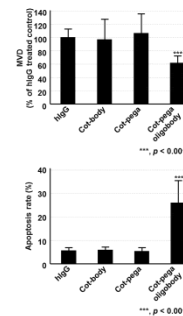
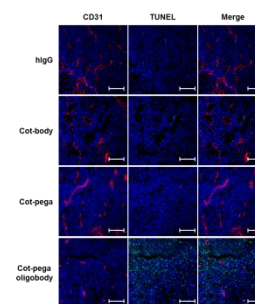
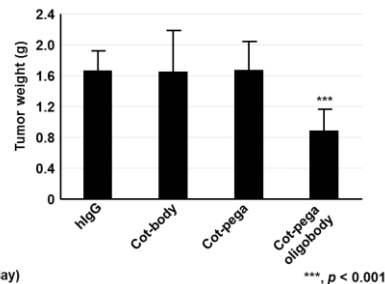
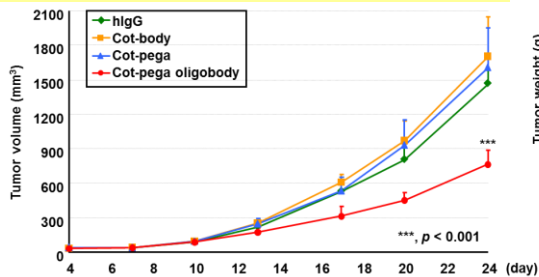


Anti-cotinine (Antibody)
Anti-VEGF (Aptamer)

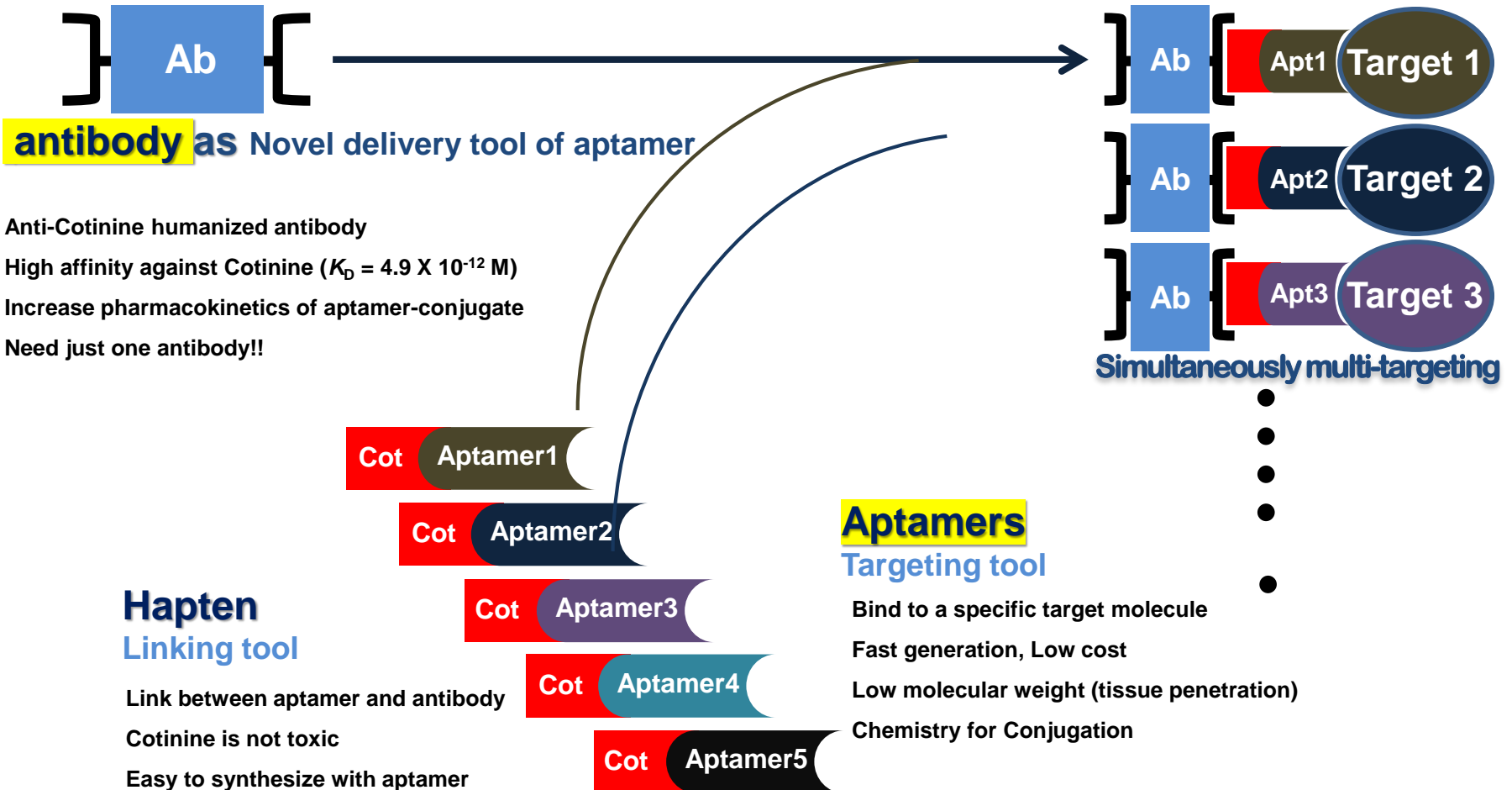
Increase tumor tissue penetration



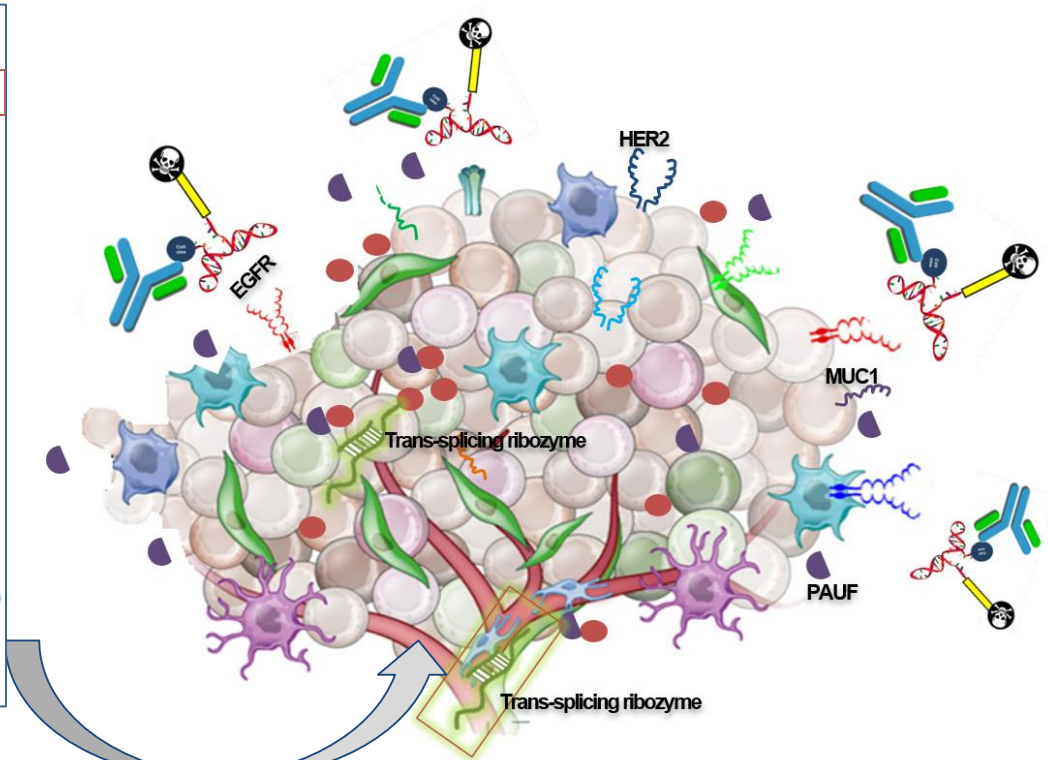
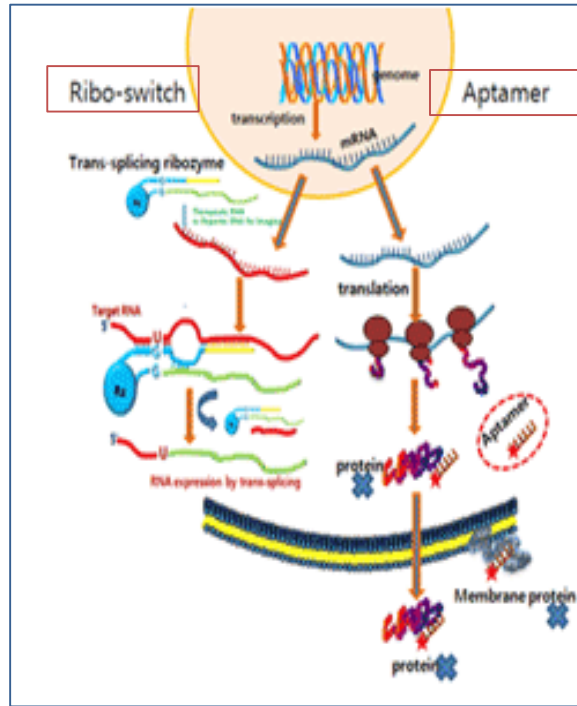
Inhibit tumor growth



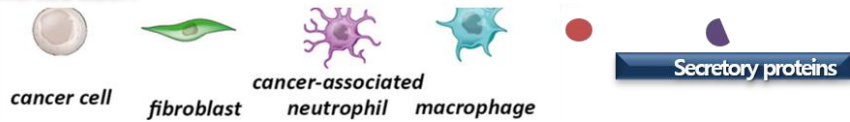
Oligobody: a novel aptamer-antibody hybrid complex



Conclusion : Nucleic acid-based High Performance Theranostics



Systemic administration



Ref: Theranostics 04: 0931, No3

Acknowledgement

<Aptamer>

- Dr. In-Hoo Kim

- Dr. Kyun Heo



- Dr. Sun Il Choi (Ph.D)

- Dr. Jun-Ho Jung (SNU)

- Dr. Jin Sook Jeong (Dong-A Univ)

- Dr. Sang-Jin Lee

- Dr. Eun Sook Lee

- Dr. Ho Jin Sung

- Dr. Kyoung Ho Choi (SNU)

- Dr. Seok-ki Kim

* All lab. members

* Research Core Center, NCC

<Pancreatic Cancer>

Dr. Sang Myoung Woo

Dr. Sang Jae Park

Dr. Woo Jin Lee,

Dr. Sung Sik Han

Dr. Eun Kyoung Hong

Dr. Sun-Young Kong

