The AptaReport™
Newsletter
Winter 2020

Molecular Subtyping of Breast Cancer with Aptamers

Breast cancer is the most commonly diagnosed cancer for US women. It is classified into four molecular subtypes: Luminal A, Luminal B, HER2-enriched, and triple-negative. Immunohistochemistry is most commonly used for breast cancer subtyping; however, the antibodies generally used in this procedure are heterogeneous, expensive, and unstable. Aptamers could potentially troubleshoot these limitations due to their high specificity, high binding affinity, and stability. Recently, Liu et al. developed a DNA aptamer-based fluorescence probe, which can distinguish HER2-enriched breast cancer from the other subtypes and healthy breast tissue.

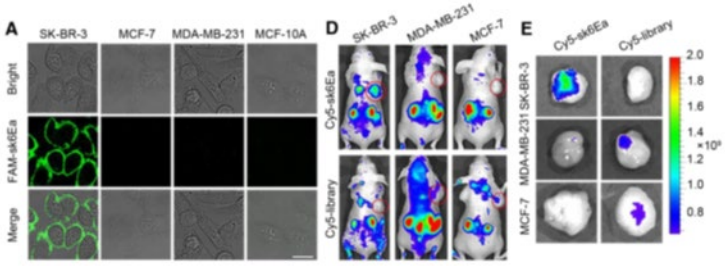

Figure (adapted)*. (A) *Molecular subtyping of different breast cancer cells Aptamer sk6Ea labeled with FAM (green)* ***(D)*** *In vivo fluorescent imaging xenografted mice. Library & sk6Ea labeled with Cy5. Tumor sites of interest circled red.* ***(E)*** *Ex vivo fluorescent imaging of breast cancer tumors from xenografted mice*

DNA aptamers for molecular subtyping breast cancer were developed via CELL-SELEX, where HER2 overexpressing SK-BR-3 breast cancer cells were the target and MCF-7 and MDA-MB-231 breast cancer cells, and MCF-10A healthy breast cells were negative controls. One aptamer (sk6Ea) demonstrated the highest affinity and was used for further experimentation and fluorescence imaging. *In vitro* and *in vivo* fluorescence imaging were used to distinguish the molecular subtypes of breast cancer in cells, tissue, and mice with breast cancer tumors. The *in vitro* fluorescent microscopy data demonstrated a greater fluorescent signal for SK-BR-3 cells than the negative control cells. For *in vivo* and *ex vivo* fluorescence imaging, Cy-5 labeled aptamers were injected in mice. SK-BR-3 tumor sites showed greater fluorescence than the other breast cancer cells, which had little to no fluorescent signals. The aptamer developed in this study has potential for subtyping breast cancer; however, other biosensors need to be developed to distinguish the other three molecular subtypes.

-M.D.

*Reference: Apta-Index™ ID # 7049

The BACE1-Specific DNA Aptamer A1 Rescues Amyloid-β Pathology and Behavioral Deficits in a Mouse Model of Alzheimer's Disease

Alzheimer's Disease (AD) is one of the most common degenerative diseases. It affects more than 50 million people worldwide. Liang et Al. have researched a DNA aptamer termed A1 that exhibits distinct inhibitory effects on BACE1 activity. BACE1 is short for β-site APP-cleaving enzyme 1, which when cleaving amyloid precursor protein (APP) proteolytically with the help of γ-secretase, produces amyloid-β (Aβ) plaque deposits in the brain. Aβ is one of the main pathological biomarkers of AD. Liang et Al. conducted E.L.I.S.A. and thioflavin S staining to test whether aptamer A1 can reduce expression of Aβ as well as the number of amyloid plaques. Based on their research A1 dramatically reduced levels of Aβ42; however, it did not significantly affect the levels of Aβ40 in the hippocampus of mice.

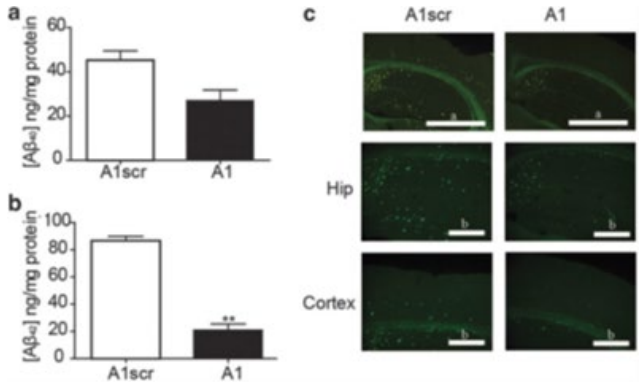

Figure (adapted). Chronic intracerebroventricular injections of aptamer A1 inhibit Ab deposition. Quantification of total (a) Ab42 and (b) Ab40 levels in hippocampal homogenates by ELISA (c) Amyloid plaques detected by thioflavin S staining in the hippocampus and cortex of mice. Scale bar: (a) 2,000 mm; (b) 500 mm.*

Lower levels of Aβ40 and Aβ42 protein were expressed in the presence of aptamer A1, which was supplied to the mouse models by chronic intracerebroventricular injections, a total of 15 with a frequency of once every three days. Aptamer A1 can effectively rescue Aβ pathology and memory deficits in mice. Also, aptamer A1 improves working memory in mice while decreasing the protein expression of BACE1 in the hippocampus of mice. BACE1-specific DNA aptamer A1 exhibited symptomatic modification in the mouse model of AD which revealed to be effective and gives the potential to be used further in clinical trials.

-B.A.

*Reference: Apta-Index™ ID # 7051

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Nucleolin Aptamer-Drug Conjugates Promise a Powerful New Strategy for Targeted Chemotherapeutics

Since the nucleolin aptamer (NucA) was serendipitously discovered in 1999, researchers have hypothesized that it may constitute a powerful and broadly applicable chemotherapeutic strategy with the potential to improve outcomes for patients facing challenging cancer diagnoses. Phase II FDA trials against metastatic renal cell carcinoma yielded anticlimactic results in 2014, forcing scientists to go back to the drawing board. Several groups have since independently developed nucleolin aptamer-drug conjugates, utilizing NucA to seek out cancer cells and deliver a covalently bound cytotoxic payload composed of a traditional chemotherapeutic. This can radically lower the effective chemotherapy dose and decrease adverse side-effects associated with chemotherapy treatment. Most recently, Pusuluri et al. constructed a nucleolin aptamer conjugated to a peptide scaffold, loaded with several molecules of doxorubicin (DOX) and captothecin (CPT) for optimal therapeutic efficacy.

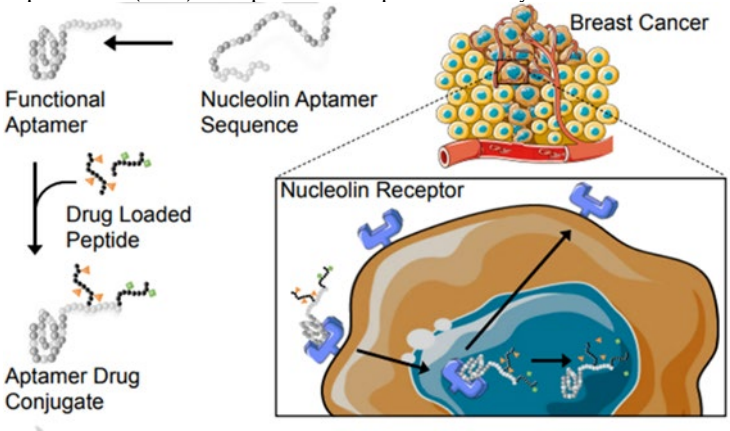


Figure (adapted): The Ap-DOCTOR conjugate utilizes peptide functionalized aptamers to deliver doxorubicin and captothecin to oncogenic cells in synergistic therapeutic ratios.*

This construct was named "Ap-DOCTOR", an acronym for Aptamer-targeted DOX and CPT in Therapeutically Optimal Ratios. Ap-DOCTOR was shown to be approximately ten times more potent than DOX or CPT on their own against breast cancer *in vivo*, and side-effects of nonspecific cytotoxicity were not observed in mice even when administered at 4x the effective dose.

-N.H.

*Reference: Apta-Index™ ID # 290

An RNA Aptamer for Detecting Glioblastoma Cells & Tissues Expressing Integrin α5β1

Glioblastoma (GBM) is a very aggressive form of brain cancer. Patients rarely live longer than 2 years, even with treatment. Researchers aim to discover GBM biomarkers, such as cell surface receptors, for the use of diagnostics and therapeutics. Integrins are cell surface receptors that regulate cell growth and intracellular signaling. The overexpression of Integrin α5β1 is associated with aggressive tumors in GBM patients.

In a study by Fechter et al. (2019), an aptamer for Integrin α5β1 (aptamer H02) was developed by hybrid SELEX. The procedure was a hybrid of cell SELEX, protein SELEX, and another few rounds of cell SELEX. Using confocal microscopy analysis, Cy5-labeled aptamer H02 and Alexa 546-labeled anti-α5-antibody IIA1 colocalized on the cell surface of cells overexpressing α5. Another experiment was done to characterize the ability of aptamer H02 to differentiate between high α5-expressing cells and low α5-expressing cells derived from patient xenografts (TC7 and TC22, respectively). In addition, immunofluorescence and aptafluorescence of these GBM cells were compared. Both histochemical assays demonstrated higher levels of integrin α5 levels in TC7 than TC22. However, the aptahistochemical assay showed greater fluorescence signal than the immunohistochemical assay. In conclusion, the aptamer in this study was able to differentiate GBM cells based on α5-expression levels, which can potentially be of use for diagnostic and therapeutic purposes.

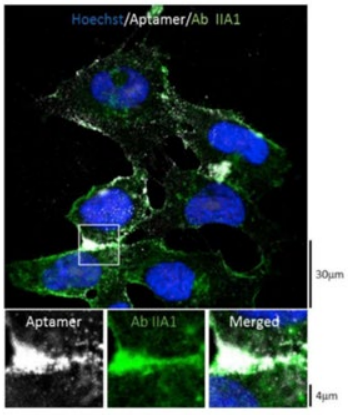
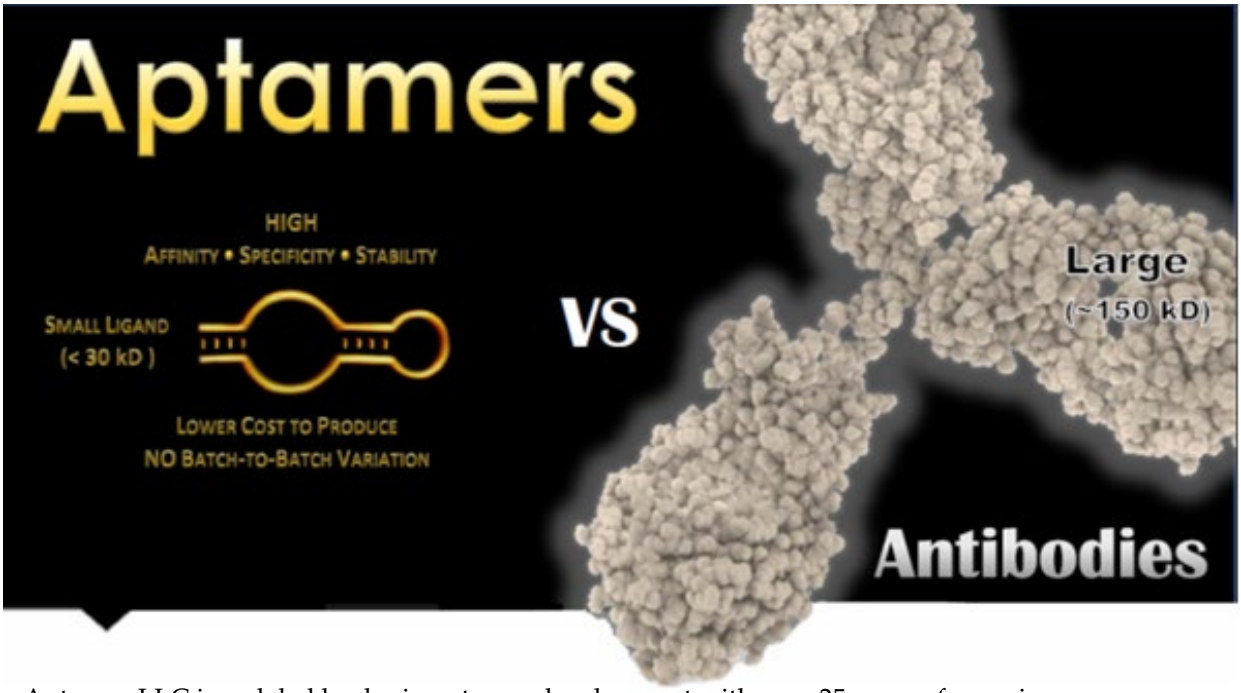


Figure (adapted)*: A) Confocal microscopy analysis with Cy5-labeled aptamer H02 (white) and anti-α5 antibody IIA1 labeled with Alexa 546 (green) on U87MG α5+ cells. The nuclei were stained with Hoechst (blue).

-M.D.

*Reference: Apta-Index™ ID # 7050



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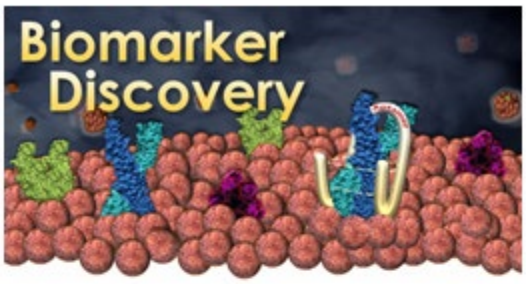

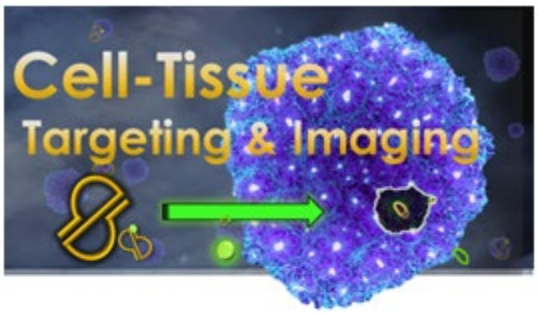

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