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Evaluation of Analytical Biochemistry by Aptamer Applications

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Abstract

Aptamers are single-stranded molecules of relatively short DNA or RNA (20–60 nucleotides) that bind to a variety of targets with high affinity and specificity. Aptamers are frequently referred to as "synthetic antibodies," despite the fact that they are simpler to acquire, less costly to manufacture, and more versatile than antibodies in several ways. Since their inception in 1990, Aptamers have been the subject of a steady increase in publications. The focus of this account was on recent original research publications, or those published between April 2019 and the time this account was written in 2020. Relevance-ranking of articles was found by conducting a Google Scholar search of this recent literature. There was no new aptamer selection methods included. Representative examples of each of the nine categories of applications are provided. Last but not least, an outlook is provided that focuses on application performance factors such as "faster, better, and cheaper" as the primary drivers for upcoming innovations in aptamer applications.

Keywords: Aptamers; Targeted delivery; Beacons; Cell imaging; Imaging RNA

Introduction Aptamers are typically defined as relatively short single-stranded DNA or RNA molecules with 20–60 nucleotides that bind to a variety of targets with high affinity and specificity, such as small molecules, peptides, proteins, cells, and tissues. Aptamers are often referred to as "synthetic antibodies", but they are easier to obtain, less expensive to produce, and more versatile than antibodies in a number of ways. These characteristics have led to the idea that aptamers could replace antibodies in many applications [1-3].

Incredibly, two labs—Tuerk and Gold and Ellington and Szostak-published the conceptually similar achievement of selecting RNA sequences that bind to specific target molecules independently in 1990, coining the term "Aptamers" two years later. Albeit to some degree different specialized approaches were utilized, both began with exceptionally complex combinations of engineered RNA having "irregular succession" (A/G/C/U) locales. As of April 2020, there are approximately 9100 citations in Google Scholar for the method that was described by Tuerk and Gold, which was referred to as "systematic evolution of ligands by exponential enrichment" (SELEX). In addition, the alternative selection strategy described by Ellington and has had a significant impact, as evidenced by the approximately 8400 citations that Google Scholar has received during the same period.

Major databases of scientific publications like PubMed, which contains over 30 million citations for the fields of biomedicine, health, and portions of the chemical sciences and bioengineering, but not patents, provide a better measure of the impact of aptamers following these two seminal publications [4]. Which currently contains over 47 million records that are maintained by Chemical Abstracts Service, contains information on patents as well as additional chemistry sources? There is some overlap between these two databases, which are freely accessible and simple to search in a variety of ways. For instance, Fig. 1 is a chart of PubMed-indexed annual publications containing the term "aptamer" from 1992 to 2019, with aptamer appearing in any field. The absence of atent literature, despite its significance for commercialization, was deliberate. Items with application-related words like sensors, electrochemical, fluorescent, targeting, delivery, diagnostics, proteins, food safety, and so on were also found through a Google Scholar search. Using a Boolean "OR" method. Since recent reviews exist for each topic, we did not directly search for methods for selecting aptamers or attaching aptamers to gold surfaces. The 500 items that were derived and ranked according to Google Scholar's algorithms were then sifted through for selection, sorting, and organization [5-7]. In addition, with apologies to the numerous aptamer researchers not cited here, the following account is by no means comprehensive and unavoidably reflects the author's subjective biases.

As previously mentioned, the 48-mer DNA aptamer can specifically recognize membrane-expressed protein tyrosine kinase. Flow cytometry, dyes for microscopic visualization, and endocytosis modulators were used to investigate Apt-Exos's cellular uptake mechanism and compare it to that of free Exos. Dox, a commonly used anticancer drug, was used as the drug model and electroporated into Exos in order to engineer the Apt-Exos as a drug delivery system [8]. Standard methods were used to demonstrate the Apt-Exos construct (APT-Exos-D)'s capacity for cell type-specific Dox accumulation and cytotoxicity. The authors came to the conclusion that Apt-Exos is a promising platform for cancer theranostics' delivery of molecular drugs and fluorophores to cancer cells. Which are a moderately new class of fluorescent carbon nanomaterials that have demonstrated to be useful assets in the field of bio imaging and biosensing because of their little size, photostability and ideal biocompatibility. Kong and others assembled CDs coated with positively charged polyethyleneimine (PEI), which then formed CDs-PEI-AS1411 Nano complexes by conjugating with AS1411 through electrostatic interactions. Two cancer cell lines, breast MCF-7 (high nucleolin level) and fibroblast L929 (low nucleolin level), were used to test the cytotoxicity of the CDs-PEI and CDs-PEI constructs. Confocal microscopy and flow cytometry were also used to examine the cellular uptake of CDs-PEI-AS1411. There was no obvious cytotoxicity and the constructs showed good photostability. In accordance with nucleolin-mediated uptake, the CDs-PEI-AS1411 non-complex was significantly

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absorbed by MCF-7 cells in comparison to L929 cells. The CDs-PEI-AS1411 construct may be useful for nucleolin-related cancer cell targeted imaging, it was concluded.

Aptamers have also made it possible to use a variety of cellular imaging applications, particularly for imaging cancer, similar to the development of antibody-based cell imaging techniques in the past. There are four recently reported applications for imaging cancer cells, three of which involve aptamers that target cell surface glycoprotein receptors, and then a dead-cell imaging example, as dead vs. live cell status is a crucial technical aspect of cellular imaging or detection that is frequently overlooked. In a variety of epithelial cancers, the transmembrane glycoprotein mucin (MUC1) is abnormally glycosylated and overexpressed which contributes significantly to the disease's progression. In terms of its biochemical properties, cellular distribution, and function, tumor-associated MUC1 differs from normal MUC1. MUC1 plays a role in intracellular signal transduction pathways in cancer cells and controls the transcriptional and post-transcriptional expression of its target genes. Advances in the use of MUC1 as a biomarker and therapeutic target for cancer, as well as the structural and functional differences that exist between normal and tumor-associated MUC1, have been reviewed.

Prussian blue staining, a common histopathology stain utilized by pathologists to detect the presence of iron in biopsy specimens, was used to observe BxPC-3 cells cultured with MUC1-SPION and SPION under various conditions. To investigate MUC1-SPION in vivo, a nude mouse model of PC and the conventional MTS assay, a colorimetric method for sensitively quantifying viable cells in cell proliferation assays, were established. Immunohistochemistry and Western blot analyses were used to look for MUC1 expression in tumor biopsies, and MRI was used to measure the transplanted tumour's signal intensity. Importantly, MUC1-SPIONs were nontoxic in vitro and could significantly reduce MRI T2-signal strength [9-10]. In the nude mouse model, in vivo MUC1-SPIONs selectively accumulated in PC tumors, suggesting that early in vivo imaging of PC in humans might be useful.

Conclusion

Finally, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) coronavirus infection known as coronavirus disease 2019 (COVID-19), which was first reported in late December 2019 and swiftly developed into a major pandemic, has prompted unprecedented global efforts to rapidly develop efficient detection, diagnostic, therapeutic, and preventative methods to combat this crisis. Aptamers for sensitive detection and possible treatments of that earlier strain of coronavirus were developed, albeit not immediately, in response to the 2003 pandemic of SARS, which was similar but less severe. It is hoped that important tools for COVID-19/SARS-CoV-2 will be developed much more quickly as a result of developments since then that have led to faster, cheaper, and better aptamer technologies. Indeed, posted a preprint in April 2020 is related to aptamers and is titled "De novo 3D models of SARS-CoV-2 RNA elements and small-molecule-binding RNAs to guide drug discovery." Additionally in April of 2020, Abbott et al. Development of CRISPR as an antiviral strategy to combat SARS-CoV-2 and influenza was the title of a paper that was published.

Declaration of Competing Interest

The authors declare that they have no competing financial interests

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