Aptamer-Based Cancer Cell Analysis and Treatment

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Aptamers are a class of single-stranded DNA or RNA oligonucleotides that can exclusively bind to various targets with high affinity and selectivity. Regarded as “chemical antibodies”, aptamers possess several intrinsic advantages, including easy synthesis, convenient modification, high programmability, and good biocompatibility. In recent decades, many studies have demonstrated the superiority of aptamers as molecular tools for various biological applications, particularly in the area of cancer theranostics. In this review, we focus on recent progress in developing aptamer-based strategies for the precise analysis and treatment of cancer cells.

1. Introduction

Cancer, a chronic disease with a high mortality rate, is a serious public health problem.\textsuperscript{[1]} Although rapid advances in molecular biotechnology and chemical biology have led to significant progress in the area of cancer theranostics, a series of challenges remains, owing to the inherent heterogeneity and complexity of cancer cells.\textsuperscript{[2]} Thus, to achieve early diagnosis and precise therapy, the development of high-performance recognition tools for cancer cells and cancer-related biomarkers is urgently required.

In recent decades, a diverse array of molecular recognition tools have been widely exploited, including antibodies, peptides, and nucleic acids, and lay an essential foundation for cancer diagnosis and therapy.\textsuperscript{[3]} Nucleic acid aptamers are single-stranded DNA/RNA oligonucleotides with a length of approximately 25–80 nucleotides that could bind with target cargos by folding into specific secondary/tertiary conformation.\textsuperscript{[4]} The concept of aptamers was first reported by Ellington et al. in 1990 and screened through a repetitive process known as the systematic evolution of ligands by exponential enrichment (SELEX).\textsuperscript{[5]} With rapid advances in the SELEX technology, aptamers against various targets, such as small molecules, peptides, and proteins, have been generated.\textsuperscript{[6]} Particularly, Tan et al. used intact living cells as the target to develop the Cell-SELEX technology and successfully selected many cell type-specific aptamers, such as sgc8 specific for acute lymphoblastic leukemia CCRF-CEM cell line,\textsuperscript{[7]} XQ-2d for pancreatic ductal adenocarcinoma PL45 cells,\textsuperscript{[8]} and TD05 for Ramos cells.\textsuperscript{[9]}

Taking advantage of the high specificity, facile synthesis, convenient modification, and high programmability, aptamers have been widely used as reliable recognition ligands in biosensing, bioimaging and bioregulation.\textsuperscript{[10]} In addition, their integration with therapeutic modules, such as small-molecule drugs, peptides, nucleic acid drugs, has attracted broad interest for cancer-targeted treatment.\textsuperscript{[11]} Meanwhile, some aptamers could even serve as therapeutics. As a typical example, the first aptamer drug, Macugen® (pegaptanib), was approved by the US Food and Drug Administration (FDA) for the treatment of wet age-related macular degeneration, and several other aptamer therapeutics underwent clinical trials.\textsuperscript{[12]} While several aptamer-related review papers have been published to date,\textsuperscript{[13,14]} in the current manuscript, we mainly focused on recent research progress of aptamer-based strategies in precise cancer analysis and targeted therapy.

2. Aptamer-based Cancer Cell Analysis

2.1. Aptamer-based Recognition and Capture of Cancer Cells

The specific recognition of cancer cells is essential for cancer diagnosis, therapy, and prognosis. Typically, circulating tumor cells (CTCs), which are shed from the primary tumor into the vasculature, provide an attractive index for evaluating tumor progression.\textsuperscript{[15]} However, because of the extraordinarily low concentration (nearly 1–10 CTCs per mL of whole blood) and high heterogeneity of CTCs, their capture and detection remain a technical challenge.\textsuperscript{[16]} To overcome this issue, aptamers, which exhibit high affinity and high specificity against surface biomarkers of CTCs, have been used as the targeting ligands.\textsuperscript{[17]}

For example, Zeng et al. reported a cancer cell-activatable probe by conjugating aptamers with paired fluorochrome-quencher molecules for one-step assay of CTCs.\textsuperscript{[18]} This aptamer probe could be specifically internalized by CTCs with over-expression of CD30 receptors, and then was degraded in lysosomes to separate the fluorophore-quencher pair, leading to fluorescence restoration. This aptamer reporter could selectively detect CTCs in whole blood and narrow aspirate samples of patients with lymphoma tumors. To improve the capture efficiency and detection sensitivity of CTCs, Ding et al. designed a magnetic nanoplateform by integrating multivalent aptamer-functionalized Ag₃S nanodots with hybrid cell membrane-coated magnetic nanoparticles (HM–Fe₃O₄@SiO₂/Tetra-DNA–Ag₃S nanoplatorm, Figure 1A).\textsuperscript{[19]} Owing to the multivalent aptamer modification and magnetic isolation, this nanoplatorm could specifically and efficiently identify target cancer cells, and a high cell capture efficiency of over 90% was obtained in whole blood sample.

While the magnetic nanoplatorm could effectively enrich CTCs, their application was time-consuming and required a...
large volume of blood samples. Microfluidics, processing small-volume fluids with high throughput, automation, and multiplexing, showed great potential in the detection and isolation of CTCs.\[19\] Zhao et al. developed a microfluidic assay using rationally designed aptamer cocktails with synergistic effects (Figure 1B).\[20\] By combining a microfluidic chip embedded with silicon nanowire substrate (SiNS), enhanced and specific capture of CTCs from non-small cell lung cancer (NSCLC) patient samples was achieved.

To further reduce the non-specific cellular adsorption, Zhang et al. synthesized leukocyte membrane-coated magnetic nanoclusters and then decorated them with aptamer SYL3C.\[21\] By loading these biomimetic nanoparticles into the microfluidic system (Figure 1C), more than 90% of rare EPCAM-positive tumor cells could be isolated from a whole blood sample and directly counted online based on fluorescence imaging within 20 min, indicating a good promise for the high-performance analysis of CTCs in complex biological samples.

2.2. Aptamer-based membrane protein imaging and regulation

Membrane proteins play pivotal roles in many biological processes, such as cell migration, signaling, and communication.\[22\] The abnormal expression and oligomerization of membrane proteins are closely associated with the occurrence of many diseases.\[23\] Developing methods for visualization and manipulation of disease-related membrane proteins could provide important information in the context of cell biology, disease theranostics, and drug discovery.\[24\]

Aptamers with excellent molecular recognition ability have been widely applied for the specific imaging of membrane proteins.\[25\] For example, Tan et al. synthesized a sgc8 probe labeled with a fluorescein isothiocyanate dye to map the cellular density and distribution of protein tyrosine kinase-7, which was regarded as a potential biomarker of cancer cells.\[26\] To image target membrane proteins at the single-molecule level, Albertazzi et al. constructed a DNA-based point accumulation approach for imaging by nanoscale topography (DNA-PAINT).\[27\] Using aptamers with a tunable affinity, they achieved single-molecule tracking of epidermal growth factor receptor (EGFR) on the cell surface (Figure 2A). They also showed that PAINT could be exploited for mapping the distribution and density of EGFR in different cancer cell lines without involvement of genetic and/or chemical modification. In addition, to realize the sensitive detection of low-abundance membrane proteins, Tang et al. developed an aptamer-based imaging strategy with combination of rolling circle amplification (RCA), which allowed the signal-amplified visualization of low-abundance EpCAM on MCF-7 cells during the epithelial-mesenchymal transition.\[28\]

Due to the extraordinary heterogeneity and complexity of cells, it is rather difficult to achieve accurate cellular identification via single-marker detection.\[29\] To address this issue, multiplex analysis of membrane proteins held great promise for improving the accuracy of cell recognition.\[30\] For example, Zheng et al. designed a range of silica nanoparticles modified...
with several different aptamers (TD05, Sgc8, and Sgd5), which enabled cancer cells to be precisely classified according to their specific molecular signature patterns. To further realize the intelligent typing of cancer cells, aptamer-based DNA circuits, which enable computation of multiple membrane proteins, have also been developed. As a typical example, You et al. developed an aptamer-encoded Boolean logic circuit that allowed programmable and higher-order profiling of multiple co-existing cell-surface markers based on AND, OR, and NOT logic gates. Besides, to further improve the accuracy of DNA computation, Chang et al. introduced a hybridization chain reaction (HCR) to develop multiple aptamer-based AND logic circuits and achieved sensitive detection of CCRF-CEM cells.

In addition to the expression level of cell membrane proteins, their oligomerization state is also related with the cellular function and behavior. Dynamic monitoring the oligomerization of surface proteins would therefore be beneficial for understanding their biological roles. By combining aptamer-based molecular recognition and proximity-induced DNA assembly, Liang et al. proposed an aptamer-based method for dynamically imaging the dimerization process of mesenchymal-epithelial transition (Met) receptor. To further improve the reliability and efficiency of protein monitoring, tetrahedral DNA nanostructures were introduced to prepare a proximity-induced fluorescence resonance energy transfer (FRET) nano-platform for visualizing dimerization of Met (Figure 2B). With enhanced biostability, this DNA tetrahedron-based nanoprobe enabled imaging of Met receptor dimers in nude mice.

Manipulating the behavior of membrane receptors in living cells would offer a valuable strategy for studying their biological function. By combining the advantage of aptamer-based molecular recognition and DNA-based receptor assembly, Yang et al. recently reported a non-genetic approach to realize the dynamic dimerization of Met receptors, and thus modulation of corresponding signal transduction events (Figure 2C). They designed aptamers as robotic arms that captured target receptors (C-Met and CD71), and DNA logic components as computer processors to handle multiple inputs. Based on DNA assembly, c-Met and CD71 were brought into close proximity, leading to interference on the ligand-receptor interactions of c-Met and thus inhibiting related biofunctions. Besides, Han et al. developed a bispecific aptamer chimera, and proved its good

Figure 1. Aptamer-based recognition and capture of CTCs in body fluids. (A) The HM-Fe$_3$O$_4$@SiO$_2$/Tetra-DNA-Ag$_5$S nanoplatform for the efficient isolation and detection of rare CTCs. Reproduced with permission from Ref. [18]. Copyright 2019, Wiley-VCH. (B) Microfluidic CTC chip is composed of an aptamer-grafted silicon nanowire substrate (SNS) and an overlaid PDMS chaotic mixer. Reproduced with permission from Ref. [20]. Copyright 2016, Wiley-VCH. (C) Working principle of the biomimetic microfluidic system for the detection of CTCs. Reproduced with permission from Ref. [21]. Copyright 2019, ACS Publications.
performance for triggering lysosomal degradation of therapeutically relevant proteins (e.g., Met receptor) through specific interaction with lysosome-shuttling receptor (IGF-IIR) (Figure 2D), indicating an attractive strategy for disease therapy.

2.3. Aptamer-based probes for bioimaging at subcellular organelles

Eukaryotic cells contain many organelles, and these are compartmentalized through individual membranes. Since many biochemical processes occur in specific organelles, their disorganization is associated with many diseases, such as neurological diseases, diabetes, and cancer. Molecular imaging at the subcellular level would be beneficial for the study of complex biological systems and accurate disease diagnosis.

Lysosomes, as one of the key cellular organelles, contain approximately 50 different degradative enzymes that are active at acidic pH levels, and abnormal acidification is closely associated with reduced digestion ability, autophagy blockage, and storage disorders.

To realize dynamic imaging of the lysosomal environment, Du et al. designed a lysosome-targeting framework nucleic acid (FNA) nanodevice by incorporating an i-motif and an adenosine triphosphate (ATP)-binding aptamer (ABA) into a DNA triangular prism (Figure 3A). After entering into the lysosomal compartment of cancer cells, this nanodevice would undergo a conformational switch to generate fluorescence output for indicating the abnormal level of pH and ATP.

The nucleus is one of the most important cellular organelles that plays a critical role in maintaining the integrity and expression of genes. Aptamer AS1411 could specifically bind with nucleolin, which is generally overexpressed on the membrane of cancer cells and can translocate between the cell membrane and the nucleus. For example, Li et al. integrated AS1411 with poly cytosine as a scaffold to synthesize AS1411-functionalized silver nanoclusters (Ag NCs) for nuclear staining. In addition, Shen et al. used the Cell-SELEX technol-
ogy to select a nucleus-targeted aptamer Ch4-1 (Figure 3B).

They demonstrated that this aptamer probe exhibited a high affinity ($K_d = 6.65 \pm 3.40$ nM) to nucleoproteins and could be applied for distinguishing dead cells from live cells.

Aptamer-based fluorescent probes have also been applied to monitor other subcellular microenvironments. For example, to synchronously map the activity of nitric oxide synthase 3 (NOS3) at the plasma membrane and in the trans-Golgi network (TGN), Jani et al. designed a fluorescent DNA probe by conjugating a nitric oxide (NO)-sensitive fluorophore with aptamers specific for either the plasma membrane or the Golgi apparatus. Because of their subcellular targetability and sensitivity to NO, these probes allowed simultaneous measurement of the NOS3 activity in these two organelles. They also used this imaging platform to investigate the selective regulators of NOS3 in different compartments. To image mitochondrial ATP in living cells, Wang et al. developed a ratiometric fluorescent DNA nanostructure (RFDN) based on hybridization chain reaction (HCR) and split aptamers (Figure 3C). The results showed that the RFDN was easily designed and assembled by HCR, which had good biocompatibility and response to mitochondrial ATP.

3.1. Aptamer-small molecule drug conjugates

It is well known that traditional chemotherapeutic drugs can cause serious side effects owing to their low selectivity. To overcome this problem, cancer-targeted drug delivery systems have been proposed and achieved significant progress in chemotherapy. For example, by conjugating doxorubicin (Dox) with aptamer sgc8c, Huang et al. developed the first aptamer-drug conjugate (ApDC). They demonstrated that this ApDC could specifically kill target acute lymphoblastic leukemia CCRF-CEM cells with limited influence on non-targeted cells. In addition, the effective release of molecular drugs from conjugates was expected to improve their therapeutic efficacy. In this context, Yang et al. used 4-nitrophenyl-4-(2-pyridyldithio) benzyl carbonate (NPDBC) and (4-nitrophenyl-2-(2-pyridyldithio)ethyl carbonate (NPDEC) as tumor microenvironment-responsive linkers to bridge an aptamer with mitomycin C (MMC) (Figure 4A). They demonstrated that this ApDC could specifically accumulate in the tumor region, where the MMC module could be effectively released owing to the reductive microenvironment of tumors, thereby leading to an enhanced drug activity compared with that of the stable conjugation design. Meanwhile, to address the heterogeneity issue of cancer cells, Zhu et al. developed a bispecific aptamer probe by connecting aptamers sgc8c and sgd5a. They demonstrated that this bispecific aptamer possessed a broader recognition capability for cancer cells than the individual monovalent probes. In addition, to enhance the biostability and drug loading capability, Deng et al. proposed a polymer-based approach by conjugating cell-targeting aptamers with a water-soluble polymer prodrug that contained a reductive environmentally-sensitive prodrug and a biocompatible brush skeleton. This high-payload aptamer poly(prodrug) conjugate (ApPDC) showed prolonged circulation time and enhanced therapeutic efficacy.

3.2. Aptamer-based Cancer Therapies

In addition to accurate diagnosis, effective treatment of cancer cells is another critical issue in the area of cancer research. The development of precise cancer treatments with minimal side effects is urgently required. In recent decades, aptamers have been widely exploited for developing cancer targeted therapeutic systems.

Figure 3. Aptamer-based probes for fluorescence imaging at the subcellular level. (A) An aptamer-based logic nanodevice for sensing ATP and pH in the lysosomal environment. Reproduced with permission from Ref. [44]. Copyright 2019, ACS Publications. (B) A nucleus-targeting aptamer probe (Ch4-1) for dead cell indication. Reproduced with permission from Ref. [48]. Copyright 2019, ACS Publications. (C) Assembly of the RFDN and its application in the ratiometric imaging of mitochondrial ATP in living cells. Reproduced with permission from Ref. [50]. Copyright 2021, ACS Publications.
To improve the synthetic controllability of ApDCs, Gao et al. designed artificial pharmaceutical solid-phase modules of Combretastatin A-4 (CA-4) for automatically synthesizing ApDCs (Figure 4B). Through solid-phase synthesis technology, this module was automatically and efficiently conjugated with an aptamer at predesigned positions. Biological studies showed that these ApDCs could maintain specific recognition ability and induce cytotoxicity against tumor cells.

3.2. Aptamer-conjugated biomacromolecular therapeutics

In addition to chemotherapeutic drugs, nucleic acids and peptides emerged as biomacromolecular therapeutics with high specificity. As a typical example, interference RNAs (iRNAs) that can specifically silence disease-related genes have attracted increasing interest in cancer therapy. Meanwhile, the lack of cell specificity has impeded the full potential of iRNA-based therapy in clinics. To address these issues, McNamara et al. developed aptamer-conjugated siRNA that enabled specific delivery of siRNA therapeutics to target cancer cells. By using an anti-PSMA aptamer as the targeting ligand, the designed siRNA could effectively reduce the tumor volume in prostate cancer xenograft models with limited systematic side effects. Besides, to improve the cellular internalization efficiency, Yoo et al. developed multivalent comb-type aptamer-siRNA conjugates (Comb-Apt-siR) by combining chemical coupling and DNA hybridization. Their results proved that the cellular internalization efficiency of Comb-Apt-siR could be improved based on the clustering effect.

Peptides, too, are attractive therapeutic biomacromolecules. As a typical example, Tan et al. designed anti-MUC1 aptamer-conjugated peptides (ApPCs) as targeted chemical sensitizers to overcome the poor cellular permeability of peptides, which specifically target the heat shock protein 70 (HSP70), and showed significant protein inhibition and chemical sensitization (Figure 4C). They also found that DOX could be loaded onto the ApPCs, which acted as both a targeted sensitizer and an anticancer agent for the treatment of drug-resistant breast cancer cells. The application of this technology enabled targeted peptides and DOX to be delivered to MCF-7/ADR drug-resistant breast cancer cells, thereby enhancing tumor growth inhibition in vivo and significantly reducing side effects.

3.3. Aptamer-conjugated nanotherapeutics

Nanoparticles have shown the potential to encapsulate and deliver anticancer drugs to tumors with enhanced efficiency. Even with the permeability and retention (EPR) effect against the tumor region, functionalization of nanoparticles with targeting ligands against cancer cells is still an intensively investigated topic. To date, many aptamer-functionalized nanoparticles have been widely developed.

For example, Cao et al. proposed aptamer-conjugated liposomes encapsulated with cisplatin, and successfully applied them for effective cancer treatment. In addition, Kim et al. developed a doxorubicin (Dox)-encapsulated liposome functionalized with two types of aptamers for separately targeting mucin 1 (MUC1) and CD44 antigen. This dual-aptamer-effect.
conjugated liposome achieved higher cytotoxicity against cancer stem cells than liposomes without or with only a single aptamer (Figure 5A).

In addition to soft nanomaterials, rigid inorganic nanoparticles possess unique material- and size-dependent physical properties, such as optic, electric and magnetism, which make them promising candidates as nanocarriers for targeted drug delivery. Jo et al. developed aptamer-modified gold nanoparticles (AuNSs), and proved their potential for effective eradication of prostate cancer cells based on the photothermal therapy effect. In addition, Jamileh et al. constructed anti-MUC1 aptamer-modified PEG-AuNPs for the loading of paclitaxel (PTX) for synergistic therapy (Figure 5B). The PTX-loaded PEG-AuNPs@antiMUC1 system could specificity bind to MUC1-positive cancer cells, achieving effective cell killing with combination of NIR irradiation.

Despite these advantages, NPs themselves cannot be used for intelligent regulation and precise drug delivery. As an alternative, structural DNA nanotechnology with programmable self-assembly and spatial addressing capabilities has showed great potential for the precise cancer therapy. For example, Wu et al. constructed an acrydite-modified DNA nanoassembly loaded with multi-drug-resistant antisense (MDR1-AS) oligonucleotides and functionalized by different aptamers (Sgc8 and KK1B10) to target leukemia cells. Moreover, to explore novel activatable theranostic agents with robust in vivo applicability, Lei et al. designed a multivalent activatable aptamer (Ntri-SAAP) and a robust DOX-functionalized DNA nanotriangle scaffold, which combined the advantages of a programmable self-assembly, the multivalent effect and target-activatable architecture. This Ntri-SAAP showed good in vitro and in vivo performance for treatment of leukemia. The assembly was capable of targeted drug delivery and silencing the role of drug-resistant P-gp protein in CCRF-CEM and K562/D cells.

In order to achieve precise and effective treatment, Wang et al. constructed a second-order DNA logic-gated nanorobot (DLGN) that could be anchored on the cell membranes to load multiple aptamers and therapeutics (Figure 5C). This DLGN allowed accurate differentiation among five different cell lines, and then triggered synergistic killing of target cancer cells.

3.4. Aptamer-based cancer immunotherapy

Cancer immunotherapy refers to the attack of cancer cells by boosting the immune system, which could avoid the side effects associated with damage to normal cells. In recent decades, immunotherapy has attracted increasing attention and has gradually become an extensive research topic in the area of cancer treatment. Many immunotherapeutic strategies, such as the immune checkpoint blockade (ICB) and adoptive T cell immunotherapy, have been designed and applied to inhibit tumor growth. For example, Gao et al. successfully isolated an anti-PD-1 aptamer PD4S using the Cell-SELEX procedure (Figure 6A), and verified its good performance for rescuing PD-1/PDL1 induced T cell exhaustion. Moreover, Du et al. prepared a highly stable multifunctional aptamer that could block the CTLA-4/B7 and PD-1/PD-L1 signaling pathways, and achieved enhanced anti-tumor immunity against liver tumors. To reduce the toxic side effect of ICB-based therapy, Yang et al. proposed an aptamer-based logic operation to achieve effective and sustained immune checkpoint blockade treatment by chemical modification of anti-PDL1 aptamers on the cell surface with limited internalization and detachment (Figure 6B). Their results indicated that incorporation of DNA logic gates could improve the accuracy and robustness of ICB therapy.

Adoptive T cell immunotherapy, such as chimeric antigen receptor (CAR) T cell therapy, was proven to be highly effective in the treatment of hematologic malignancies, but it was...
challenged by complicated ex vivo engineering and high systemic side effects. To address the above issues, Yang et al. reported an aptamer-based “recognition-then-activation” strategy, where naive T cells could be specifically recruited by cancer cells and then activated in situ to effectively kill cancer cells (Figure 6C). This strategy was a universal and economical approach, providing a prototype of on-shelf T cells for cancer immunotherapy. Moreover, Zhang et al. proposed an aptamer-equipped strategy to generate specific, universal, and permeable natural killer (NK) cells for enhanced adoptive immunotherapy in solid tumors (Figure 6D).

4. Conclusion and Outlook

Aptamers, which are reliable recognition units, have been incorporated into various emerging devices for biosensing, imaging, and bioregulation due to their high programmability, affinity, and selectivity. In recent years, rapid progress has been made in the fields of aptamer-based devices and aptamer-conjugated drugs for accurate cancer detection and targeting treatment. However, the development and application of aptamers are in their initial stages, and many challenges remain to be solved.

First, natural nucleic acids have a limited biological stability and a short half-life in vivo in aptamer-mediated therapy. While advances in nucleic acid chemistry and biotechnology have made major breakthroughs to circumvent these challenges, there is still much room for improvement. Second, the number of available aptamers is still very limited. Thus, new strategies that enable the selection of good-performance aptamers with high efficiency would be desirable. Third, many aptamer-drug conjugates have failed to realize their potential in clinical studies, suggesting that challenges remain in the rational design of such conjugates, including improving drug delivery capacity and efficiency in targeting established oncogenes. Overall, while aptamers have shown great promise in the areas of bioanalysis and cancer therapy, intensive efforts are still required in this exciting research field, and future studies should focus on the design of novel configuration-activated aptamer probes for recognition in complex environments.

Acknowledgements

This work is supported by the National Key Research Program (2021YFA0910101), the National Natural Science Foundation of China (NSFC 21922404, 22174039 and 21827811), and the Science and Technology Project of Hunan Province (2021RC4022, and 2019SK2201).

Conflict of Interest

The authors declare no conflict of interest.
