Review

Current Advances in Aptamer-based Biomolecular Recognition and Biological Process Regulation

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The interaction between biomolecules with their target ligands plays a great role in regulating biological functions. Aptamers are short oligonucleotide sequences that can specifically recognize target biomolecules *via* structural complementarity and thus regulate related biological functions. In the past ten years, aptamers have made great progress in target biomolecule recognition, becoming a powerful tool to regulate biological functions. At present, there are many reviews on aptamers applied in biomolecular recognition, but few reviews pay attention to aptamer-based regulation of biological functions. Here, we summarize the approaches to enhancing aptamer affinity and the advancements of aptamers in regulating enzymatic activity, cellular immunity and cellular behaviors. Furthermore, this review discusses the challenges and future perspectives of aptamers in target recognition and biological functions regulation, aiming to provide some promising ideas for future regulation of biomolecular functions in a complex biological environment.

Keywords Aptamer; Molecular recognition; Biological process regulation

1 Introduction

Most biological processes involve the accurate regulation of biological functions to maintain normal physiological activities[1−6]. The precise regulation of biomolecular functions is important for understanding biological processes[7,8]. Specifically, biomolecules interact with their target ligands to complete various physiological processes, such as intracellular signal transduction and cellular response to environmental stimuli[9−12]. Thus, designing molecules that mediate the interaction between biomolecules and their ligands is of great significance for regulating biological functions.

To investigate the regulation of biomolecular functions, it is necessary to design a series of ligands with specific recognition for biomolecules. Aptamers are short oligonucleotide sequences generated from an *in vitro* method known as SELEX(systematic evolution of ligands by exponential enrichment)[13,14]. Folding into distinct spatial structures, aptamers can recognize their target molecules with high affinity and specificity[13,15]. Furthermore, aptamers recognizing biological target molecules can activate or inhibit relevant signal pathways[16,17]. These characteristics make aptamers powerful tools for regulating biological functions.

In the past ten years, aptamers in molecular recognition and biological process regulation have become a hot spot for researchers[7,18]. However, there are few reviews on this aspect. This review presents the recent advancements of aptamers in molecular recognition and biological process regulation. Firstly, we introduce some methods to enhance the affinity of aptamers, such as structural optimization, multi-aptamer combination and chemical modification[19−22]. Then, the regulation of biological functions based on molecular recognition of aptamers is summarized. Given aptamers recognition characteristics, we mainly review the progress of aptamers in regulating the function of biomolecules, including enzymatic activity, cellular immunity and cellular behavior. Finally, the challenges and future perspectives of aptamers in target recognition and biological function regulation are discussed. It is expected to provide new ideas for future research in this field.

2 Improving Binding Affinity of Aptamers to Biological Targets

Owing to many favorable features, such as high stability, small size, minimal immunogenicity, easy synthesis and modification, aptamers have attracted substantial research interest for biosensing, chemical biology, disease diagnosis and drug discovery[20,23,24]. Currently, it has been reported that nucleic acid sequence changes and chemical modifications can transform the three-dimensional conformation of aptamers and enhance their interactions with biomolecules, being beneficial for the biological process regulation of aptamers on target biomolecules[16,24,25]. In this part, we will discuss how to enhance the recognition between aptamers and target biomolecules by optimization of aptamer spatial structures,

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construction of multivalent aptamers and chemical modification of nucleotides in detail.

2.1 Optimization of Aptamer Spatial Structures

The complex three-dimensional configurations of aptamers including duplexes, hairpins, stem-loops, bulges, false knots and quadruplexes, ensure their interactions with biomolecules by van der Waals contacts, hydrogen bonds and hydrophobic effect[26,27]. Among the various methods to enhance aptamer recognition, structural optimization can provide a simple strategy to improving aptamer specificity and affinity. The following section summarizes the enhanced recognition of aptamer from cyclization, truncation, fixing termini, and adding intercalated motif(i-motif).

Circular structures of aptamers are beneficial for maintaining conformational stability owing to a reduction in flexibility[28−30]. Using click chemistry reaction, Ji *et al.*[28] cyclized Spiegelmer(RNA aptamer) to obtain a significant improvement in binding affinity and selectivity toward the target protein. Specifically, the electrophoretic mobility shift assay(EMSA) showed that the binding affinity of circular RNA aptamer was increased ten-fold than that of the linear. Interestingly, a circular DNA library based on a 15-nucleotides(nt) thrombin binding aptamer(TBA_{15}) with two symmetrical random regions and two constant regions was designed by Mao and co-workers[29]. They successfully selected the circular aptamer CTBA4T-B1 containing a central quadruplex and two long stem-loops[Fig.1(A)]. According to the stability assay, the circular aptamer CTBA4T-B1 depending on the cross linking of bases showed high stability. Besides, the affinity of circular aptamer CTBA4T-B1 increased about 1000-fold compared to the linear mother sequences. Moreover, Li *et al.*[30] reported that cyclization *via* base complementary pairing could facilitate transferrin receptor(TfR) aptamer-induced permeability of the blood-brain-barrier and enhance the binding ability of Tau aptamer, showing the broad prospects of circular aptamers in diagnosis and therapy. These studies above indicated that cyclization is a direct and effective strategy to improving the target recognition properties of aptamers.

It has been widely reported that the primer sequences and partial random region sequences of selected full-length aptamers are usually not beneficial for recognizing targets[31]. These redundant sequences may even interfere with the binding of aptamers to the target[26,32]. Therefore, rational truncation of redundant sequence from the full-length sequence is an important method to increase aptamer binding affinity. Zhao *et al.*[26] obtained a 24-mer aptamer and a 10-mer aptamer by truncating a 15-nt at the site of the primer sequences. According to the simulated new secondary structure, the 24-mer aptamer retained a hairpin structure the same as the parental full-length(39-mer) aptamer. And the binding affinity between the truncated 24-mer aptamer and phthalate esters(PAE) was improved by 1.5-fold than that of the original 39-mer aptamer. But the truncated 10-mer aptamer could not form a stable secondary structure due to its short sequence, which seriously affects its activity to bind PAEs. Considering the higher affinity and stability of aptamers with quadruplex structures, Liu *et al.*[32] truncated a 19-nt primer of the aptamer A-9 to obtain A-9S with G-quadruplex structures. The real-time qPCR results showed that the resulting aptamer A-9S bound to the E antigen of hepatitis B enhanced the binding affinity by 19 times. These showed that reasonably truncating the redundant sequence of aptamers could effectively increase the binding affinity of aptamers.

In addition, fixing the termini of the aptamer can also achieve higher affinity recognition *via* stabilizing its folded conformation. For example, Tan's group[33] reported a terminal fixation design that a triple helix, a polyT linker and a tetrahedron fixed the termini[Fig.1(B)]. The end of the aptamer was fixed by the triple helix structure to form a recognition

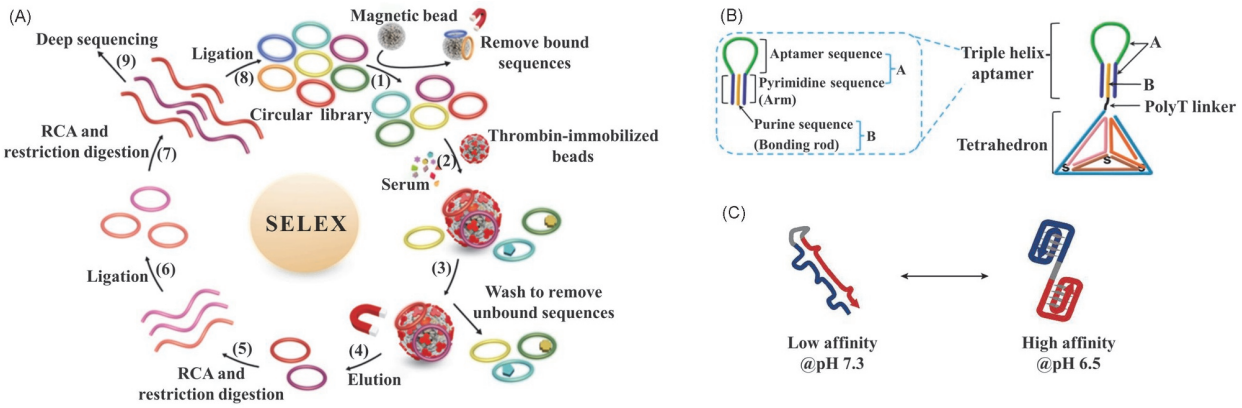

Fig.1 Bead-SELEX for obtaining a circular thrombin binding DNA aptamer(A), schematic illustration of the terminal fixation design(B) and the switchable structure of aptamer with i-motif(C)

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loop, and then the authors utilized the tetrahedron to further stabilize the aptamer folded structure. They found that tetrahedron-assisted triple helix-aptamers generated a ten-folds enhancement in affinity.

The intercalated motif(i-motif) is a DNA sequence rich in cytosine and it can simultaneously form a quadruplex structure at physiological acidic pH[23]. Because of the pH responsiveness of i-motif, Li *et al*.[23] added a split i-motif to both ends of the Sgc8 aptamer to regulate the formation of a high-affinity structure of aptamer, because the split i-motif was unstructured at physiological pH, preventing aptamer Sgc8 from folding into recognition loops. However, the split i-motif could simultaneously fold into a quadruplex structure at acidic pH, inducing aptamer Sgc8 folding to the recognition loop and enabling the aptamer to specifically recognize target receptors. In another study, Li *et al*.[34] designed a different structure-switching aptamer(SW-Apt) *via* adding an i-motif to the termini of a nucleolin binding aptamer AS1411, while i-motif as a modulating element and AS1411 as a recognition element. As shown in Fig.1(C), the SW-Apt forms a stable long-stem structure at physiological pH, and it can form a double-quadruplex structure in the acidic microenvironment. This design of SW-Apt not only improves aptamer-specific recognition of targets under complex biological conditions, but also provides a practical approach to improving aptamer binding ability.

2.2 Construction of Multivalent Aptamers

When monovalent aptamers target the biomolecules in complex matrices, their limited binding affinity is generally hard to meet the practical requirements[35]. Inspired by natural multivalent interactions, many strategies involving hybridization and polymerization have been applied in constructing multivalent aptamers[36]. Unsurprisingly, the multivalent aptamers can interact with multiple target receptors simultaneously, showing higher affinity and specificity than monomer aptamers[35−38]. Therefore, the rational construction of multivalent aptamers has received extensive attention in enhancing the specific recognition of aptamers.

Yang *et al*.[39] engineered a bivalent aptamer B72 by rational sequence truncation and ligation of anti-Aflatoxin B1(AFB1) binding aptamer B50[Fig.2(A)]. According to the predicted spatial structure and active domain, B72 exhibited two recognition sites to AFB1, showing a great optimization in the affinity toward toxicants in theory. Afterward, the microscale thermophoresis(MST) assay affirmed consistently that the affinity of B72 increased 188-fold compared with that of B50, revealing a significant improvement in affinity. Moreover, a hybrid bivalent Sgc8 aptamer binding toward T lymphoblastoid CCRF-CEM cells was successfully constructed by Kuai *et al*.[40]. Compared to mono-aptamer, the hybrid bivalent aptamer possessed a 2-fold higher binding ability, better thermal stability and a lower degradation rate *in vivo*, due to the effect of complementary base pairs on stabilizing the aptamer structure in the biological media. This research promoted the application of aptamers in clinical diagnosis and therapy.

Loading polyvalent aptamers on the surface of hydrogels *via* a hybridization chain reaction, Richards *et al*.[41] synthesized a platform with sufficient reaction sites for enhancing cell capture efficiency during clinical diagnostics[Fig.2(B)]. Similar to the aforementioned study, a multivalent aptamer modified nanointerface was used to capture cells and the capture efficiency was enhanced by over 300 % than that of the monovalent aptamer-modified nanointerface[42]. In addition, Sun *et al*.[43] developed a unique cocktail therapy, in which three cocktail neutralizing aptamers recognizing the different receptor-binding domains(RBDs) of coronavirus were anchored on one gold nanoparticle to block the virus infection. The dissociation constant value of cocktail neutralizing aptamers was 3.90 pmol/L and the affinity of three aptamers modified gold nanoparticles was approximately

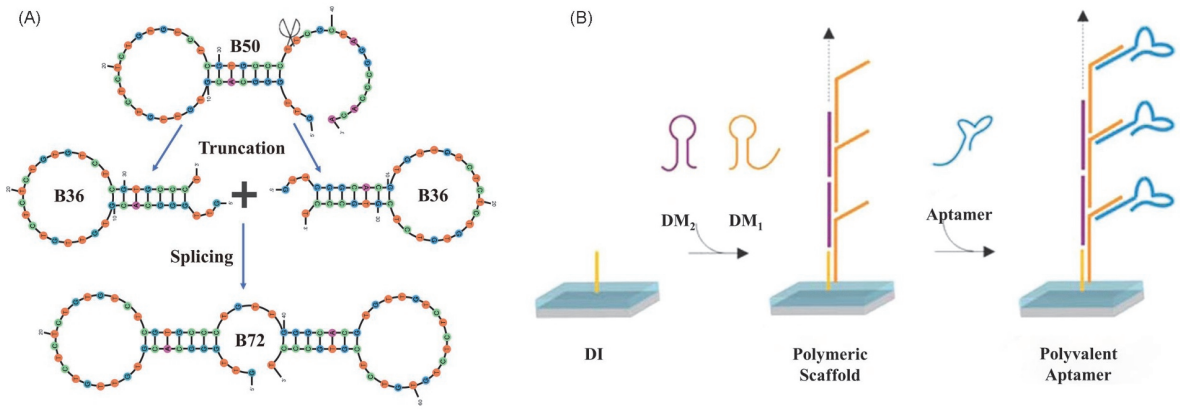

Fig.2 Formation process of bivalent aptamer(A) and hybridization chain reaction for polyvalent aptamer(B)
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(1—8)×10³ folds higher than that of the single aptamer. So far, there are not many aptamers that can recognize different epitopes of the same target, but it is worth affirming that this study supplies novel guidance for improving the aptamer affinity.

2.3 Chemical Modification of Aptamers

Although aptamers have specific recognition, their conformation and function are limited by four kinds of natural nucleotides(A, T, G, C or U)[44—46]. The introduction of chemical modifications in nucleotides endows aptamers with richer spatial configurations and further offers new interactions between aptamers and their targets[1,45,47]. Recently, it has already been reported that a series of chemically modified aptamers may have a higher nuclease resistance, stronger affinity and higher selectivity than natural nucleotide consisted aptamers. Next, we mainly discuss the effect of chemical modification to improve aptamer affinity toward the target.

In early studies, chemically modified aptamers with improved affinity were generated by replacing conventional elements in the natural nucleotide structure of aptamers. For example, Abeydeera *et al.*[48] replaced the one non-bridging phosphate oxygen atom in the aptamer with sulfur, achieving a significant increase in aptamer affinity from nanomolar to picomolar[Fig.3(A)]. The reason was that sulfur endowed the RNA aptamer with additional hydrophobicity and strengthened the hydrophobic effect of the aptamer on the target protein. Furthermore, the sugar ring is also an important position for element substitution. Pagratis *et al.*[49] and Ruckman *et al.*[50], one after another, presented the same strategy to obtaining higher affinity aptamers by substituting the 2′ hydroxyl of sugar ring with fluoro(F), amino(NH_2) and methoxyl(OMe)[Fig.3(B)—(D)]. They respectively isolated F modified RNA aptamers binding to human vascular endothelial growth factor(VEGF) from libraries of RNA substituted by 2′ NH_2, 2′ F and 2′ OMe. Based on their research, NeXstar Pharmaceuticals developed the first therapeutic RNA aptamer(Pegaptanib) that had been approved by the US Food and Drug Administration(FDA). Recently, a 2′ F-pyrimidine RNA aptamer specifically binding to HIV-1 reverse transcriptase(RT) was isolated from the different structure modified RNA aptamer libraries[51]. The affinity of the 2′ F-pyrimidine RNA aptamer toward RT was increased about two-fold compared to the natural RNA aptamer. In these studies, such elements-replaced aptamers obtained not only increased binding affinity but higher stability and nuclease resistance[52,53], being widely applied to *in vivo* imaging and therapy.

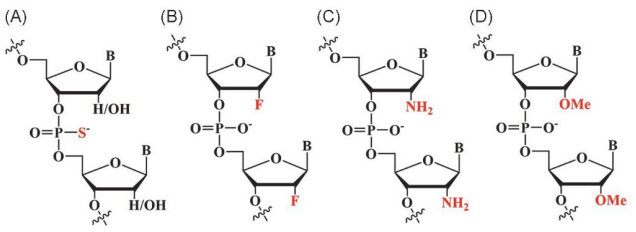

Fig.3 Chemical structures of modified aptamers
(A) Phosphorothioate-DNA/RNA; (B) 2′ F-RNA; (C) 2′ NH_2-RNA; (D) 2′ OMe-RNA.

Interestingly, some scholars have increased the binding ability of natural aptamers by introducing new bases. For instance, Kimoto *et al.*[54] replaced original adenine using hydrophobic pyridine derivative Ds and screened aptamers targeting VEGF-165 or interferon-γ(IFN-γ) from the nucleic acid library containing the Ds artificial nucleotides. The binding assay demonstrated that the affinity of Ds modified aptamers was about 2—500 times higher than that of the aptamers with original bases. According to the hydrophobic base Ds before, Matsunaga *et al.*[55] obtained a higher affinity DNA aptamer targeting dengue protein and the K_d value of the DNA aptamer was 27—182 pmol/L. In other studies, mimic amino acid side-chains were modified on 5′-position of pyrimidine bases to expand aptamer chemical diversity. Based on this strategy, a new class of aptamer named the slow off-rate modified aptamer(SOMAmer) was created that endowed the aptamer with protein-like properties and showed high affinity(K_d<1 nmol/L) with the target protein[56]. These examples reveal that introducing chemical modification on bases is very effective in enhancing aptamer affinity.

3 Aptamers for the Regulation of Biological Functions

The realization of biological functions depends on the recognition of biomolecules[5]. Recognitions and interactions among proteins, nucleic acids and carbohydrates are achieved by their specific structural complementarity[25,57,58]. Moreover, these interactions exist in cellular biological processes that mediate various cellular events in living organisms[2]. Aptamers as high affinity recognizing modules can form distinct three-dimensional configurations and specifically bind to the active site of target biomolecules, triggering the downstream signal transduction and then regulating biomolecular functions[11,59]. The following part elaborates on the wide applications of aptamers in regulating enzyme activity, cellular immunity and cellular behaviors.

3.1 Regulation of Enzyme Activity

Enzymes are proteins or RNAs exhibiting high catalytic activities and participate in almost every biological process[60—62]. Aptamers recognize the active site of enzymes to

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allow for efficiently regulating enzymatic activity, making it significant in the prevention of diseases associated with enzymatic activity[2,61].

Thrombin is the central factor in the function of hemostasis and its' prime function is to catalyze the formation of fibrin, promoting hemostasis and wound healing[63−65]. Yuan's group[63] constructed a closed-loop structure with two thrombin binding aptamers TBA15 and TBA29 that recognized and blocked different sites of thrombin to critically inhibit its catalytic activity *via* the synergistic effect. As shown in Fig.4, the two aptamers based closed-loop structure will liberate thrombin after the temperature rises. Subsequently, the inhibited catalytic function of thrombin gets efficiently recovered. This strategy provides a robust approach for protein activity regulation and photocatalytic reaction, being a great boost to the discovery of protein inhibitors.

The function of aspartyl proteases BACE1 is to catalyze the metabolization of amyloid precursor protein(APP) into amyloid β peptide(Aβ)[66]. The APP and Aβ are related to the pathogenesis of Alzheimer's disease(AD), and therefore BACE1 becomes a key drug target of AD[66−69]. Liang *et al.*[68] obtained DNA aptamers A1 that could specifically bind to the extracellular domain of BACE1 and suppress the activity of BACE1, decreasing APP expression and Aβ production. This provides preliminary feasibility of developing a potential BACE1 binding aptamer inhibitor. Simultaneously, two DNA aptamers against BACE1 were identified by Cai's group[69]. The two identified aptamers interacted with BACE1 and significantly inhibited the activity of BACE1 in a fluorescence resonance energy transfer(FRET) assay, avoiding Aβ-induced neuronal deficiency. Recently, Gasse and co-workers[70] utilized a 5-Chlorouracil modified aptamer(single-stranded xenonucleic acid, ssXNA) recognizing the BACE1 ectodomain to realize the regulation of BACE1 catalytic activity. Interestingly, the FRET assay shows that the manner of the selected ssXNA sequences modulating BACE1 is a dose-dependent effect. There is a decrease in BACE1 activity at the low concentrations of ssXNA but an increase in BACE1 activity at the high concentrations of ssXNA due to a loss of effective inhibition caused by self-aggregation. In a word, ssXNA enables to regulate the activity of BACE1 and treat neurodegenerative diseases.

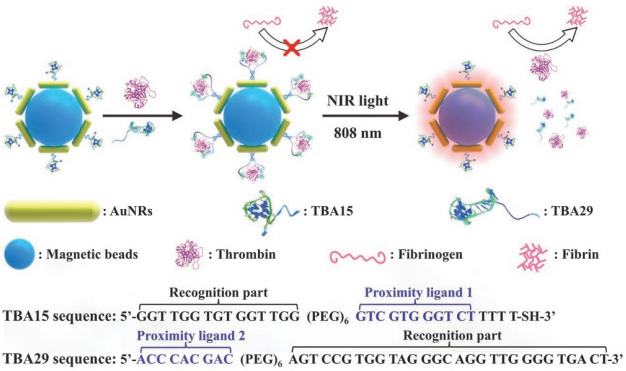

Fig.4 Inhibition and recovery of thrombin by the closed-loop structure under near-infrared ray(NIR) irradiation

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Most receptor tyrosine kinases(RTKs) are located in the intracellular domains of transmembrane receptors, playing a significant role in regulating signal transduction pathways in cells[71]. Under the stimulation of extracellular ligand, the receptor is dimerized to induce activation and phosphorylation of intracellular tyrosine kinases[72]. To regulate the kinase activity of the receptor, Ueki and Sando *et al.*[73] designed a DNA aptamer Apt_46 that was useful for the inhibition of the aberrant dimerization of fibroblast growth factor receptor 2b(FGFR2b, a member of RTKs). According to the average phosphorylation levels of FGFR2b using Western blotting, they found that Apt_46 could strictly block the activation of FGFR2b endogenous tyrosine kinase and substantially reduce the phosphorylation level of the kinase. In summary, aptamers can regulate kinase activity, which is of great importance in cellular signal transduction and cancer therapy.

3.2 Regulation of Cellular Immunity

The immune system can identify and eliminate abnormal cells to maintain the relatively stable immune function of the organism[74−76]. If the immune system is dysfunctional, the organism may occur to generate diverse inflammation-driven pathologies, such as cancer[75,76]. Unsurprisingly, aptamers are used to intervene in the binding of immune checkpoints to their ligands, thereby regulating cellular immune responses[77]. In addition, aptamers can participate in cellular immune regulation by enhancing the target recognition of non-specific immune cells[78].

Cancer cells are capable of evading host immune surveillance owing to the function of coinhibitory ligands, such as programmed-death ligand 1(PD-L1)[79,80]. These coinhibitory ligands interact with immune checkpoints to inhibit immune responses. In particular, the binding of PD-L1 to PD-1(programmed-cell-death protein 1) on T lymphocytes is considered one of the major pathways involved in tumor immune evasion. Yu *et al.*[79] reported the application of threose nucleic acid(TNA) aptamers in cancer immunotherapy [Fig.5(A)]. TNA aptamers can effectively block the binding of PD-1 to PD-L1 *in vitro* and activate the innate immunity of Balb/c mice to inhibit tumor growth. Additionally, Tan's group[81] also investigated how to intervene in the interaction of PD-1 with PD-L1 to realize the immune checkpoint blockade(ICB) therapy. They proposed a method that the antagonizing aptamer(aPDL1) of the immune checkpoint was

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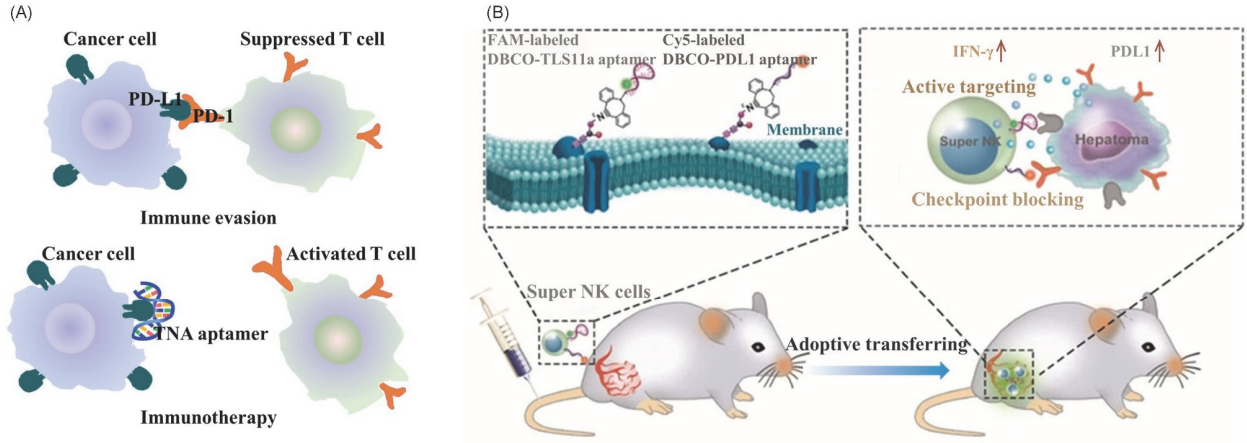

Fig.5 *In vitro* selected TNA aptamer inhibiting PD-1/PD-L1 interaction(A) and bispecific aptamer-engineered NK cells for checkpoint blocking(B)

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covalently modified on the cancer cell surface, with the purpose of achieving precise and sustained ICB therapy. Subsequently, D-aPDL1 was constructed by the aPDL1 aptamer modified with dibenzocyclooctyne(DBCO), and the PD-L1 was labeled with azides *via* metabolizing. Finally, the D-aPDL1 aptamer could covalently conjugate azide-labeled PD-L1 *via* click reaction to precisely inhibit the activation of PD-L1 and mediate antitumor immunotherapy.

Apart from blocking the immune checkpoint, aptamers modified innate immune cell is an effective strategy to perform targeted cancer immunotherapy and regulate the immune response. Yang *et al.*[82] constructed aptamer-engineered natural killer cells(ApEn-NK) with synthetic CD30-specific aptamers anchored on NK cells, triggering apoptosis of CD30-expressed lymphoma cells. In another similar research, Shi *et al.*[83] synthesized a polyvalent antibody mimic(PAM) composed of supramolecular aptamer *in situ*. They found that the killing rate of NK cells conjugated with PAM was improved 2.73 times than that of the natural NK cells. Moreover, a strategy of loading aptamers on the innate immune cells was also proposed by Zhang *et al.*[78] to improve adoptive immunotherapy[Fig.5(B)]. Two aptamers, specifically HepG2 cell binding aptamer TLS11a and PD-L1 aptamer were tightly coupled on the surface of the NK cell. On one hand, aptamers-equipped NK cells induced more secretion of immune cytokines and thus enhanced adoptive immune responses compared with parental NK cells. On the other hand, PD-L1 aptamer located on the NK cell could interact with PD-L1 that was highly expressed on the membrane of HepG2 cell, suppressing PD-1/PD-L1 signal and enhancing immunotherapy. These studies demonstrate that aptamers can regulate cellular immunity by blocking immune checkpoints or modifying non-specific immune cells.

3.3 Regulation of Cellular Behavior

Cellular behaviors in living organisms are strongly associated with the emergence and progression of diseases[59,84]. Thus, precisely regulating cellular behaviors is essential in understanding the pathological mechanism and achieving personalized treatment[59,14]. At present, there are many studies on aptamers used in regulating cell migration, adhesion and cell-cell interactions.

Cellular behaviors generally depend on the sensing and response of the receptors to extracellular ligands, and therefore the dimerization of receptors plays a fundamental role in regulating cellular behaviors[59]. Take mesenchymal epithelial transition(Met) receptor as an example, it will dimerize and subsequently occur to autophosphorylation after activating by its ligand hepatocyte growth factor(HGF), further regulating various cellular behaviors including proliferation and migration[85]. To modulate Met receptor-mediated relevant cellular behaviors, Sando *et al.*[85,86] have made great efforts to develop HGF mimetics and successfully produced the dimerized aptamer Di-SL1 to substitute HGF. Concretely, the dimerized aptamer Di-SL1 could bind to two Met receptors, thereby allowing Met dimerization and phosphorylation to stimulate cell migration and proliferation[85]. Subsequently, Sando and co-workers[87] developed a photoreactive molecular glue ^{BP}Glue-N_3 in order to continuously enhance the covalent binding of Di-SL1 with Met. This work showed that ^{BP}Glue-N_3 increased the suppression of Di-SL1 on cell migration. In addition, Wang *et al.*[88] proposed an efficient method to selectively regulate the dimerization of receptors and designed the bispecific aptamer probe that was consisted of a Met aptamer, a TfR aptamer and a complementary aptamer. This bispecific

aptamer probe induced the close pairing of Met receptor with TfR on the cell membrane, allowing for inhibiting the activation of Met receptor and regulating cell migration *via* the present strong steric hindrance effect.

Consisting of an α-subunit and a β-subunit, integrins are transmembrane heterodimeric cell-adhesive molecules that provide connections and regulate interactions between cells and extracellular matrices[1,89]. Tan *et al.*[1] isolated aptamer ZAP-1 targeting integrin alpha3(ITGA3) from artificial-nucleotide-expanded libraries[Fig.6(A)]. The binding of ZAP-1 to ITGA3 blocked the interaction between ITGA3 and its ligand laminin 10, thus inhibiting breast cancer adhesive [Fig.6(B)] and metastatic activity in preclinical research. In another study, a chemical modified aptamer PNDA-3 was synthesized to recognize the active site of periostin with nanomolar affinity and disrupt the binding of periostin to integrin, efficiently inhibiting the adhesion and invasion of breast cancer cells[89].

Precisely regulating cell-cell interactions will not only offer great potential for studying various mechanisms of biological functions but also be of great significance in understanding disease progressions and promoting the development of theranostics[7]. Liu *et al.*[90] presented a strategy that multivalent, bispecific aptamers were linked to DNA nanoscaffolds for regulating cells interactions. They found that these aptamer-DNA scaffolds were available to promote the T-B cell interactions to generate an effective immune response. In addition, the specificity and biocompatibility of aptamers

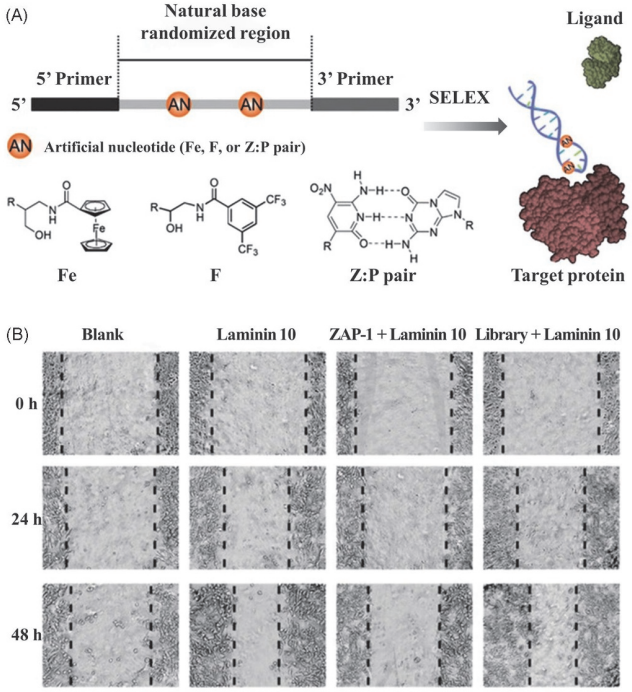

Fig.6 Design of artificial aptamers targeting integrin(A) and cell migration result modulated by ZAP-1(B)

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on DNA nanoscaffolds enabled them to be applied to biomedical fields. Similar to the above work, Li *et al.*[7] designed a DNA nanoplatform to initiate intercellular communication *via* aptamer-based molecular recognition. The DNA nanoplatform consisting of an amphiphilic DNA tetrahedron and one DNA aptamer was anchored on the cell membrane, further triggering intracellular signaling cascades by enhanced intercellular communication. Moreover, combining the inherent high flexibility and diversity of DNA aptamer, this DNA-based platform holds great promise to become a powerful tool to study multicellular communication networks and cellular biology. These studies indicate that the specific recognition of aptamers is critical to regulating cell-cell communications and other biological processes, avoiding metabolic disorders and autoimmune diseases.

4 Summary and Outlook

The research on the properties and applications of aptamers has gradually matured after more than thirty years[91−93]. Based on those studies, this review mainly describes the effect of structural optimization, multivalent aptamer assembly and chemical modification on enhancing aptamer recognition. At the same time, the biological process regulation of aptamers in enzymatic activity, cellular immunity and cellular behavior is concluded. We found that aptamers have made a lot of progress, but there is still a little space for improvement. First, the kinds of high-affinity aptamers are limited. Due to the long period of screening and complex environmental factors, such as pH, temperature and concentration, some selected high-abundance sequences are not highly specific. Second, methods to improve the affinity of aptamers need to be improved. Taking chemical modification as an example, there are few types of replacement elements and artificial bases of aptamers, and chemically modified aptamers are also confined by the compatibility of traditional screening techniques. Third, poor stability and short circulation time *in vivo* restrict the long-term development of aptamers in biological process regulation.

Despite these challenges, aptamers in property and biological process regulation will receive further developments in the near future. Especially, new advances that improve the screening efficiency and aptamer property have emerged. For instance, the recent popular microfluidic screening incorporating high-throughput sequencing technologies may be possible to effectively shorten the screening time and develop more high-affinity aptamers. Expanding the genetic alphabet and assembling aptamers that recognize different epitopes of the same target should be useful to enhance aptamer affinity. Besides, aptamers functionalized nanomaterials have largely overcome the

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difficulties of aptamers in the biological application. In short, with the advancement of screening technology and the above-mentioned methods for enhancing aptamer affinity, the application of aptamers in biological target recognition and function regulation will be a broader prospect.

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Conflicts of Interest

The authors declare no conflicts of interest.

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