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HIGHLIGHT

DNA origami nanodevice with spatial regulation of CD95 signaling for rheumatoid arthritis treatment

KEY WORDS

DNA origami;
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CD95 death-inducing signaling;
DNA nanostructure;
Drug delivery;
Receptor−ligand interaction;
Cell signaling;
Drug intervention

Recently, a work jointly studied by Ling Li and coworkers[1] was published in *Nature Materials*, describing a reconfigurable DNA origami nanodevice designed to regulate CD95 death-inducing signaling of immune cells. The researchers utilized the DNA origami nanodevice to establish selective local immune tolerance and demonstrated its ability to alleviate rheumatoid arthritis (RA) in the inflamed synovial tissue of mice without causing any obvious side effects (Fig. 1). This approach presents a novel idea for the development of drug interventions involving ligand-receptor interactions.

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterized by synovitis. Despite significant progress in the treatment of RA over the past two decades, achieving long-term disease remission without serious side effects remains a major challenge[2]. One potential approach for the effective treatment of RA is to inhibit autoimmune responses by eliminating activated immune cells in inflamed synovial tissue[1]. The CD95/CD95 ligand (CD95L) signaling pathway could induce cell-specific apoptosis, leading to the elimination of activated lymphocytes and playing a crucial role in regulating immune responses[3]. The interaction between CD95/CD95L can promote the establishment of local immune tolerance, offering great potential for reversing and treating RA. In its resting or "non-signal" state, the CD95 receptor forms a large hexagonal network on the cell surface. Upon binding with CD95L, this complex further forms a hexameric cluster with a molecular spacing of approximately 10 nm[4]. It has been identified that the CD95L ligand array with an optimal molecular spacing of 10 nm is an effective method for activating CD95 death-inducing signals[1,4]. However, current synthesized scaffolds for regulating cell signaling often struggle to accurately control ligand valency, spacing, and spatial arrangement. This highlights the necessity of exploring new strategies that can provide nano-precision spatial arrangement for ligand arrays to address this limitation.

To investigate the complex and controllable spatial arrangement of CD95L, Li and colleagues[1] utilized DNA origami technology to construct rectangular DNA nanosheets (referred to as NS-empty). The intermolecular distance of CD95L was manipulated with poly-T complementary chains by controlling the position of the protruding poly-A chain on the surface of the DNA origami. To examine the impact of CD95L interspacing on triggering the CD95 receptor, they designed three spatially patterned DNA origami nanosheets with intermolecular distances of 5, 10, and 30 nm (referred to as NS-5, NS-10, and NS-30) for CD95L. Furthermore, six pairs of i-motif-based DNA fasteners were additionally incorporated into DNA nanosheets to achieve dynamic structure conversion of pH response in controlling the activity of CD95L arrays (referred to as ND). The pH-dependent fluorescence emission spectra experiment confirmed the reversible closing and opening function of the DNA origami nanodevice. The researchers observed that DNA origami nanosheets exhibited excellent stability and resistance to degradation in biofluids.

Generally, T and B lymphocytes play crucial roles in the onset and progression of RA[5,6]. To investigate the impact of the spatial

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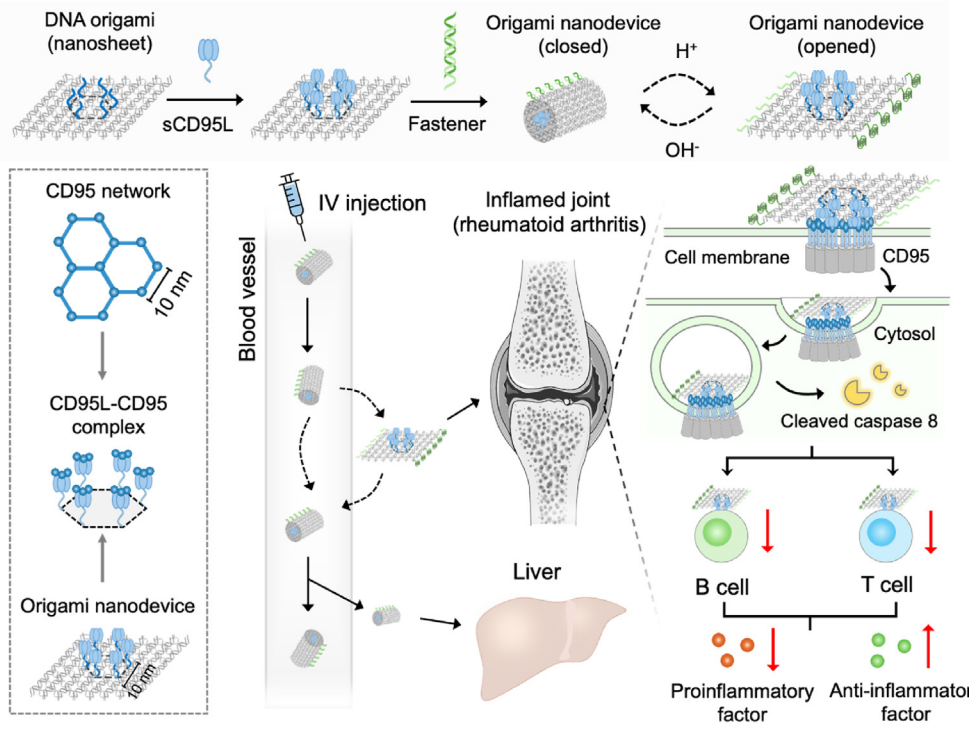

Figure 1 Reconfigurable DNA origami nanodevices spatially control CD95 death-inducing signaling in immune cells to alleviate rheumatoid arthritis (RA). Reprinted with permission from Ref. 1. Copyright © 2024 Springer Nature.

arrangement of the CD95L array on apoptosis induction, cytotoxicity analysis was conducted on A20 and Jurkat cells following treatment. The results revealed that DNA nanosheets with CD95L arrays exhibited significantly higher activity in inducing apoptosis compared to soluble CD95L (sCD95L), which had very low activity. Notably, the DNA origami nanodevice (ND-10) demonstrated the highest potency under acidic conditions when the intermolecular spacing of CD95L was 10 nm; conversely, no apparent apoptosis was observed under neutral conditions. The binding of CD95L to the CD95 receptor triggers their aggregation on the cell surface, subsequently activating downstream signals through endocytosis[7]. To elucidate the potential mechanism underlying the exceptional performance of NS-10, an examination of CD95 receptor distribution post-NS-10 treatment was conducted. The stimulation by NS-10 led to highly aggregated CD95 receptors on the cell surface, whereas no discernible change occurred after NS-empty or sCD95L treatment. Interestingly, these aggregated spots did not appear on the cell surface but rather in the cytoplasm. Consequently, further exploration into endocytosis of CD95 receptor post-NS-10 stimulation took place. Colocalization studies with endosomal markers suggested that most receptor-ligand complexes would be internalized and directed to early endosomes after aggregation, thereby activating downstream caspases 8. Furthermore, apoptotic cells were also detected to be cleared by bone marrow-derived macrophages and promote the release of anti-inflammatory cytokine TGF-β.

Both near-infrared fluorescence imaging and *ex vivo* imaging of the CIA mouse model demonstrated that the fluorescence signal in inflamed joints and paws, following injection of cy5.5-labeled DNA origami nanodevices, was significantly higher than that observed in healthy mice. These findings suggest that DNA origami nanodevices are capable of effectively penetrating and accumulating within inflamed tissues while maintaining their pH-responsive structural transitions *in vivo*. Importantly, due to the controllable conformational changes and immunologically inert DNA origami, this nanodevice has the potential to mitigate the risk of liver injury to a certain extent.

Based on the clinical arthritis score and joint swelling measurement data of CIA mice, the treatment effect of ND-10 treated mice was comparable to that of the positive control group, significantly delaying disease progression. Moreover, results from micro-computed tomography (microCT) imaging and hematoxylin and eosin (H&E) staining confirmed that ND-10 treatment could effectively inhibit bone erosion and cartilage degradation at the joint. The researchers also investigated the immune cell profile and inflammatory cytokine profile in inflamed synovial tissue after ND-10 treatment. Immunohistochemical analysis revealed a significant enrichment of $CD95^+$ cells in inflamed synovial tissue, which decreased notably after treatment. Additionally, there was a marked decrease in pro-inflammatory cytokine expression levels in synovial tissue following ND-10 treatment, accompanied by an increase in anti-inflammatory cytokines. This may be attributed to phagocytes promoting a local immune tolerance environment through the phagocytosis of apoptotic cells, thereby facilitating immunosuppression.

The hexagonal CD95L array-based DNA origami, featuring a precise recognition pattern, has been shown to exhibit the most effective CD95 receptor signaling. Additionally, the nanodevice can undergo reversible configuration conversion in response to pH changes, resulting in the specific exposure of CD95L and ultimately alleviating chronic inflammation in the CIA mouse model. In summary, this study has successfully achieved precise control

over the spatial regulation of cellular signals and presents a promising tool for the treatment of RA.

DNA nanostructure offers the advantages of excellent biocompatibility and high addressability. It can accurately integrate drug molecules and functional components, such as targeting ligands and stimuli response modules, in a nanoscale controllable spatial arrangement[8]. The DNA origami nanodevice developed by Li[1] provides a unique approach for long-term RA treatment, expanding the understanding of pharmaceutical interventions in ligand-receptor interactions. It has been reported that the pathogenesis and progression of cancer and autoimmune diseases fundamentally involve the dysregulation of cellular signaling pathways[9,10]. This reconfigurable DNA origami nanodevice with precise spatial arrangement provides a general paradigm for regulating cell signaling, facilitating the development of drug interventions for disease. However, the complex folding behavior of DNA origami may become a limiting factor for its wide application and production; therefore, it is necessary to further improve the assembly yield of target structures. Optimization and quality control of guest molecules placed on DNA origami are equally important, including labeling efficiency and purification efficiency of functionalized DNA structures.

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Author contributions

Miao Mao: Conceptualization, Writing − original draft. Zhe Pu: Writing − original draft. Yuanqing Zhang: Supervision, Writing − review & editing.

Conflicts of interest

The authors have no conflicts of interest to declare.

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