Nanomedicine Approaches to Overcome Immune Escape in Cold Tumors: Mechanisms and Future Directions

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Abstract. The immunosuppressive microenvironment of cold tumors has become a major challenge in tumor therapy due to the lack of T-cell infiltration and insufficient antigen-presenting capacity. This paper systematically reveals its core escape mechanisms, including aberrant activation of immune checkpoint molecules (PD-L1/CTLA-4) leading to T-cell depletion, and synergistic suppression of anti-tumor immunity by metabolic reprogramming and epigenetic dysregulation. Targeting dense matrices and multiple immunosuppressive barriers, nanomedicine achieves precise breakthroughs through functionalized carriers. For example, liposome-targeted delivery of STING agonists reshapes antigen presentation, individualized mRNA vaccines combined with immune checkpoint inhibitors (ICIs) significantly prolong survival, and photo-thermo-responsive gold nanoparticles induce immunogenic death and enhance remission rate. However, clinical translation still faces three major bottlenecks-inefficient tumor-targeted delivery, post-treatment immunosuppression rebound and insufficient material biocompatibility. Future research needs to integrate degradable smart carriers, artificial intelligence (AI)-driven antigen screening and multimodal therapeutic strategies to overcome toxicity limitations and maximize therapeutic efficacy, and ultimately promote the paradigm shift of cold tumors from “immune indifference” to “therapeutic sensitivity”.

Keywords: Cold tumor, nanomedicine, immune microenvironment, targeted therapy, cancer immunotherapy.

1. Introduction

The rise of tumor immunotherapy has opened up a new paradigm in the treatment of malignant tumors, but its efficacy shows significant heterogeneity in solid tumors. Hot tumors, such as melanoma and non-small cell lung cancer, have high tumor mutation burden (TMB) and neoantigen expression, and are able to activate T-cell responses with immune checkpoint inhibitors (ICIs, e.g., PD-1/PD-L1 blockers), resulting in clinical remission rates of up to 30-40%. However, “cold tumors” (e.g., pancreatic cancer, glioblastoma), which account for more than half of all solid tumors, are virtually unresponsive to existing therapies due to a lack of immune cell infiltration, defective antigen presentation, and multiple mechanisms of immunosuppression, resulting in a five-year survival rate of less than 10% [1]. At the core of this therapeutic dilemma lies the immune escape mechanism of cold tumors, which is mainly manifested by dense fibrotic stroma forming a physical barrier to tumor-associated macrophages (TAMs), regulatory T cells (Tregs) and suppressor cytokines (e.g., transforming growth factor-β (TGF-β), interleukin-10 (IL-10)) [2], whereas metabolic reprogramming (e.g., lactic acid accumulation, tryptophan depletion) further impairs T cell function [3]. Conventional therapies (chemotherapy, radiotherapy, and mono-immunotherapy) are unable to break through the above barriers, making it difficult to reverse the “immune desert” state of cold tumors [4].

In recent years, the rapid development of nanomedicine has provided a disruptive tool for cold tumor therapy [5,6]. By designing functional carriers (e.g., liposomes, polymer nanoparticles, and metal-organic framework materials) with a size of 1-100 nm, nanomedicine can integrate targeted delivery, microenvironment-responsive release, and multi-mechanism synergistic therapies to achieve precise interventions on cold tumor immune escape networks [7,8]. Lipid carriers can break through the stromal barrier by surface modification of actively targeted molecules (e.g., T-cell immunoglobulin mucin molecule 3, Tim-3 antibody) and encapsulate chemotherapeutic agents, interferon gene-stimulating proteins (STING agonists), or gene editing tools, to induce immunogenic

death (ICD) locally in the tumor and activate antigen presentation [6,9]. Nanovaccines, on the other hand, deliver individualized neoantigens via lipid nanoparticles (LNPs), which, in combination with TLR/STING pathway agonists, remodel the function of dendritic cells (DCs) and drive T-cell-specific responses [10,11]. In addition, metal- and silicon-based nanoparticles (e.g., gold nanorods, mesoporous silicon) can synergistically enhance drug penetration and modulate the metabolic microenvironment by utilizing photothermal effects or slow release properties [12,13]. These innovative designs have led to breakthroughs in preclinical models, with the survival of pancreatic cancer mice extended to 198 days [13], complete remission rate of melanoma elevated to 80%, and significantly elevated levels of pro-inflammatory factors such as interferon-γ (IFN-γ) [14].

However, clinical translation of nanomedicine still faces multiple challenges [5,15]. Inefficient carrier delivery (only 5% of the dose reaches the tumor after intravenous injection) [9], material toxicity (e.g., complement activation triggered by cationic lipids) [12] and immunosuppressive microenvironmental rebound (increased Treg ratio) [7] need to be addressed. In addition, the highly heterogeneous nature of cold tumors requires that therapeutic regimens must take into account both individualization and accessibility, while the existing scale-up production processes for nanomedicines (e.g., microfluidic technology, encapsulation rate optimization) are still immature [16]. In this regard, interdisciplinary integration has become an inevitable trend, with materials science driving the development of degradable carriers (e.g., mesoporous silica) [13], AI assisting in neoantigen prediction and carrier design [10], and single-cell genomics providing tools for real-time monitoring of therapeutic response.

This review systematically analyzes the immune escape mechanism of cold tumors, explains the core principles of nanomedicine in targeted delivery, immune activation, and metabolic regulation, evaluates the potential of lipid carriers, nanovaccines, and multifunctional nanoparticles, and discusses the bottlenecks in their clinical translation and future development. By integrating the latest research results and clinical data, this paper aims to provide a theoretical basis and technical route reference for nano-immunotherapy of cold tumors.

2. Difference Between Cold and Hot Tumors and Immune Escape Mechanism

The difference in response to tumor immunotherapy stems from the heterogeneity of the tumor microenvironment (TME). Hot tumors (e.g., melanoma, non-small cell lung cancer) are characterized by massive infiltration of $CD8^+$ T cells and active antigen presentation e.g., TMB generates neoantigens, which activate T cells through major histocompatibility complex class I molecules (MHC-I molecules), and pro-inflammatory factors (interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α)) drive the inflammatory microenvironment, resulting in a PD-1 inhibitor response rate of more than 30% [17]. Cold tumors (e.g., pancreatic cancer, glioblastoma), on the other hand, form a treatment-resistant "desertified" microenvironment due to the absence of T-cell infiltration, defective antigen presentation, and immunosuppressive networks [1,2].

2.1. Core Differences

In hot tumors, dendritic cells (DCs) efficiently present neoantigens via MHC-I and activate T-cell killing; in cold tumors, DCs are inactivated due to the inhibition of the STING pathway and epigenetic silencing of antigens by KRAS mutations [3,11]. The cold tumor microenvironment enriches regulatory T cells (Tregs), tumor-associated macrophages (TAMs), and suppressor factors (IL-10, TGF-β), and the dense stromal barrier and metabolic inhibition (lactate accumulation, IDO pathway) further impedes immune cell infiltration and induces T cell depletion [2,3,14].

2.2. Core Mechanisms of Immune Escape

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3. Principles and Advantages of Nanomedicine in Cold Tumor Therapy

3.1. Targeted Delivery and Intelligent Release Mechanism of Nanocarriers

The core principle of nanomedicine is to utilize the size effect of nanoparticles (1-100 nm) and surface functionalization modification to achieve precise drug delivery [6,8]. The dense stroma and abnormal vascular structure of cold tumors limit the penetration of conventional drugs, while nanocarriers can be enriched in the tumor by passive targeting (EPR effect). The large vascular endothelial gap (100-800 nm) and lack of lymphatic drainage in tumor tissues make nanoparticles more likely to be retained [7]. For example, liposomal or polymeric nanoparticles (~100 nm in diameter) can accumulate 10-30 times higher in pancreatic cancer than in normal tissues through the EPR effect [18].

To further enhance the targeting efficiency, the surface of nanoparticles can be modified with active targeting molecules (e.g., antibodies, peptides) that specifically recognize tumor cell or immune cell surface markers [7]. Nanoparticles targeting PD-L1 can precisely bind to tumor cells with high PD-L1 expression while delivering the loaded drug to TAMs to reverse their immunosuppressive phenotype [6]. In addition, the nanocarriers can be designed to be microenvironment-responsive, triggering drug release in the presence of tumor acidic environment (pH 6.5-6.9), high concentrations of enzymes (e.g., MMP-2), or external stimuli (light, heat) to reduce systemic exposure toxicity [15].

3.2. Multi-mechanism Synergistic Remodeling of The Immune Microenvironment

While immune escape from cold tumors involves multiple barriers and single therapies are often difficult to be effective, nanomedicine can achieve synergistic treatment through multi-component co-loading and combined physico-chemical strategies [2,14]. Liposomes can simultaneously harbor chemotherapeutic drugs (e.g., gemcitabine), STING pathway agonists (e.g., cGAMP), and light-heat conversion materials (e.g., gold nanorods) [9]. Chemotherapeutic agents directly kill tumor cells and release antigens; STING agonists activate DCs and enhance antigen presentation [11]; and near-infrared light irradiation of gold nanorods generates localized hyperthermia (42-45°C), which induces immunogenic cell death (ICD) of tumor cells, further releases tumor antigens and recruits T cells. This tri-modal synergy of “chemotherapy-immune activation-physical therapy” can significantly enhance the immunogenicity of cold tumors [14].

3.3. Breaking Through the Physical and Metabolic Barriers of Cold Tumors

The dense fibrotic stroma and metabolically inhibited microenvironment of cold tumors are the main barriers to immune cell infiltration, in which the physical barrier of 60% collagen and metabolic abnormalities, such as lactic acid buildup, limit the therapeutic response. Nanomedicine breaks through these barriers through innovative design. In terms of matrix penetration, nanoparticles with surface-modified hyaluronidase- or matrix metalloproteinases (MMPs)-responsive shells can target collagen degradation and significantly reduce matrix stiffness. Polymeric nanoparticles carrying MMP-2-sensitive peptides increased the depth of tumor penetration threefold in a pancreatic cancer model, creating spatial channels for drug delivery. In response to metabolic suppression, nanoparticles containing IDO inhibitors or targeting lactate transporter protein (MCT-4) remodeled the immune microenvironment, and studies have shown that such delivery systems reduce levels of tryptophan metabolites (kynurenine) in the TME by up to 80%, thereby reversing suppression of T-cell function and restoring anti-tumor immune activity. These strategies work synergistically to

weaken the physical and metabolic barriers to cold tumors, setting the stage for subsequent immune activation.

Nanomedicine provides a revolutionary tool for cold tumor therapy through targeted delivery, multi-mechanism synergy, and barrier-breaking strategies [8,19]. Its core advantages lie in precision, low toxicity and efficient synergy, and in the future, combined with gene editing and personalized design, it is expected to completely reverse the immunosuppressive fate of cold tumors.

4. Assessment of The Potential For Nanomedicine Applications

4.1. Lipid Carriers

With its biocompatible and multifunctional design, lipid carriers have demonstrated unique applications in cold tumor therapy. the JOT-Lip system developed by Zhang's team successfully achieved cold TME-specific targeting by coupling Tim-3 monoclonal antibody via an MMP-2-sensitive linker, encapsulating oxaliplatin (OXA) and the STING agonist, cGAMP [2]. In vitro experiments demonstrated that the vector showed a 2.5-fold increase in uptake efficiency in B16F10 melanoma cells compared to unmodified liposomes ($P<0.001$) and significantly induced the release of DAMPs (HMGB1, ATP) and activated antigen presentation [9]. In a B16-OVA melanoma mouse model, the JOT-Lip treatment group showed a 73% reduction in tumor volume and a 4.8-fold increase in the density of intratumoral CD8+ T-cell infiltration, confirming that it remodels the immune microenvironment by blocking Tim-3-mediated T-cell depletion [2]. Nevertheless, the clinical translation of lipid carriers still faces challenges. although Cheng's team's TT3 lipid nanoparticles achieved 95% mRNA transfection efficiency in vitro and a 12-fold increase in IFN-γ levels and 89% tumor inhibition rate in mice with hepatocellular carcinoma, only 5% of the dose reaches the tumor site after intravenous injection and cationic lipids may trigger toxicity related to complement activation [9]. To address these issues, current research focuses on intelligent design (e.g., MMP-2-sensitive linkers) and combination therapies (e.g., combined radiotherapy to enhance penetration) with the aim of breaking through the delivery efficiency bottleneck [18,19].

4.2. Nanovaccines

Nanovaccines has emerged as a key strategy to reverse cold tumor immunosuppression through precise delivery of tumor antigens and immune adjuvants. The individualized neoantigen mRNA vaccine (LNP package) developed by Sahin's team screened for immunogenic epitopes based on the patient's tumor mutational profile, and induced an 8-fold increase in the expression of dendritic cells (DCs) maturation markers, CD86/CD80, in in vitro experiments and drove the T cell proliferation stimulation index (SI) up to 15.2 [10]. In the KPC mouse model of pancreatic cancer, this vaccine in combination with a PD-1 inhibitor prolonged median survival from 54 to 198 days ($P<0.001$) with a 40% complete remission rate [13]. However, only 15% of patients in their clinical trial (NCT04161755) showed an imaging response, which was mainly limited by the accuracy of neoantigen prediction, with only 1.5% of tumor mutations yielding valid epitopes [10]. To this end, Guo's team designed a STING-activated nanovaccine (PC7A), which achieved a 60% complete remission rate in a TC-1 HPV-associated tumor model by co-loading tumor antigens with STING agonists with no recurrence within 100 days [6]. The core mechanism lies in the activation of the STING pathway within DCs (12-fold increase in the level of IRF3 phosphorylation), but it was noted that this strategy may lead to a 2-3-fold increase in the proportion of Treg cells [7]. Future studies need to incorporate deep learning models (e.g. NeoRanker boosts neoantigen prediction to 82%) or combine IDO inhibitors to balance immune activation and suppression microenvironment [20].

4.3. Other Nanocarriers

In addition to lipid-based carriers and vaccines, innovative designs of metal- and silicon-based nanoparticles provide new ideas for cold tumor therapy. Chen's team [12] developed near-infrared light-responsive gold nanorods (surface-modified with PD-L1 antibody and loaded with IL-2) to

achieve an 80% complete remission rate in B16F10 melanoma mice. The mechanism relies on the photothermal effect to trigger IL-2 release (>90%) and induce immunogenic death, but the long-term accumulation rate of gold nanorods in the liver is as high as 70%, limiting their clinical translation. Similarly, Santos' team's mesoporous silica nanoparticles (PSi) loaded with adriamycin via a ROS-sensitive lipid coating resulted in a 65% reduction in tumor volume and a 50% reduction in cardiotoxicity in an MCF-7 breast cancer model [13]. Despite the highly promising slow-release properties and degradability (no toxic residue in vivo) of PSi, its high cost of production at scale and the proposed strategy of co-delivery of TLR7/9 agonists (10-fold enhancement of CD8+ T-cell infiltration density) further suggests that combining physical therapies (e.g., photothermal) with immunomodulators may be a key direction to break through the limitations of the material [3,14].

Nanomedicine has demonstrated the triple potential of targeted delivery, immune activation and physical modulation in cold tumor therapy through the synergistic design of lipid carriers, vaccines and multifunctional nanoparticles. The TME responsiveness of JOT-Lip, the neoantigenic specificity of individualized mRNA vaccines and the photothermal effect of gold nanorods have confirmed their clinical value [2,13], but the delivery efficiency (<5% tumor accumulation), immunosuppressive rebound (Treg upregulation) and material toxicity (hepatic accumulation) remain common challenges [7,9,12]. In the future, a combination of intelligent carrier design (e.g., deep learning to guide neoantigen screening), interdisciplinary technologies (e.g., microfluidic process to optimize encapsulation rate) and clinical data-driven strategies are needed to promote nanomedicine to make a substantial leap from the laboratory to the clinic.

5. Discussion

Nanomedicine provides a breakthrough tool for cold tumor therapy, but its clinical translation still faces the triple challenges of delivery efficiency, immunosuppression and material toxicity. Lipid carriers activate anti-tumor immunity through targeted delivery (e.g., 73% tumor shrinkage with JOT-Lip) [2] and gene modulation (89% tumor suppression with TT3 liposomes) [9], but only 5% of the dose reaches the tumor after intravenous injection and cationic lipids may trigger complement activation [12]. Nanovaccines enhance T-cell response through precise presentation of neoantigens and STING pathway activation, but increased Treg cell ratio weakens efficacy. Metal/silicon based carriers break through the barrier through physical effects, but liver accumulation and process cost limit the application.

To address these challenges, future research needs to integrate the strengths of multiple disciplines, including materials science, AI and immunology. Reduce long-term toxicity through degradable PSi carriers, combine with deep learning models to accurately screen new antigens, or optimize liposome encapsulation rate to 95% using microfluidics. For combination strategies, photothermal therapy enhances drug penetration, while IDO inhibitors reverse Treg-mediated immunosuppression. Clinical translation requires designing individualized regimens based on existing data and developing a "diagnosis-delivery-monitoring" closed-loop system, which will ultimately increase the cold tumor response rate to more than 50%.

6. Conclusion

Cold tumors have become a core challenge in the field of tumor immunotherapy due to their unique immune escape mechanism and complex microenvironmental characteristics. This paper systematically analyzed the core mechanisms of immune escape in cold tumors, including aberrant activation of PD-L1/CTLA-4 signaling pathway driving T cell depletion, metabolic reprogramming (e.g., lactic acid accumulation, tryptophan depletion) inhibiting immune cell function, and dense stromal barrier and epigenetic regulation further weakening immune recognition and response. In response to the above mechanisms, nanomedicine has demonstrated significant potential through targeted delivery, microenvironment-responsive release, and multi-mechanism synergistic strategies.

For example, lipid carrier-mediated STING agonist delivery, individualized mRNA vaccines, and photo-thermal-responsive nanoparticles have been used in preclinical models to achieve tumor volume reduction, prolonged survival, and immune micromodification.

The significance of this study is that it provides a systematic framework from mechanism analysis to technology implementation for cold tumor therapy. By integrating the results of nanomedicine with immunology and material science, this paper not only reveals new targets for cold tumor therapy (e.g., metabolic regulation, matrix degradation), but also proposes a synergistic therapeutic paradigm of "precise delivery-immune activation-metabolism intervention", which lays a theoretical foundation for the development of highly efficient and low-toxicity nanomedicines in the future. In addition, the in-depth analysis of clinical translation bottlenecks (e.g., delivery efficiency, immunosuppressive rebound) provides a clear direction for optimizing carrier design and balancing efficacy and safety.

However, existing studies still have limitations. First, the targeting efficiency of nanomedicines has not yet fully broken through the limitations of physical barriers and tumor heterogeneity; second, the efficacy validation of most strategies relies on mouse models and lacks the support of large-scale clinical data; and lastly, the long-term toxicity of nanomaterials and the dynamic modulation of the immune microenvironment are still insufficiently investigated. Future research may benefit from exploration in the following directions. First, to further optimize the design of intelligent carriers (e.g., degradable materials or external field-responsive nanoparticles), and try to enhance the targeting efficiency by dynamically regulating the drug release; second, to combine AI and multi-omics technology to gradually improve the matching accuracy between neoantigen prediction and individualized therapy; third, to strengthen interdisciplinary collaboration, seek potential synergistic effects in emerging fields such as gene editing and microbiome intervention, and gradually verify the feasibility of multimodality therapy systems. modality therapeutic system feasibility. Although nanomedicine shows certain potential in cold tumor treatment, its clinical translation still requires long-term and systematic research support. Through continuous accumulation of basic research and feedback of clinical data, we may be able to understand the interaction between nanomaterials and the immune microenvironment more comprehensively, and thus make progressive breakthroughs in the balance between safety and efficacy.

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