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REVIEW

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A strategy for synergistic enhancement of immune circulation in head and neck squamous cell carcinoma by novel nucleic acid drug therapy and immunotherapy

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Abstract

Studies have shown that in the pathogenesis of head and neck squamous cell carcinoma, immune circulation obstruction caused by various factors including metabolic abnormalities, gene mutations, and matrix barrier, is a critical factor for the induction of tumor development and progression. Therefore, the immunotherapy strategy of killing head and neck squamous cell carcinoma cells by an enhanced immune circulation mechanism has attracted much attention. In addition, the rapid development of new nucleic acid drug therapy, such as mRNA, oligonucleotide and small guide RNA (sgRNA), has taken immunotherapy of head and neck squamous cell carcinoma (immune checkpoint inhibitors, tumor vaccines, cellular immunotherapy, cytokines and adjuvants, etc.) to a new level. The combination of nucleic acid therapy with immunotherapy developed for its therapeutic properties has brought a new direction for the diagnosis and treatment of head and neck squamous cell carcinoma, and the combination of the two has had considerable curative effect to patients with refractory/recurrent head and neck squamous cell carcinoma. In this review, we summarized the latest progress of nucleic acid therapy applied to conventional immunotherapy for head and neck squamous cell carcinoma, discussed its mechanism of action and efficacy, and looked into the future development trend.

Introduction

Head and neck squamous cell carcinoma (HNSCC) is a tumor originating from the epithelial tissues of head and neck sites, including oral cavity, throat, nasal cavity and larynx, with a very high degree of malignancy, and is usually closely related to smoking, excessive alcohol consumption and human papilloma virus (HPV) infection [1–3]. Its early symptoms are insidious, mostly manifested as erythema and leukoplakia of oral mucosa or multiple neck masses of unknown origin, and in clinical practice, there is often an awkward situation of "late detection and late treatment" [4–6]. According to the latest statistics, the five-year survival rate of patients with advanced HNSCC is usually between 30% and 50% [7],

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while the five-year survival rate of patients with R/M HNSCC (recurrent/metastatic HNSCC) is even less than 10% [7, 8], which is the top priority in the current treatment of head and neck tumors. The mainstream treatment modalities include surgical therapy, radiotherapy, chemotherapy, targeted therapy and immunotherapy [9, 10]. Among them, surgical therapy can achieve a cure rate of 70–90% for early HNSCC [11]. However, due to the extensive invasion of the surrounding important organs or tissues by advanced HNSCC, surgical therapy is usually unable to completely remove the tumor foci, and will lead to the impairment of normal physiological functions of organs or tissues and serious postoperative complications instead [12, 13]. In addition, radiotherapy, chemotherapy and targeted therapy are the most important treatment modalities for advanced HNSCC [14, 15]. However, the severe toxic and side effects of radiochemotherapy, poor tolerance of some patients and chemoradiotherapy resistance due to long-term treatment will seriously affect the therapeutic effect, and lead to the recurrent/metastatic transformation of head and neck tumors [16, 17]. For example, some targeted drugs, such as cetuximab, can prolong the Progression Free Survival (PFS) and Overall Survival (OS), but the response rate for R/M HNSCC patients is still low [18, 19].

In order to address the above-mentioned issues in the clinical treatment of HNSCC, immunotherapy, with excellent potential and prospects, has gradually become a new benchmark in the treatment of advanced and refractory/recurrent HNSCC [13, 20]. At present, immunotherapy for head and neck tumors is mainly divided into four directions: immune checkpoint inhibitors (PD-1/PDL1, CTLA4 inhibitors, etc.) [21], tumor vaccines (whole cell vaccines, antibody tumor vaccines, etc.) [22], cellular immunotherapy (CAR-T, CAR-NK, etc.) [23–25], and cytokine and adjuvant therapy (IL-2, IFN-γ, etc.) [25, 26]. In addition, immunotherapies, in essence, are designed to address the immune circulation obstruction caused by various factors including metabolic abnormalities [27], gene mutations [28], and matrix barrier by activating, enhancing or regulating the immune cycle, so as to enhance the immune response to tumors. The theory of "cancer-immunity cycle" also emphasizes the iterative enhancement effect in the complete immune cycle: that is, any link can have an amplified effect on the next link, and form a complete "closed loop system", thus greatly enhancing the tumor killing ability and the effect of the entire immunotherapy, and even the complete remission of tumors may be achieved [29, 30].

Conventional immunotherapies, such as tumor vaccine and CAR-T cell therapy, has significant effect in certain specific tumors with definite etiology (such as HPV-positive HNSCC), but has limited effect in the treatment of R/M HNSCC [22, 31]. This is because the single HNSCC immunotherapy often can not construct an efficient "closed loop system" to completely kill the HNSCC cells, but make the residual tumor cells with weak immunogenicity produce "immunoediting" effect, and eventually lead to immune escape or tolerance [32, 33]. Moreover, the high heterogeneity of R/M HNSCC and the immunosuppression induced by multiple mechanisms lead to the lack of universality of single conventional immunotherapies, making it difficult to cope with tumor immune escape, and there is also a lack of effective biomarkers to predict the effect of immunotherapy [34]. It has also been found in clinical studies that conventional single immunotherapies are often faced with such challenges as limited clinical response rate, immune tolerance, high resistance rate and frequent immune-related adverse events [35]. For example, when PD-1/PD-L1 inhibitors are used alone, some immune checkpoint molecules, such as CTLA4, LAG-3 or TIM-3, are often up-regulated, providing additional immune escape pathways for head and neck tumor cells [36, 37]. In addition, treatment with tumor vaccines in cases of head and neck squamous cell carcinoma often activates immunosuppressive cells and induces depletion or dysfunction of T cells [38, 39]. Therefore, with the development of new nucleic acid drug therapies, such as mRNA (mRNA vaccine, mRNA drug, etc.), oligonucleotide (siRNA, miRNA, ASO, etc.) and sgRNA, their therapeutic efficiency and flexibility of combination provide a new treatment strategy for traditional immunotherapy of head and neck squamous cell carcinoma [40–42]. The application of these nucleic acid drug therapies in combination with the above immunotherapy strategies not only solved the problems of resistance to some drugs and systemic toxicity, but also better promoted the process of "cancer-immunity cycle" and achieved considerable antitumor effects. In addition, LNP (Lipid Nanoparticle)-mRNA recently studied by our scientific research team activated endogenous CAR-T cells and dendritic cells (DCs) for the treatment of HNSCC, respectively (Fig. 1).

In this review, we will summarize the application of these novel nucleic acid drug therapies in combination with different immunotherapies for HNSCC, and explore how to construct a complete closed-loop pathway of "tumor immune cycle" to enhance the iterative effect of tumor immunotherapy and achieve efficient inhibition of head and neck squamous cell carcinoma. At the same time, we look forward to the development trend of basic and clinical studies in the future, hoping to provide better therapeutic strategies for HNSCC patients and bring more promising curative effects.

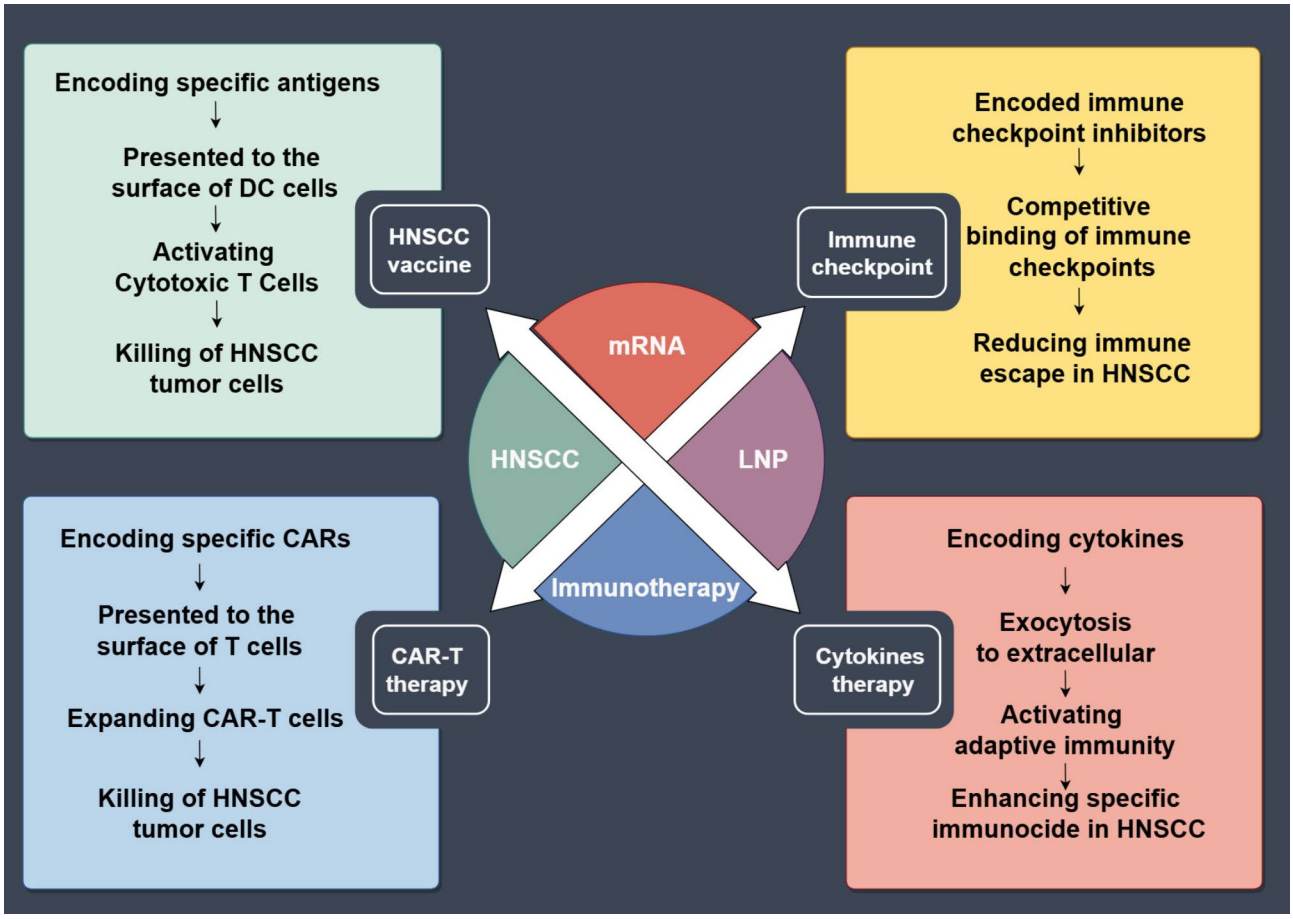

Fig. 1 Immunotherapy of HNSCC Using LNP Encapsulated Target mRNA. It is mainly embodied in the four aspects: coding personalized tumor vaccine, CAR-T cell therapy, immune checkpoint inhibitors, cytokines and adjuvant

Application of mRNA drug therapy in conventional immunotherapy of head and neck squamous cell carcinoma

The mRNA therapy is a novel type of biomedical therapy that uses messenger RNA (mRNA) to induce cells to produce therapeutic proteins [43] (Fig. 2). Studies have shown that various clinical applications, including those of tumor vaccines, gene editing and protein therapy, could be achieved by delivering mRNA expressing cancer antigens, gene editing components or disease-related therapeutic proteins [44–47]. The application of mRNA technology in the conventional immunotherapy of HNSCC is more prominent in the fields of tumor vaccine, cellular immunity, coding cytokine and adjuvant.

Application of mRNA therapy to HNSCC vaccines

There are many difficulties in the research and development of HNSCC vaccines, such as weak expression of tumor antigens, phenotypic and intratumoral heterogeneity, immunosuppressive microenvironment, and lack of effective vaccine delivery system [48, 49]. However, mRNA therapy can effectively addresses the problems of a low tumor surface antigen expression level in the research and development of HNSCC vaccines [22, 50]. For example, mRNA encoding specific tumor antigen information can be delivered to tumor cells or dendritic cells, which can effectively improve the expression level of antigen and activate more specific CD8 + T cells [22, 44, 51]. In addition, by designing mRNA sequences of different antigen information for joint delivery, the problem of tumor heterogeneity can be well addressed. At the same time, the development of mRNA therapy has also promoted the rapid development of vaccine delivery vectors such as lipid nanoparticles (LNP) and extracellular vesicles (EVs). The advantage of mRNA vaccine compared with polypeptide vaccine is that the antigen expressed by it can be presented by normal cells in the body, and will not be weakened by tumor cells in the way of downregulating MHC (Major Histocompatibility Complex), which effectively evades the immune escape mechanism of head and neck tumors [52]. In addition, mRNA vaccines encode peptide antigens in DCs and present them on the surface of DCs to activate a large number of effector and memory T cells, which

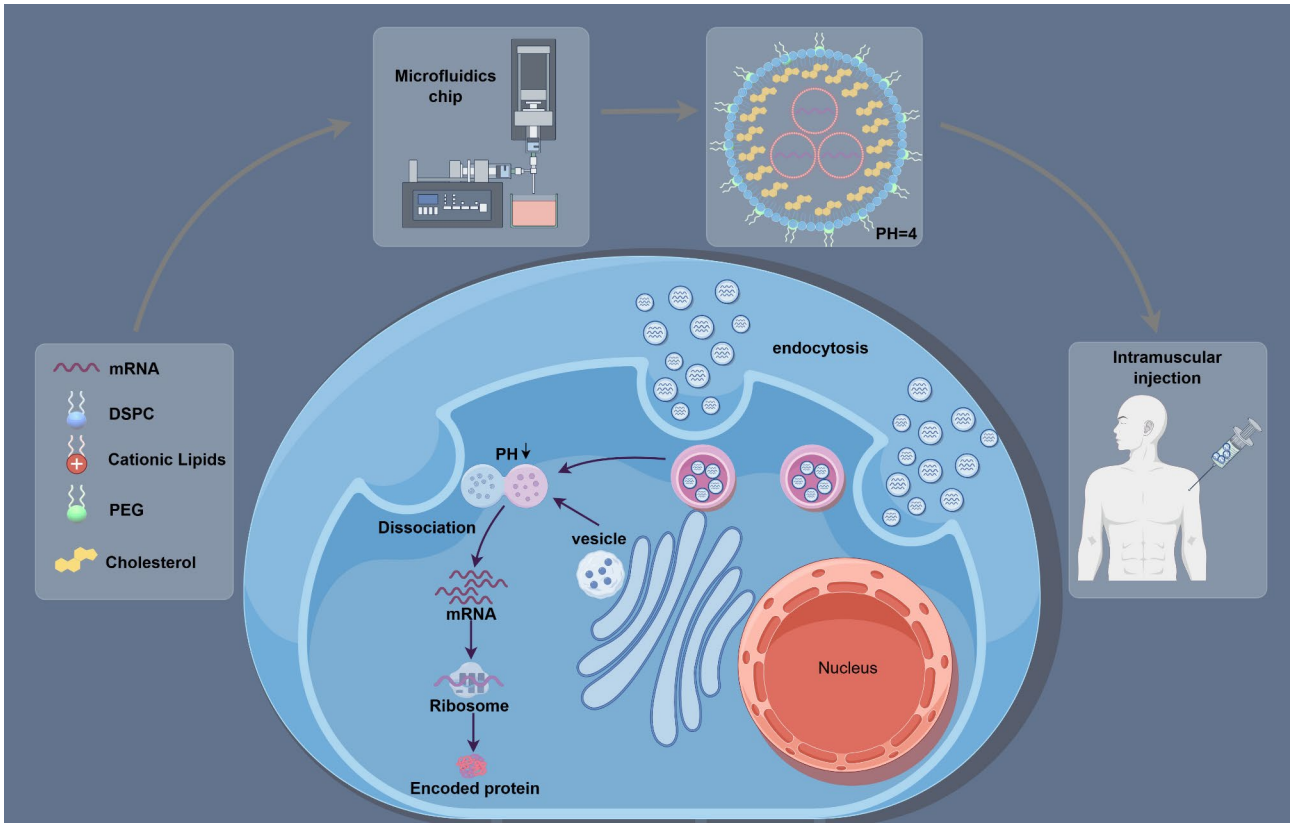

Fig. 2 mRNA Therapy. The delivery carrier for mRNA is composed of four lipid components: ionizable cationic lipids (Cationic Lipids), phospholipids (DSPC), cholesterol, and PEG-lipids (PEG). Under acidic conditions (PH = 4), cationic lipids can be protonated and bind to negatively charged mRNA by electrostatic interaction to form lipid nanoparticles (LNPs) loaded with mRNA. Based on the ratio of 6:1 nitrogen to phosphorus, the formulation is put into the Microfluidics chip, and the lipid solution and the mRNA solution are allowed to form LNP with uniform particle size in the micro-mixer sufficiently and rapidly. The mRNA can be injected subcutaneously or intramuscularly for better efficacy and delivered in vivo to the target cells (e.g., APC cells or tumor cells). With the help of LNP, mRNA enters cytoplasm through endocytosis, lysosomal escape and mRNA release, and initiates translation of target protein after binding to ribosome

also solves the problem of establishing immune memory in the development of HNSCC vaccines [52, 53]. There are several HNSCC vaccines on the market that have entered clinical trials. For example, the mRNA vaccines for HPV16-related HNSCC developed by Moderna, USA has entered the phase 3 clinical trial, which mainly uses mRNA encoding E6/E7 protein (HPV major pathogenic protein) to stimulate immune response and kill HPV-positive related HNSCC [54]. Hpv-negative HNSCC is a huge challenge in the field of vaccine therapy, so the identification of tumor antigens and immune subtypes in head and neck squamous cell carcinoma to customize personalized mRNA vaccines is currently a hot treatment [55, 56]. For example, in a phase I clinical trial of a new personalized cancer vaccine, mRNA-4157, the disease control rate against solid tumors such as head and neck squamous cell carcinoma was as high as 90% [57, 58]。.

Application of mRNA therapy to cellular immunity, cytokines and adjuvants

Cellular immunotherapy mainly includes several main therapeutic types such as CAR-T (Chimeric Antigen Receptor T), iNKT (invariant Natural Killer T), CIK (Cytokine Induced Killer) and DC-based immunotherapy, and mRNA therapy has been applied in some of these therapeutic areas [59, 60]. Among them, CAR-T cell therapy, especially in the treatment of hematoma, is considered to have great potential in the treatment of head and neck tumors [61]. However, the complex preparation process and high cost of CAR-T cell therapy makes its popularization and application difficult. Moreover, due to the lack of specific T cell receptor (TCR) in the treatment of solid tumors, the efficacy is often not recognized [62]. For example, in the treatment of head and neck tumors, once enter the chronic confrontation phase of the tumor, the resulting immunosuppressive microenvironment and other factors often lead to the depletion of CAR-T cells, making it difficult to maintain the therapeutic effect [63]. The mRNA-encoded CAR-T cell

therapy has the advantages of favorable safety and rapid preparation, and is expected to solve the limitations of conventional CAR-T and promote the rapid development of CAR-T technology. At present, there have been successful cases of mRNA-encoded CAR-T cells targeted therapy for solid tumors on the market, paving the way for the treatment of CAR-T + mRNA vaccine for HNSCC. For example, BioNTech company in the United States used mRNA encoding antigen targeted by CAR T cells in a study, so that antigen presenting cells in lymph nodes expressed antigen targeted by CAR T cells to stimulate the expansion of CAR T cells, and the final tumor control rate reached 86% [64].

Cytokines and adjuvants encoded by mRNA technology have been proved to be effective in many solid tumors, including HNSCC. However, they are usually used in combination with tumor vaccines or cellular immunotherapy because of the severe immune stress induced by the therapeutic doses required for their use alone [65]. For example, in head and neck cancer cells, mRNA encoding survivin and IL12 was introduced by electroporation into DC activated survivin-specific T cells, which not only enhanced the activity and proliferation of survivin-specific T cells, but also activated a stronger innate immune response, and achieved excellent effectiveness in the treatment of HNSCC [66].

Strategies, limitations, and challenges of mRNA therapy for cancer

The potential of mRNA therapy in the treatment of malignant tumors is extensive. With technological advancements, therapeutic strategies have been significantly refined. Currently, the strategies endorsed by the academic community in preclinical or clinical trials primarily encompass nanoparticle delivery systems, targeted ligand modification, localized injection, cell-mediated delivery, and stimuli-responsive delivery systems. The development of stable nanoparticle delivery systems, such as lipid nanoparticles, safeguards mRNA from degradation and facilitates its accumulation at the tumor site via enhanced permeation and retention (EPR) effects. Additionally, targeting ligands (e.g., antibodies or peptides) can be conjugated to the surface of nanoparticles to enhance their specificity at the tumor site, thereby increasing nanoparticle enrichment and reducing systemic toxicity associated with off-target mRNA effects. For instance, a study utilizing nanoparticles to treat aggressive orthotopic glioblastoma demonstrated that intraperitoneal injection of EGFR-targeting sgPLK1-cLNPs achieved up to 80% efficacy in vivo, markedly inhibiting tumor growth and improving survival rates by 80% [67]. However, a limitation of this approach is that most solid tumors lack suitable ligand receptors on their cell surfaces, which limits the effectiveness of targeted therapies. In the context of skin cancer and certain superficial solid tumors, the approach of local injection (such as intratumoral injection) of mRNA drugs allows for the direct administration of the mRNA delivery system into the tumor site. This method can circumvent systemic toxicity while significantly increasing the local concentration of mRNA within the tumor, thereby enhancing therapeutic efficacy. In a clinical trial evaluating the intratumoral injection of mRNA-2416 for advanced solid tumors, including ovarian cancer, head and neck squamous cell carcinoma, and lymphoma, most patients experienced tumor shrinkage and disease stabilization with minimal side effects [68]. However, this local injection strategy has limitations, particularly for deep-seated tumors such as pancreatic or brain tumors, or those with metastasis. Additionally, well-vascularized tumor tissues may be prone to drug diffusion, and accurate positioning and technical proficiency are essential for successful local injections. Cell-mediated delivery strategies leverage the natural homing ability of immune cells (e.g., T cells, macrophages) or stem cells to migrate to tumor tissue via the bloodstream, utilizing their interaction with the tumor microenvironment to achieve targeted delivery [69]. Stimulus-responsive delivery systems, akin to targeted ligand modifications on the surface of nanoparticles, leverage the distinctive attributes of the tumor microenvironment, including acidic conditions (low pH), high reducibility, overexpression of specific enzymes (e.g., matrix metalloproteinases), and hypoxic conditions. By engineering carrier materials that are responsive to these stimuli, precise and targeted release of mRNA can be achieved. For instance, pH-sensitive nanoparticles will disassociate and release mRNA in the acidic tumor milieu [70], whereas enzyme-responsive vectors will disassemble and liberate their mRNA cargo upon encountering specific enzymatic activities [71].

The aforementioned strategies for mRNA therapy in the treatment of malignant tumors encounter several limitations and challenges that restrict their clinical application. For instance, the significant objective heterogeneity of tumors poses a major obstacle for mRNA therapy. Tumor cells exhibit substantial variability in gene expression, phenotype, and microenvironment, leading to potential inadequacies in delivery systems that fail to target al.l tumor cells, particularly in the context of mRNA vaccines. Specifically, CD8 + T cells activated by mRNA delivered to antigen-presenting cells may not eliminate all tumor cells, thereby diminishing therapeutic efficacy [72]. Additionally, low delivery efficiency is a critical issue. mRNA is prone to nuclease degradation in vivo and encounters difficulties in penetrating cell membranes, which hampers its effective delivery and expression [73]. Moreover, the immunogenicity of both the mRNA itself and its delivery vectors can elicit an immune

response, causing inflammation or rapid clearance of the delivery system by the immune system, further reducing efficacy [73]. Safety concerns, especially with systemic administration, as well as toxicity or side effects due to off-target mRNA effects, are also notable limitations [74]. From an economic standpoint, the complexity and high cost associated with the preparation process of mRNA therapy further limit its widespread clinical application. Addressing these limitations will require ongoing technological innovation and interdisciplinary collaboration to facilitate the practical implementation of mRNA therapy in cancer treatment.

Application of oligonucleotide drug therapy in conventional immunotherapy of head and neck squamous cell carcinoma

The oligonucleotide drug, as a novel nucleic acid drug, has become a hot spot in recent years. It regulates gene expression by binding and degrading target mRNA, including small interfering (si) RNA, microRNA (miRNA), antisense oligonucleotides (ASOs), CpG oligonucleotides, aptamers, and ribozymes [75]. The first four nucleic acid drugs are widely used in the field of HNSCC immunotherapy, and provide excellent therapeutic options for the treatment of HNSCC. However, aptamers are mainly used as sensitive and selective biomarkers for early diagnosis and detection of HNSCC, as well as targeting and inhibiting key proteins in the progression of HNSCC, such as EGFR, vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs), etc [76]. And then ribozymes are often used to couple with carriers targeting HNSCC cells (such as nanoparticles), which are internalized by tumor cells to degrade cellular DNA, and are used in combination with radiochemotherapy to induce apoptosis of tumor cells and enhance the killing effect on HNSCC cells [77]. In addition, ribozymes, as an important part of CRISPR/Cas9 technology, have also been used in gene editing therapy of HNSCC.

SiRNA drug therapy

Small interfering RNA (siRNA) therapy, which is based on the mechanism of RNA interference (RNAi), has been widely used in immunotherapy of HNSCC. siRNA is a double-stranded RNA molecule typically generated through the enzymatic cleavage of exogenous double-stranded RNA by Dicer. Unlike miRNA therapy, which induces mRNA degradation via partial complementary pairing, siRNA can achieve complete complementarity with target mRNA, thereby guiding the RNA-induced silencing complex (RISC) to specifically degrade the target mRNA. Consequently, siRNA exhibits higher specificity in gene regulation. By selectively degrading the mRNA of critical genes, siRNA can precisely silence the regulatory genes involved in proliferation, survival, and metastasis signaling pathways within tumor cells [78]. Therefore, in the treatment of HNSCC, it is possible to specifically design siRNA that pairs with the mRNA sequences of proto-oncogenes or oncogenes to inhibit the expression of proto-oncogenes or oncogenes, playing a role in fighting against tumor development and progression [79]. The application of siRNA therapy in HNSCC immunotherapy mainly focuses on regulating all aspects of the immune response, including the activation of immune cells, the expression of immune checkpoint inhibitory proteins and the control of inflammatory response [80].

Directly or indirectly modulating the expression of immune checkpoint inhibitory proteins (PD-L1 on the tumor cell surface or PD-1 on the immune cell surface) by small interfering RNA (siRNA) therapy is a promising approach for the treatment of HNSCC [81]. Due to the transient nature of siRNA in gene regulation, it is not easy to directly knock down the expression of PDL1 on tumor cells. However, transiently knocking down the expression of PD1 on immune cells through siRNA to activate transient immune equivalents is currently a research hotspot [82]. For instance, in one study, a co-delivery platform was used to deliver chimeric antigen receptor (CAR) mRNA targeting PD-1 and siRNA to ex vivo primary human T cells, and strong CAR expression and PD-1 knockdown were observed, achieving breakthroughs in the treatment of solid tumors including head and neck squamous cell carcinoma [82]. At present, indirect regulation of immune checkpoint inhibitory proteins is more widely used to treat HNSCC, because the expression of many genes in HNSCC can directly or indirectly affect the expression of immune inhibitory proteins in tumor or immune cells, such as HFG, STAT1, TRIM24 and IFN-g signal transduction related genes [83]. For example, in a study using siRNA to modulate PD-L1 expression, the study personnel detected total proteins and phosphorylated STAT1, STAT3, and p65 proteins by Western blotting in HNSCC with PD-L1 + TME (Tumor microenvironment) and knocked down the genes encoding these proteins with siRNA [81]. PD-L1 cell surface proteins and mRNA were also detected by flow cytometry and real time fluorogenic quantitative PCR, respectively, and the proximal promoter region of PD-L1 (CD274) was sequenced to assess the relationship between these genes and PD-L1 expression [81]. The results showed that after knockdown of STAT1, IFN-g- and IL-27-induced PD-L1 protein expression on tumor cells was significantly inhibited, and the immune escape capability of HNSCC was attenuated [81]. In addition, in another preclinical and clinical finding of HGF (Hepatocyte growth factor) gene-induced PD-L1 expression in head and neck cancer, the study personnel used two different Met-specific siRNA constructs to treat three HNSCC cell lines, Detroit 562,

FaDu and SCC-9, and the results showed that silencing Met-related genes by siRNA regulated HGF/MET signal transduction, which in turn regulated the expression of PD-L1 protein in HNSCC immune checkpoint, thereby solving the problem of immune tolerance in HNSCC [84].

MiRNA pharmacotherapy

MicroRNA (miRNA) therapy represents an emerging nucleic acid-based therapeutic approach that has demonstrated significant potential in complex gene regulation. miRNAs are typically derived from endogenous primary miRNAs (pri-miRNAs) processed by the Drosha and Dicer enzymes, leading to partial complementarity with target mRNAs, which results in mRNA degradation or translational inhibition. Unlike siRNA, which degrades mRNA through fully complementary double-stranded pairing, the mechanism of partial complementary binding of miRNAs allows them to regulate multiple target genes and participate in a more intricate gene regulatory network. Additionally, the single-stranded structure of miRNAs is less likely to be recognized by cells as foreign entities, providing higher stability and making it suitable for long-term gene regulation studies. By targeting a diverse set of cancer-related genes, miRNAs can inhibit tumor cell proliferation and metastasis over extended periods compared to siRNA therapy (for instance, miR-34 has been shown to suppress the growth of various cancer cells) [85]. Due to their ability to target multiple mRNAs, excellent biocompatibility, and low immunogenicity, miRNAs hold great promise in the immunotherapy of head and neck cancer, particularly in adjunctive therapies involving immune cell modulation and the suppression of immunosuppressive proteins within the immune microenvironment [86].

In a study, the regulatory effects of Extracellular Vesicles-miRNAs (EV-miRNAs) on HNSCC microenvironment and immune system were investigated, and EV-miRNAs was used to amplify γ δ T cells in HNSCC microenvironment [87]. In addition, studies have demonstrated that miR-21-rich exosomes derived from OSCC (Oral squamous cell carcinoma) in hypoxic environment enhance the inhibitory function of MDSC (Myeloid-derived suppressor cells) through miR-21-mediated down-regulation of PTEN levels and up-regulation of PD-L1 expression, leading to depletion of γ δ T cells, inhibition of miR-21 exosomes in OSCC, down-regulation of immunosuppressive protein expression, and thus inhibition of tumor [88]. Distinct from siRNA, miRNA possesses the capability of long-term regulation of the degradation of multiple mRNAs. Hence, aside from the aforementioned facilitation of tumor killing by immune cells through modulating the expression of PD1, miRNA has currently been employed in the regulation of PDL1 on the surface of tumor cells. For example, in a study, the let-7 family of microRNAs was exploited to inhibit the immune escape of head and neck squamous cell carcinoma by promoting the degradation of PD-L1 [89].

Antisense oligonucleotide drug therapy

Antisense nucleotide therapy (ASO) is a kind of therapies that use short DNA or RNA molecules to bind to a specific mRNA, block its translation or promote its degradation, thereby reducing or regulating the production of a target protein, and has been applied in the research of head and neck squamous cell carcinoma [90]. This therapy inhibits tumor growth by preventing the transcription and translation of cancer-related genetic information, such as genes involved in cancer growth and metastasis (EGFR, CDCA1, MYC and TWIST1, etc.) and genes involved in tumor angiogenesis (VEGF, FGF and TGF-β, etc.) [91]. Antisense nucleotide therapy (ASO) has also been used in the traditional immunotherapy of head and neck squamous cell carcinoma, mainly in the field of cellular immunotherapy. According to a study, local injection of myeloid cell-targeted STAT3 antisense oligonucleotide (CpG-STAT3ASO) activated human DC/macrophages and promoted CD8 + T cell recruitment, thus preventing UM-SCC1 head and neck tumor growth [91]. Of course, some studies have reported some examples of the use of ASOs as vaccine adjuvants, such as the regulation of regulatory T cells in head and neck tumors, the binding of specific sequences to target RNA, and the regulation of the expression of immunosuppressive proteins through a variety of different mechanisms [92].

CpG oligonucleotide drug therapy

Oligonucleotides containing unmethylated guanine cytosine dinucleotide (CpG ODN) can activate T and B lymphocytes, antigen presenting cells (APC), and induce immune cells to produce Th1-type inflammatory cytokines such as IFN-mediated [93, 94]. CpG ODN has the ability to activate innate and adaptive immune responses, and has an excellent prospect in adjuvant therapy of head and neck squamous cell carcinoma vaccine. CpG nucleotide therapy in head and neck squamous cell carcinoma immunotherapy is mainly embodied in the research of head and neck squamous cell carcinoma vaccine and nucleic acid vector, for example, 2 nanosheets based on functionalized MoS CpG delivery nanoplatform can enhance anti-tumor immunity of head and neck squamous cell carcinoma, CpG-loaded nanosheets of CpG@M-PL can promote the maturation, antigen presentation ability and proinflammatory cytokine production of bone marrow-derived dendritic cells (DCs) [95]. In addition, DNA methylation is one of the characteristics of early changes in malignant tumors. Therefore, in the study of DNA methylation associated with head and

neck squamous cell carcinoma, different therapeutic methods should be developed based on the functional changes of different gene loci. In addition, in a study on the effect of gossypol acetate on the growth of human tongue squamous cell carcinoma Tca8113 cells and on human mismatch repair genes, the treatment of hMLH1 gene, which is highly methylated, was investigated using CpG nucleotide therapy [96].

sgRNA nucleic acid drug therapy in traditional immunotherapy for head and neck squamous cell carcinoma

sgRNA is one of the core components of the CRISPR/Cas9 gene editing system, which is often designed to direct Cas9 protein to precisely edit target genes. CRISPR/Cas9 is a revolutionary gene editing technology [97]. Because of its high editing efficiency and simple operation, CRISPR/Cas9 is the most widely used gene editing therapy in the treatment of head and neck squamous cell carcinoma [97]. Its principle is to use the CRISPR-Cas9 system derived from bacteria to firstly recognize the target sequence of DNA by small guide RNA (sgRNA), and then use Cas9 nuclease (oligonucleotide drug) to cleave DNA to achieve gene editing (Fig. 3). At present, CRISPR/Cas9 technology provides a broad application prospect for immunotherapy of head and neck squamous cell carcinoma (CAR-T cell therapy) and immune checkpoint gene silencing [98].

While CAR-T therapy is the second most effective and personalized immunotherapy for hematologic malignancies, it lacks an equally effective response rate in solid tumors. The application of CRISPR/Cas9 gene editing technology in CAR-T therapy of solid tumors such as head and neck squamous cell carcinoma solves this problem very well [99]. CAR-T cells express both antigen recognition structures and immune checkpoint antagonists on the surface of CAR-T cells, which can maximize the ability of CAR-T to kill solid tumors. For example, in head and neck squamous cell carcinoma, CAR-T cells with high expression of hyaluronan receptor CD44v6 (a subtype of CD44, which is highly expressed in solid tumors) were constructed by CRISPR/Cas9 technology, which showed excellent killing effect on head and neck squamous cell carcinoma cells with CD44 subtype of hyaluronan receptor, and also proved that the application of immune checkpoint antagonists at the same time had a better tumor remission effect [100].

In tumors with high incidence of immune escape, such as head and neck squamous cell carcinoma, immune checkpoint gene silencing was constructed by using CRISPR/Cas9 technology. For example, the CRISPR/Cas9 system has been used in some studies to precisely remove immunosuppression-related genes on T cells, such as PD-1 and CTLA-4, which enhanced the activity and killing ability of T cells and improved the efficacy of combination therapy for tumors including head and neck squamous cell carcinoma [101]. Furthermore, modification of CD8 T cells with CRISPR/Cas9 technology can delay tumor growth, including HNSCC, and improve survival of HNSCC patients, through simultaneous genetic ablation of the immune checkpoints PD-1, LAG-3, and TIM-3 [101]. For its ligand PD-L1, there are also studies that knock down the expression of PD-L1 and other immunosuppressive molecules on tumor cells and immunosuppressive cells to improve the activity of T cells and reduce the depletion of T cells, so as to provide a supplement for immune checkpoint inhibitors in the treatment of tumors with high incidence of immune escape, such as head and neck squamous cell carcinoma [102]. In addition to directly-targeted editing of PD-1/PD-L1, CRISPR/Cas9 technology has been used to edit genes that indirectly affect PDL-1 expression in the treatment of head and neck squamous cell carcinoma. For example, deletion of Aryl Hydrocarbon Receptor (AhR) in oral head and neck squamous cell carcinoma cells by CRISPR/Cas9 technology results in decreased expression of multiple immune checkpoint immunosuppressive proteins in oral squamous cell carcinoma models, and resulting in the production of fully protective tumor immunity [103].

In addition, the CRISPR/Cas9 system has also contributed to the research and development of HNSCC vaccines, which can enhance the therapeutic effect by constructing targeted nucleic acid vaccine. In some studies, knock-in of tumor antigen genes such as HPV-related genes E6 and E7 into antigen presenting cells (such as dendritic cells) effectively stimulated specific cellular immune response and inhibited tumor growth. Moreover, the expression of MHC class I or MHC class II molecules in HNSCC can be upregulated by targeted editing of some genes by the CRISPR-Cas9 system. For example, the expression of MHC class I molecules on the membrane surface and antigen presentation can be enhanced by CRISPR-Cas9-encoding EZH2-deficient HNSCC cells, while effectively inducing antigen-specific CD8T cell proliferation, IFN γ production and tumor cytotoxicity [104].

Application of DNA peptide vaccines in routine immunotherapy for head and neck squamous cell carcinoma

DNA peptide vaccines represent an extension of nucleic acid drug therapy, integrating the advantages of both DNA coding and peptide-based vaccines. The fundamental principle is to utilize DNA to encode specific antigenic peptides, which are then expressed in vivo to activate the immune system. For instance, DNA peptide vaccines have demonstrated objective efficacy in the newly developed COVID-19 DNA peptide vaccine [105] within the realm of infectious diseases, as well as in personalized

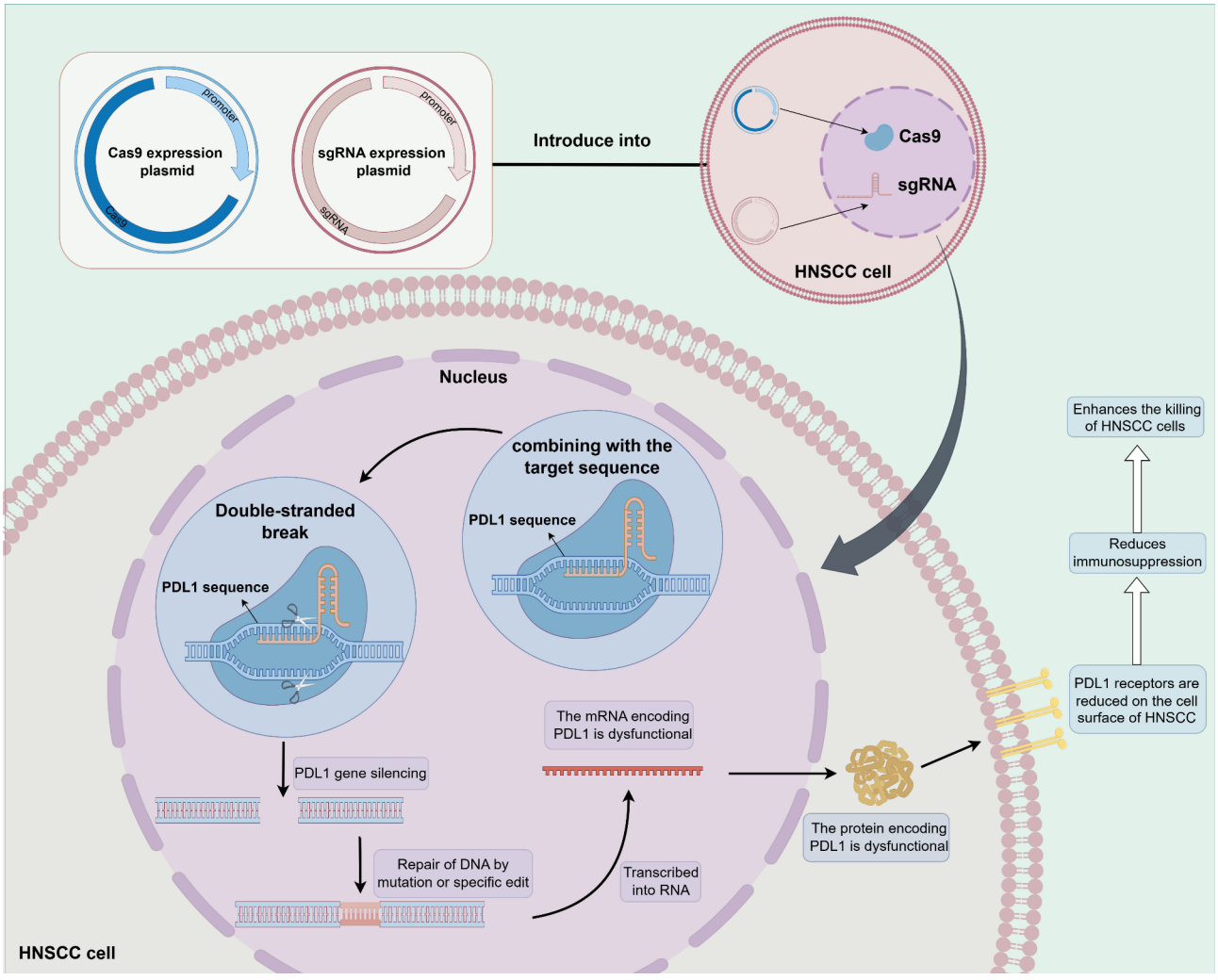

Fig. 3 CRISPR/Cas9 Gene Editing System. The target gene PDL1-specific sgRNA (single guide RNA) sequence is designed, which contains a part that matches the target gene sequence to guide the Cas9 protein localization on the DNA, and a skeleton sequence that binds to the Cas9 protein. Cas9 protein and sgRNA are delivered to HNSCC cells by transfection (plasmid or viral vector), transduction, or microinjection. The sgRNA directs the Cas9 protein to its matching target DNA sequence, and the Cas9 protein cuts the DNA double strand near the target sequence, causing a double strand break. Finally, the broken DNA double strand is repaired by means of non-homologous end joining (NHEJ) or homologous directed repair (HDR). By introducing silencing PDL1 related gene sequences in this way, the expression of PDL1 on the surface of HNSCC cells is inhibited, thereby weakening the immune escape of tumor cells

neoantigen vaccines for solid tumors such as melanoma in oncology [106]. Given their high safety, good stability, comprehensive immune response, and significant potential for personalized treatment, DNA peptide vaccines hold excellent prospects for the immunotherapy of head and neck squamous cell carcinoma (HNSCC). Previous studies on DNA peptide vaccines for HNSCC have primarily concentrated on HPV-associated cases. However, the personalized vaccine treatment for HPV-independent HNSCC remains in its exploratory phase, limited by the feasibility of neoantigen screening and antigen coding. For instance, in a Phase 1/2 clinical trial designated MEDI0457, a DNA vaccine encoding the E6 and E7 proteins of HPV16/18, administered via electroporation, successfully elicited HPV-specific T cell responses [107]. Notably, HPV-independent head and neck squamous cell carcinoma (HNSCC) exhibits higher incidence and recurrence rates, suboptimal treatment outcomes, and increased resistance to chemotherapy. Currently, the development of personalized DNA peptide vaccines for treating HNSCC is an area of significant interest. In a study, targeting the expression of MAGED4B and FJX1 in HNSCC using a DNA peptide vaccine not only controlled tumor growth but also enhanced T-cell infiltration within the tumor microenvironment. When combined with PD-1 checkpoint inhibitors (CPI), this approach significantly improved CPI efficacy, leading to tumor clearance in approximately 75% of mice [108].

Innovation in packaging and delivery systems is crucial for the efficient utilization of nucleic acid therapies

In clinical applications, nucleic acid drugs such as mRNA vaccines, siRNA, and CRISPR gene editing tools are susceptible to degradation in vivo. Consequently, enhancing the overall efficacy of these therapies hinges on developing more stable packaging and delivery systems. Single nucleic acid drugs encounter various in vivo challenges, including rapid serum and renal clearance, low cell membrane penetration efficiency, and inadequate organ targeting. To address these issues, several innovative packaging and delivery methods have emerged, including Lipid Nanoparticles (LNPs), exosome-like Engineered Extracellular Vesicles (eEVs), adeno-associated viral vectors (AAV vectors), and DNA origami. Notably, iterative innovations in lipid nanoparticles (LNPs) have significantly advanced the clinical translation of nucleic acid drugs. For instance, fourth-generation ionizable lipids, such as SM-102, now achieve drug loading efficiencies exceeding 90%. By optimizing the proportion of PEG lipids (from 5 to 1.5%) and incorporating surface ligand modifications (such as GalNAc for hepatocyte targeting or RGD for tumor targeting), LNPs can better promote drug enrichment in specific tissues [109, 110]. Engineered Exosome-like Vesicles (eEVs) integrate the biological properties of natural exosomes with the programmability of artificial carriers, exhibiting lower immunogenicity and enhanced biocompatibility compared to lipid nanoparticles (LNPs). This reduced immunogenicity is attributed to the retention of host cell membrane proteins, such as CD47, on their surface, which facilitates immune evasion. Furthermore, eEVs can be engineered for organ- or cell-specific targeting through surface ligand modifications, including mannose, GalNAc, RVG peptide, among others. For instance, glycan-modified glioblastoma-derived extracellular vesicles (EVs) have demonstrated significantly improved receptor-mediated targeting of dendritic cells [111]. Additionally, adenovirus-based vectors, such as those derived from adeno-associated virus (AAV), leverage the natural infection capability of adenoviruses to deliver nucleic acid drugs more efficiently to tumor or immune cells than LNPs, thereby eliciting a stronger immune response. In a T-engineered tumor study, the targeted delivery of chimeric antigen receptors (CARs) to T cells was achieved using a dual AAV6 transduction system via CRISPR-mediated homology-directed repair, which successfully enhanced CAR expression on T cells [112]. DNA origami leverages the spontaneous assembly of short and long DNA strands into nanoscale structures during thermal cycling. Compared to lipid nanoparticle (LNP) packaging, DNA origami offers a smaller volume (< 100 nm) and enables more precise gene regulation. Furthermore, DNA origami technology can precisely control drug loading and release by designing cavities or binding sites, facilitating the co-delivery of multiple nucleic acid drugs. For instance, in a study addressing multidrug resistance in tumors, DNA origami was utilized to co-deliver two linear small hairpin RNAs (shRNAs) targeting MDR-related genes and controlled-release elements into MCF-7R cells, achieving synergistic inhibition of tumor growth without causing significant systemic toxicity [113].

Strategies for constructing a complete "immunopotentiating circulation" in head and neck squamous cell carcinoma

The complete "immunopotentiating circulation" mainly includes seven core stages, namely antigen recognition and release, antigen presentation, T cell activation, T cell migration, T cell infiltration, T cell recognition and T cell-mediated cytotoxicity, as well as two influential factors, namely regulatory immunosuppression and memory response [114]. It is the key to start highly effective immunotherapy, which shows the positive feedback mechanism in immunotherapy (Fig. 4). This mechanism enhances and optimizes the immune system to recognize and attack the tumor through a variety of ways, forming a continuous and diversified cycle process. Therefore, finding the breakthrough point of new technology combined with immunotherapy, and constructing a complete "immunopotentiating circulation" by analyzing and summarizing the blocking factors in different stages of immune cycle is the development direction of the treatment of head and neck squamous cell carcinoma in the future [114, 115].

Mechanism of immune circulation obstruction in head and neck squamous cell carcinoma

Studies have shown that metabolic abnormalities, gene mutations, immune dysfunction and other factors are the important factors leading to the obstruction of immune circulation in head and neck squamous cell carcinoma [116]. Analysis of the obstruction mechanism of these factors can help us select targeted treatment and formulate new strategies.

Metabolic reprogramming of head and neck squamous cancer cells results in acidification, hypoxia and nutrient deprivation of the tumor microenvironment, thus creating an immunosuppressive microenvironment, and leading to the functional loss of immune cells [117]. According to the "Warbug effect" of tumors, HNSCC cells rely on glycolysis for energy production, while producing large amounts of lactic acid. Once it becomes difficult to effectively excrete lactic acid from tumor tissue, it will lead to the accumulation of lactic acid in tumor, forming an acidic microenvironment, which will further obstruct the immune circulation of HNSCC by inhibiting

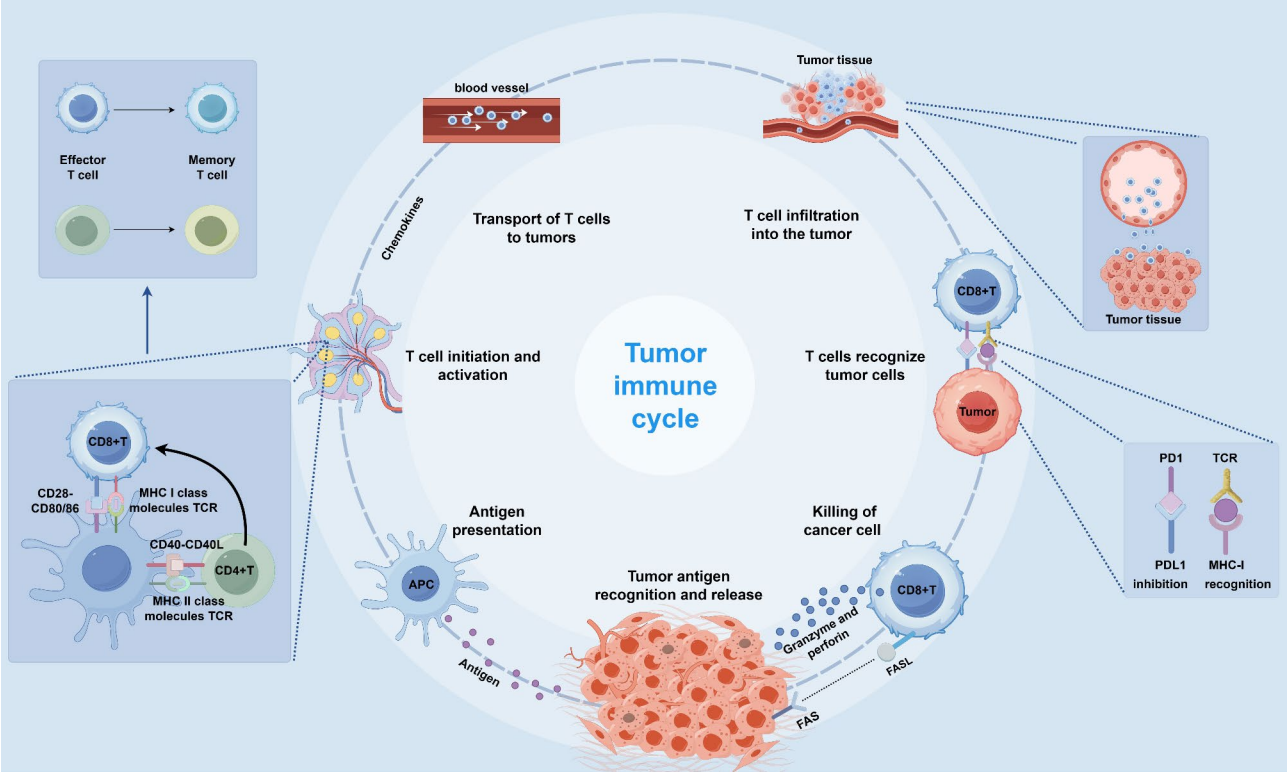

Fig. 4 Schematic illustration of the head and neck tumor immune circulation that effectively kills tumor cells through a multistep physiological mechanism, with sustained and iterative expansion. In the first step, head and neck tumors produce and release neoantigens, which are endocytosed and captured by dendritic cells (DCs), generating anti-tumor T cell responses accompanied by specific immune signals. Next, DCs are delivered to lymph nodes by the lymphatic system, presenting cancer-specific antigens captured by MHC I and MHC II molecules to CD8 + and CD4 + T cells, respectively, leading to the generation and activation of effector T cells. Activated effector T cells are delivered through the blood and infiltrate the tumor bed, specifically recognizing and binding cancer cells through interactions between T cell receptors (TCRs) and MHC I-bound cognate antigens, and killing cancer cells. At the same time, additional tumor-associated antigens are released to continuously increase the breadth and depth of the response in the immune circulation

the activation and infiltration of T cells, impairing T cell cytoskeleton and function, inducing the proliferation of regulatory T cells, and promoting the polarization of tumor-associated macrophages to M2 phenotype [118]. The rapidly proliferating squamous cell carcinoma of head and neck is often in a state of hypoxia, which can induce the expression of HIF-1α, thus promoting angiogenesis, up-regulating the expression of PD-L1 and other immune checkpoint molecules, and promoting the secretion of immunosuppressive factors such as TGF-β and IL-10 [119, 120]. Hypoxia can also alter glycolysis and tricarboxylic acid cycle metabolism, resulting in the production of more lactic acid, which aggravates the accumulation of lactic acid. During the proliferation of HNSCC, nutrients such as sugars, amino acids and fatty acids are greatly consumed, which leads to the lack of nutrients in the tumor microenvironment, thus affecting the proliferation, differentiation and function of T cells, and also leading to the obstruction of dendritic cell maturation and antigen presentation, as well as the decrease of NK cell activity (Fig. 5) [121, 122].

Under the "editorial" pressure of the immune system, head and neck squamous cancer cells gradually acquire the ability to evade immune killing through genetic mutations or phenotypic changes [123]. HNSCC is one of the malignant tumors with the highest gene mutation rate. It interferes with antigen processing and presentation, down-regulates MHC molecules, activates escape pathways, recruits inhibitory cells and blocks interferon signals in various ways, thus destroying the anti-tumor immune monitoring mechanism of the body, which is the key reason for the obstruction of immune circulation [124]. For example, mutations in immune-related genes such as β2-microglobulin and transporter-associated antigen processing protein may affect the processing and loading of tumor-associated antigens onto MHC molecules, thus reducing the immunogenicity of tumor cells [125]. Mutations in genes such as MLH1 can lead to the down-regulation of MHC molecules on the surface of tumor cells, making them escape the recognition and killing ability of cytotoxic T cells [126]. Enhanced mutation of some oncogenes (such as RAS and MYC) can activate downstream signaling pathways and induce

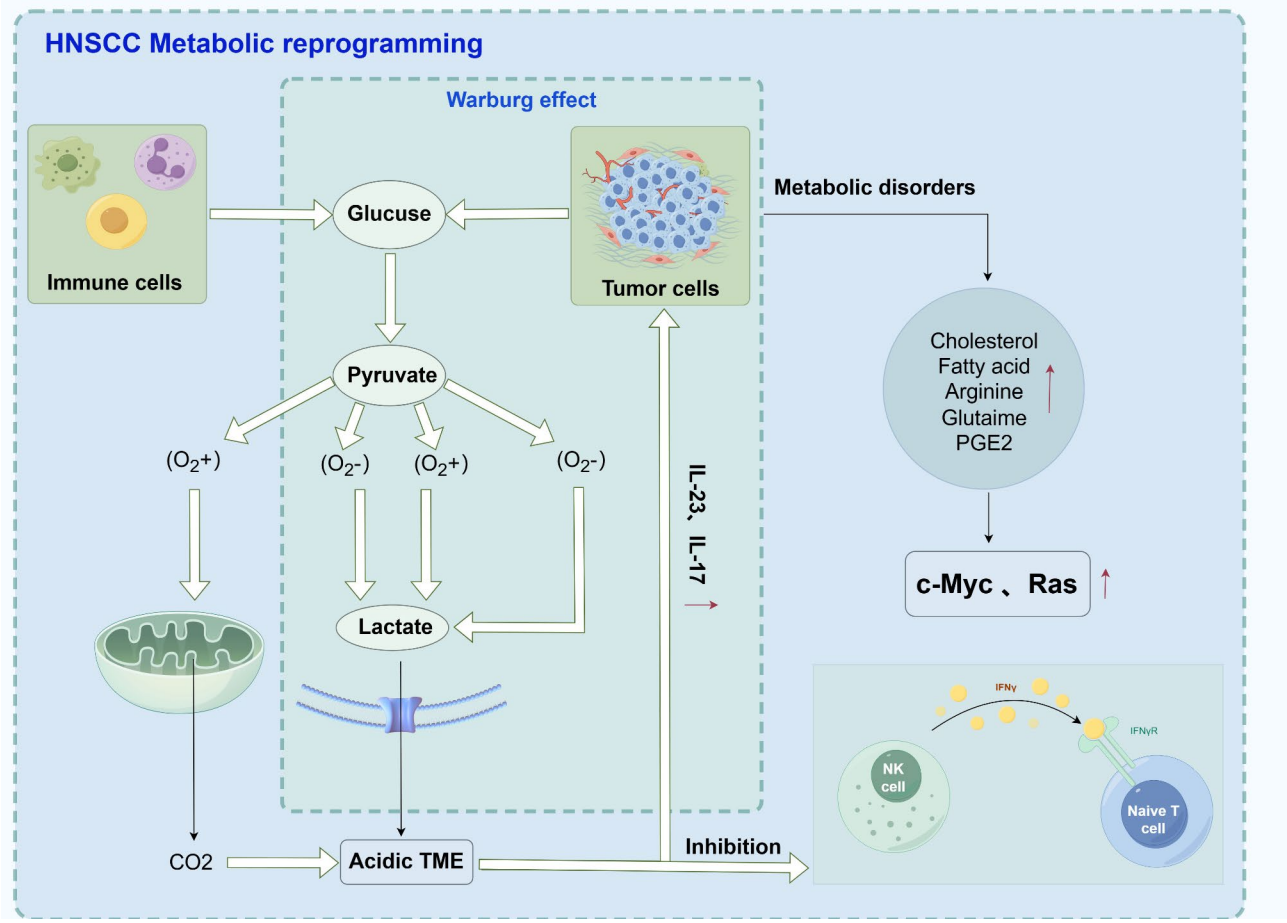

Fig. 5 Warburg Effect of HNSCC. HNSCC cells tend to produce energy by anaerobic glycolysis, rather than by more efficient aerobic respiration, even under oxygen-replete conditions. Due to massive anaerobic glycolysis, tumor cells can produce large amounts of lactic acid, resulting in acidification of the tumor microenvironment. This acidified environment promotes the upregulation of IL-23 and IL-7, thereby promoting an immunosuppressive microenvironment that suppresses immune cell function (e.g., reduces NK cell release of INF-γ and T cell activation) and helps tumor cells evade immune system surveillance. In addition, the high energy demand of head and neck tumor cells can also cause them to compete with immune cells for glucose, resulting in delayed proliferation and growth of immune cells, and aggravated immunosuppressive microenvironment. At the same time, the internal "metabolic reprogramming" of tumor cells starts. In addition to influencing glucose metabolism through Warburg effect, tumor cells readjust the utilization pathway of amino acid (Glutamine), fatty acid and cholesterol, such as increasing the utilization of Glutamine and Arginine, so as to support the synthesis of nucleic acids and proteins to promote tumor growth

up-regulation of immunosuppressive factors (such as PD-L1 and TGF-β), and mutation inactivation of tumor suppressor genes (such as p53 and PTEN) can also lead to up-regulated expression of immunosuppressive factors [127].

Homeostasis of the immune system is the key to anti-tumor immunity, but in patients with HNSCC, immune dysfunction may occur in many aspects of the immune system, thus hindering the effective anti-tumor immune response. Dysfunction of antigen presenting cells, such as dendritic cells, results in inability to efficiently capture, process and present tumor-associated antigens, leading to inability to properly activate T cells. There are many kinds of immunosuppressive factors (such as TGF-β, IL-10, etc.) in the tumor microenvironment caused by immune dysfunction, which can inhibit the activation, proliferation and effector function of T cells, and finally result in the depletion of T cells and lead to the inability to kill tumor cells effectively and continuously [128, 129]. Immune dysfunction also leads to an increase in immunosuppressive cells and high expression of immune checkpoint molecules, such as regulatory T cells (Treg) and bone marrow-derived suppressor cells (MDSC), which can inhibit effective anti-tumor immune response, while tumor cells and tumor-infiltrating immune cells overexpress immune checkpoint molecules such as PD-L1, which can also inhibit T cell activation and effector function [130].

Combination of multiple immunotherapy is key to enhancing immune circulation in head and neck squamous cell carcinoma

With the development of immunotherapy, the efficacy of single immunotherapy is being explored more thoroughly. For example, tumor vaccine therapy, as the most potential therapy for head and neck cancer treatment, utilizes enhanced antigen presentation during the immune cycle, thus enhancing CLT (Cytotoxic T lymphocytes) cell activity and reducing immune evasion. However, with the further study on the mechanism of refractory/relapsed HNSCC, it is not difficult to find that the complex immune microenvironment leads to the limited therapeutic potential of single immunotherapy. Therefore, considering "combined therapy" can break the immune barrier of refractory/relapsed HNSCC, remove obstacles in the immune circulation, and achieve strong immune killing and tumor inhibition [131].

It has been reported that the combination of immunotherapy and conventional therapy is a new trend in cancer therapy, including: immunoduodenal combination, immunotherapy combined with chemotherapy, immunotherapy combined with targeted therapy, immunotherapy combined with radiotherapy [132]. Some of these therapies have been confirmed by preclinical experiments. Specifically, the combination of dual or multiple immunotherapy in HNSCC is the key to constructing a complete immune enhancement cycle. For example, in a phase II clinical study for patients with incurable human papillomavirus 16-related cancer (some were patients with HNSCC), the combination of HNSCC vaccine and immune checkpoint blockade (especially PD-1/PD-L1 blocking drugs) resolved a number of steps, including antigen recognition, antigen presentation, T cell activation and regulatory immunosuppression from the perspective of immune cycle, and resulted in good preclinical efficacy [133].

The combination therapy of head and neck tumor vaccine and cytokine/adjuvant helps enhance the efficacy of head and neck tumor vaccine, which not only activates antigen-related specific immunity, but also enhances adaptive immunity in tumor microenvironment. The full activation of "double immunity" is beneficial to the formation of a complete immune cycle. For example, In a clinical study, the efficacy of a DNA vaccine targeting HPV-16/18 E6/E7 antigens in conjunction with an IL-12 adjuvant (MEDI0457), when combined with a PD-L1 inhibitor, was evaluated. This immunotherapeutic approach was found to augment HPV-specific T cell responses and improve clinical outcomes in patients with recurrent or metastatic HPV-16/18-associated HNSCC [134].

In addition, CAR-T cells have certain advantages for the treatment of recurrent/metastatic head and neck cancer, which can greatly solve the problem of expansion and depletion of CAR-T after combined application with cytokines and checkpoint inhibitors, addressing some difficult problems in the treatment of solid tumors. For example, in a study in which the antitumor activity of HER2-specific CAR-T cells was enhanced by pretreatment of tumors with a binary oncolytic adenovirus (CAd) to locally express immunostimulatory molecules and treatment with a construct encoding a PD-L1 blocking antibody and IL-12p70 (CAd12_PD-L1), such a "triple immunization" therapy was found to improve survival to over 100 days relative to approximately 25 days with either approach alone [135]. These examples provide more research ideas for the difficult field of refractory/recurrent HNSCC, and suggest that therapeutic strategies aimed at constructing a stable and complete "closed loop pathway" of immune circulation in HNSCC can obtain better tumor inhibitory effect.

Novel nucleic acid drugs used in immunotherapy to address the blocked immune circulation in HNSCC

The numerous studies mentioned above have shown that the therapeutic advantages of novel nucleic acid drug therapies can amplify the efficacy of traditional immunotherapy and play the most potential and powerful role in the "immunopotentiating circulation" of HNSCC. In order to construct a more efficient, stable and complete "closed loop pathway" of immune circulation in HNSCC, we believe that the combination of the advantages of novel nucleic acid drug therapy and multiple immunotherapy may be the key to the treatment of refractory/recurrent HNSCC [136]. The synergy of novel nucleic acid drug therapy and multiple immunotherapy does not mean that only one nucleic acid drug therapy and one immunotherapy are paired to study their efficacy and potential. The combination is more flexible, and more consideration needs to be given to how to match them cleverly so that the "closed loop pathway" of immune circulation in HNSCC reflects high efficacy, stability and integrity. Therefore, we come to the hypothesis that the combination therapy strategy of one nucleic acid drug therapy applied to multiple immunotherapy, or the combination therapy strategy of multiple nucleic acid drug therapies applied to different immunotherapies, may be one of the most effective methods to solve the problem of blocked immune circulation.

For example, a recent study using LNP-mRNA for simultaneous coding delivery of tumor cells and DCs for the treatment of refractory/recurrent HNSCC has well validated this hypothesis [137]. In this study, lipid nanoparticles (LNPs) were utilized to deliver mRNA expressing CD40L, inducing immunogenic cell death (ICD) and CD40L expression in tumor tissues. CD40-overexpressed bone-marrow-derived dendritic cells

(CD40-BMDCs) were then constructed using another lipid nanoparticle (LNP) to deliver CD40 mRNA, which were adoptively transferred intratumorally. These DCs are activated by CD40L in tumor tissue, and the activated DCs promote presentation of tumor-associated antigens (TAAs) and activate effector T cell responses against cancer cells, ultimately fully enhancing the tumor immune circulation. When analyzing its therapeutic strategy, it is not difficult to find that it centers on the advantages of encoding mRNA, a novel nucleic acid drug therapy, applied to a neoantigen vaccine for HNSCC and the DC adoptive therapy. Because of the antigen release from carcinoma in situ and the input of highly active DCs, the abundance of DCs and T cells is greatly enhanced, which promotes the killing of tumors through multiple links, and ensures the high efficiency, stability and integrity of the immune circulation in HNSCC. Regression of the primary tumor is well achieved in the treatment of refractory/recurrent HNSCC, and the tumor inhibition rate is more than 80–90%. Therefore, the efficacy of this method is worthy of recognition [137].

In the combination of nucleic acid drug therapy and multiple immunotherapies, we also provided some "double attack" strategies with therapeutic potential. For instance, the potential of mRNA technology to effectively program immune cells for the expression of specific antigens, cytokines, and adjuvants can be harnessed to develop dendritic cell stimulants (DCS) from HNSCC patients. This can be achieved by designing two mRNA fragments—one encoding a specific antigen and the other a toll-like receptor agonist—which are then linked and encapsulated within liposomes. Consequently, this approach facilitates a robust dual immune response against tumor cells: the activation of specific immunity and the enhancement of innate immunity. The synergistic effect of these mechanisms allows for improved activation of specific immunity and an increase in both the quantity and quality of specific CTL. For example, gene knockout technology can be used to knock out the HNSCC-related proto-oncogenes (such as TP53, EGFR, PIK3CA, etc.), and then through the mRNA expression of anti-tumor targeted drugs, can enhance the anti-tumor effect of drugs; After knocking out the genes related to immunosuppression of HNSCC (such as CD274, TGF-β, LAG-3, etc.), and then using mRNA technology to encode antigens to enhance the anti-tumor ability of immune cells, the two therapies cooperate to achieve a "double attack". In addition, the second therapeutic strategy is to combine immune "self-repair" and "efficient killing", by knocking out the immunosuppression-related genes (such as CD274, TGF-β, and LAG-3, etc.) of HNSCC to achieve "self-repair" of immune cells, and at the same time, by sequencing and other methods to find highly expressed tumor antigens in HNSCC patients and design individualized mRNA vaccines to address the efficient killing of tumors.

Therefore, in the future treatment of HNSCC, we need to pay more attention to each link of tumor immune circulation. When designing immunotherapy scheme, we need to consider various therapeutic means, make full use of the diversity and complexity of the immune system, and flexibly select appropriate combination, so as to form an efficient, stable and complete immune circulation "closed loop pathway" in the treatment, which plays a vital role in better treatment of HNSCC.

Novel nucleic acid drug therapy to correct metabolic reprogramming in HNSCC

A large number of studies have found that metabolic reprogramming is a key factor in the progression of HNSCC to recurrent/refractory HNSCC once the treatment of HNSCC enters the chronic phase [138]. Enhancement of glucose uptake and glycolysis as well as regulation of amino acid, fat and nucleic acid metabolism by tumor cells can not only promote the growth of tumor cells, but also form a complex tumor immunosuppressive microenvironment. At present, there are few novel nucleic acid drug therapies that directly treat the metabolic reprogramming of patients to prevent the deterioration of head and neck cancer, but they have potential therapeutic prospects in the treatment of HNSCC, especially in the postoperative recurrence prevention and treatment of patients. It has been found that the activation of HGF/Met signal transduction in HNSCC can affect glucose metabolism and thus significantly change the tumor microenvironment and reverse the tumor immune microenvironment [139]. A viable therapeutic effect can be achieved by encoding mRNAs that affect signaling pathways or by editing genes related to signaling pathways directly through CRISPR/Cas9 technology. Furthermore, studies have shown that pyruvate kinase (PKM2) can differentially alter metabolic characteristics in head and neck carcinogenesis, which may have tumor suppressor effects, and PKM2 mRNA expression is inversely correlated with tumor staging, more importantly, PKM2's mRNA expression is higher in HNSCC populations with higher recurrence-free survival rates [140]. This gives us good treatment guidance to reverse the gain of the "Warburg effect" in HNSCC cells through mRNA encoding enzymes involved in metabolism, especially those in the tricarboxylic acid cycle. Studies in recent years have shown that lipid metabolic reprogramming is an emerging hallmark of malignant tumors, and the focus of treatment of HNSCC by inhibiting metabolic reprogramming should be placed on genes that target lipid metabolic reprogramming. For example, a lipid metabolism-related gene LRPS was screened in a study, and its high expression group was significantly associated

with perineural invasion of tumor, cancer-related pathways, high mutation rate of TP53, infiltration proportion of natural killer T cells (NKTs), dendritic cells, monocytes, Treg, M1 and M2 macrophages in HNSCC tumor tissues. It may be a new therapeutic direction for HNSCC to affect its expression at gene level or protein level by novel nucleic acid drugs [141, 142].

Summary and prospect

In the field of conventional immunotherapy, the introduction of nucleic acid therapy is gradually advancing the progress and development of malignant tumor treatment strategies (Supplemental Table). The application of mRNA therapy provides a new way for personalized immunotherapy of HNSCC, which can enhance the immune system's attacking effect on tumor through customized vaccines, CAR-T cells and immunomodulatory proteins. In addition, the application of oligonucleotide therapy in the field of immunotherapy of HNSCC mainly focuses on the regulation of various aspects of immunological reactions, including the activation of immune cells, the expression of immune checkpoint inhibitory proteins and the control of inflammatory reaction. It also provides a complement to the field of immunotherapy of HNSCC and the field of vaccine vector design. Gene editing technology also provides an innovative means to enhance the ability of immune cells to recognize and attack HNSCC, as well as to interfere with the immune escape mechanism of tumor cells. In general, the synergistic application of novel nucleic acid drug therapies adds more options and advantages to the traditional immunotherapy strategy, and brings new hope for improving the efficacy of tumor treatment and patient survival rate. In the future immunotherapy of HNSCC, a closed-loop immune enhancement system must be constructed by considering every step of the immune cycle. This means that intervention is not only necessary in the recognition stage of tumor cells, but also in the activation, proliferation and memory immune response of immune cells. For example, through the use of immune checkpoint inhibitors to enhance the activity of T cells, the use of tumor vaccines to activate and expand immune cells, and the use of immunotherapy and other interventions in different links to improve the killing ability of immune cells to tumors, a diverse and complete "immunopotentiating circulation" can be formed. This comprehensive intervention method can maximize the therapeutic effect and provide important guidance for the implementation and optimization of immunotherapy. Therefore, the diversity and complexity of the immune system should be taken into account in the formulation of immunotherapy schemes, so as to make better use of various therapeutic methods and achieve a comprehensive therapeutic effect on tumors.

In conclusion, nucleic acid therapies, such as mRNA, oligonucleotide and gene editing, plays a synergistic role in immunotherapy of HNSCC, promoting the "immunopotentiating circulation" mechanism of HNSCC, and helping patients with HNSCC to achieve better efficacy by constructing a complete closed loop pathway during immunotherapy.

Supplementary Information

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Supplementary Material 1

Author contributions

Bowen Li and Qinglin Li: Conceptualization. Yangjian Hong: Writing- Original draft preparation. Yanyang Liu: Visualization, Investigation. Huize Shen: Supervision. Qinglin Li: Funding. Bowen Li: Writing- Reviewing and Editing.

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Declarations

Ethics approval and consent to participate

Declarations.

Consent for publication

Declarations.

Competing interests

The authors declare no competing interests.

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