Metabolic reprogramming in non-small cell lung cancer: from mechanism to drug therapy

Yu Wang*

School of statistics, Renmin University of China, Beijing, China

*Corresponding author: 161843312@masu.edu.cn

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Abstract: Metabolic reprogramming affects the development and metastasis of non-small cell lung cancer (NSCLC), in which oxidative stress, endoplasmic reticulum stress, and epigenetic modification all play key roles. From these aspects, the mechanism of metabolic reprogramming in non-small cell lung cancer was analyzed. The key regulatory protein in oxidative stress is NRF2, which has multifaceted effects on lung cancer, RRBP1 protein plays an important role in endoplasmic reticulum stress, that is vital in tumor survival as well as endoplasmic reticulum stress. Autophagy may be activated through the AMPK-mTOR pathway. These findings provide important directions for subsequent tumor therapy. Cancer-associated fibroblasts play an important role in the epigenetic modification of non-small cell lung cancer, which also explains that the annexin A6 molecule secreted by CAFs can lead to the resistance of anticancer drugs. T cells (including CD4+ T cells, CD8+ T cells, and regulatory T cells) play an important role in the epigenetic regulation of tumor cells. Therefore, future NSCLC treatment will focus on resistance in immunotherapy and targeted therapy problem, from which T cell therapy will provide more directions. More importantly, combination therapy has become an important direction to solve metabolic reprogramming.

1. Introduction

The tumor microenvironment (TME) plays an important role in tumor malignant progression, immune escape, and treatment tolerance. The development of safe and effective immunotherapy according to the characteristics of TME is a hot spot in current anti-tumor technology research. However, there are still many unsolved problems in the field of tumor immunotherapy, such as being effective only for some specific tumors, low overall clinical response rate, and recurrence after tumor immunotherapy.

As one of the most common malignant tumors in the world, lung cancer has become the leading cause of death from malignant tumors in China's urban population, of which non-small cell lung cancer (NSCLC) is the most important type. In recent years, more and more drugs have been developed for the treatment of NSCLC, and its remission rate has been greatly improved. TME signatures remain a major cause of lung cancer immunotherapy tolerance. In current study, the metabolic reprogramming in the TME that has the greatest impact on NSCLC, mechanisms such as oxidative stress, endoplasmic reticulum stress and epigenetic have been illustrated comprehensively. The genetic modifications are elaborated one by one and further explore how new therapeutic strategies can be employed to avoid and solve the problem of metabolic reprogramming in NSCLC.

2. The mechanism of metabolic crosstalk in the occurrence and development of NSCLC

The microenvironment of malignant tumors often affects signaling pathways, signaling molecules and metabolic enzymes due to changes in metabolites, resulting in their abnormal expression. These changes will promote the metabolic reprogramming of tumor cells.

Hoibin Jeong[1] and his partner found that tumor-associated macrophages (TAM) can enhance the glycolytic capacity of NSCLC cells by secreting TNF-α, and promote deficiency through AMP-activated protein kinase and PGC-1α activation. Oxygen enhancement. Furthermore, depletion of

TAM increases T-cell infiltration and PD-L1 expression in tumor cells, which favors PD-L1 blockade in NSCLC. These results all reflect the changes in the immune microenvironment caused by TAM metabolites, and in turn, tumor cell-derived lactic acid, succinic acid, etc. can promote the formation of pre-tumor TAMs and accelerate tumor progression, thus indicating that the immune microenvironment also Metabolic effects are known as metabolic crosstalk.

Oxidative stress, endoplasmic reticulum (ER) stress, and epigenetic modification all play a very important role in the tumor microenvironment. A clear understanding of these effects on metabolic crosstalk and/or metabolic reprogramming will facilitate the development of new drugs or treatments to halt tumor progression.

2.1 Oxidative stress

Excessive ROS, RNS can cause oxidative damage to lipids, proteins and DNA, what we call oxidative stress, which in turn leads to genetic and/or epigenetic changes that may further lead to cell death.

The study by Nicole E. Scharping and colleagues[2] completed an experiment using B16 tumor-bearing mice, they infused the mice with a hypoxia tracer (pimonidazole) before sacrificing them, and then treated the mice with anti-pimonidazole and anti-Hif1α antibodies. Depleted T cells were found to be the most hypoxic, and it was found that T cells that were continuously stimulated in hypoxia appeared extremely exhausted, and that this continued activation induced an exhaustion-like dysfunctional state in CD8+ T cells. Sequential activation under oxygen conditions also leads to Blimp-1-mediated inhibition of PGC1α (a transcriptional co-activator that coordinates mitochondrial biogenesis and antioxidant activity to avoid T cell depletion), increases mitochondrial ROS, and ultimately drives exhaustion-like dysfunctional program. These results reveal an important role of oxidative stress in the tumor microenvironment.

During the development and progression of NSCLC, oncogene activation, increased metabolism, hypoxia, and dysfunction of mitochondria and peroxidase all lead to high levels of ROS in tumor cells [3]. Many genes, such as genes encoding NRF2, NFE2L2 (nuclear factor erythrocyte 2-related factor 2) and negative regulator KEAP1 of NFE2L2, are involved in antioxidant regulatory mechanisms [4].

One of the most important oxidative stress protective pathways is Kelch-like ECH-associated protein 1 (KEAP1)-NFE2-related factor 2 (NRF2)-antioxidant response element (ARE) or KEAP1-NRF2-ARE signaling pathway. NRF2 is a master transcription factor that regulates antioxidant response element (ARE-)-mediated expression of antioxidant enzymes and cytoprotective proteins[5].

NRF2 has both good and bad sides in NSCLC. Under physiological conditions, NRF2 can act as a master regulator of antioxidant mechanisms by activating several transcriptional programs within cells in response to oxidative stress[3]. In the early stage of tumorigenesis, NRF2 plays a crucial role in TME, and it can participate in antioxidant reactions as a major substance. NRF2 can regulate the catalytic subunit of glutamate-cysteine ligase (GCLC), glutamate cysteine ligase (GCL) expression to maintain cellular redox homeostasis, therefore, activation of the NRF2 pathway can avoid DNA damage and the occurrence of precancerous lesions[6], however, certain roles of NRF2 can promote carcinogenesis in advanced NSCLC, and the nausea phenotype of lung cancer cells is highly dependent on the NRF2 pathway, a process known as NRF2 addiction[7].

2.2 ER stress

ER is the central organelle for the synthesis, folding and modification of secreted and transmembrane proteins. Misfolded and unfolded protein responses (UPRs) in the ER are activated when nutrients are undersupplied (conditions such as hypoxia) and nutrients are oversupplied (glucose, cholesterol, fatty acids, etc.), which lead to tumor progression and metastasis. The UPR is essential for promoting or inhibiting cancer progression in cells in the tumor microenvironment. Therefore, studies have shown that approaches target the UPR either individually or in combination therapy could provide a promising therapeutic approach for the treatment of tumors[8].

In their study, H-Y Tsai and colleagues found that ER ribosome-binding protein 1 (RRBP1) overexpression is often observed in lung cancer patients and attenuates intracellular stress-induced

apoptosis by enhancing GRP78[9]. By finding that RRBP1 is overexpressed in human NSCLC and that RRBP1 protein is upregulated in most primary lung cancer tumors compared to normal lung epithelial cells, most of the samples in this study belonged to stage I and II early stage tumors, therefore, it can be stated that overexpression of RRBP1 is an early event in the occurrence and progression of lung cancer, and overexpression of RRBP1 protects cells from ER-induced immune responses. The authors therefore propose that RRBP1 may be a key factor in maintaining tumor cell survival under stressful conditions.

Wen-Yue Xie and colleagues published a study[10] in 2016 that investigated ER stress-induced autophagy in NSCLC, they treated human NSCLC cell lines (A549 and NCI-H460) and applied GRP78 siRNA to study heat-induced autophagy and endoplasmic The relationship between ER stress and GRP78 protein levels was found to be reduced by western blot analysis after targeted siRNA transfection. Thus, it was confirmed that thermal treatment of lung cancer cells triggers protective autophagy mediated by ER stress, so it is believed that inhibiting autophagy may be a strategy to enhance the effect of hyperthermia in lung cancer patients. The study also confirmed that ER stress may activate autophagy through the AMPK-mTOR pathway.

2.3 Epigenetic modifications

Metabolic reprogramming and epigenetic changes are two important characteristics of tumors. Recent studies have shown that there is a very extensive mutual regulatory relationship between the two. On the one hand, changes in cancer metabolism, tumor microenvironment, and dietary interventions can affect the activities or substrate concentrations of epigenetic modifying enzymes and their cofactors by changing metabolite levels, thereby participating in epigenetic regulation; on the other hand, Changes in the expression or activity of epigenetic modifying enzymes can also have a wide range of direct or indirect effects on cellular metabolism[11].

Epigenetic modifications are processes that regulate gene expression without altering the DNA sequence itself. Epigenetic dysregulation in cancer often involves mutations and/or aberrant expression of epigenetic modifying enzymes and alterations in the levels of associated cofactors that lead to altered gene expression by altering chromatin structure and dynamics, ultimately promoting tumor progression, occur and evolve[12].

Regarding epigenetic modification, researches on DNA and RNA methylation and protein acetylation emerge in an endless stream. In recent years, cancer-associated fibroblasts (CAF) have also become a research hotspot. Multiple studies find that fibroblasts play a key role in cancer development[13,14].

CAF can enhance the malignant transformation of cancer by secreting various factors. Japanese researchers found that higher levels of CAF in tissues can worsen the condition of gastric cancer patients. The study also found that extracellular vesicles contained in the Annexin A6 molecules secreted by CAFs are taken up by gastric cancer cells, leading to resistance to anticancer drug therapy[14].

A study published in 2015 analyzed the expression differences of 15 microRNAs and found that miR-101 was the most down-regulated miRNA in CAFs, which inhibited the proliferation and metastasis of lung cancer by targeting CXCL12, suggesting that cancer cells and fibroblasts crosstalk between cells[15].

A study published in the journal Nature in 2019 revealed that (nicotinamide N-methyltransferase) NNMT is the master metabolic regulator of cancer-related fibroblasts[16]. A study published in the international journal Cancer Discovery pointed out that MicroRNAs can regenerate normal fibroblasts. Programmed into cancer-associated fibroblasts[17]. This series of studies has demonstrated that tumor-associated fibroblasts (CAFs) in the tumor microenvironment can modulate the sensitivity of tumor cells to therapy.

Researchers from the University of Southampton found that in a variety of tumor tissues, including head and neck tumors and rectal tumors, the more CAF cells are distributed, the lower the survival rate of tumor patients. The transformation of fibroblasts into CAF cells depends on the NOX4 enzyme (which belongs to the NADPH oxidase family). The study found that a significant increase in the

expression level of The NOX4 enzyme is observed in a wide variety of tumor cells and correlates with the number of CAF cells in the tumor tissue. strong correlation. Inhibition of NOX4 expression either at the gene level or with small-molecule compounds can alter the phenotype of CAF cells, including reduced cell surface α-smooth muscle actin (α-SMA) expression, and, in addition, in tumor-bearing small cells of various tumor types. *In vivo* experiments in mice found that inhibiting the expression of NOX4 was associated with preventing CAF cells from accumulating in tumor tissues, and could inhibit tumor growth and reduce tumor volume by 30.6%-64.0%. Using the NOX4 enzyme on CAF cells as a target, a compound drug that can inhibit NOX4 is developed, which may be widely used in clinical treatment of various tumors in the future[18].

Cancer cells are able to interact with fibroblasts to promote deterioration. Scientists at the German Cancer Research Center and the Heidelberg Institute for Stem Cell Technology and Experimental Medicine have now discovered that metastatic tumor cells release two inflammatory signaling molecules, interleukins, which stimulate fibroblasts in the lungs to release two other inflammatory molecules into the microenvironment Signaling molecules: CXCL9 and CXCL10. These molecules, in turn, attach to receptor molecules carried on the surface of several aggressive migrating cancer cells, marking a decisive step in their development into metastatic foci. Thus, these aggressive breast cancer cells directly benefit from inflammation as well as the signaling molecules CXCL9 and CXCL10. They therefore believe that some cancer cells stimulate the connective tissue cells in the surrounding environment to release transmitters that promote metastasis. The discovery plays a key role in better understanding how these metastatic cancer cells arise[13].

T cells (including CD4+ T cells, CD8+ T cells, and regulatory T cells) play an important role in the epigenetic regulation of tumor cells, and their plasticity is regulated by epigenetic marks, targeting their master regulatory protein EZH2 can develop different antineoplastic drugs. A meta-analysis study explored the prognostic value of EZH2 in NSCLC. The researcher's meta-analysis studies including 2180 patients and found that EZH2 was highly expressed in NSCLC patients, and EZH2 was found in KRAS-positive lung cancer. The expression was significantly higher, and EZH2 was weakly correlated with EGFR expression in lung squamous cell carcinoma patients. These findings provide a reference for the development of therapeutic drugs for lung cancer[19].

These relationships in the tumor microenvironment are often directly related to each other. Some drugs that trigger immune cell death (ICD) induce ER stress leading to damage-associated molecular patterns (DAMPs) such as calreticulin (CRT), secreted adenosine triphosphate (ATP), high mobility group box proteins b1 protein (HMGB1) and heat shock protein, etc., to initiate anti-tumor immune responses. These drugs in turn lead to ER stress indirectly by generating oxidative stress. Therefore, many chemotherapy drugs have this effect. DAMPs were also able to further drive ICD-induced antitumor CD8 cytotoxic T cell reactivity[20].

Epigenetic regulation and ER stress response are interconnected, partly by mechanism. When many misfolded proteins appear in the ER due to metabolic stress, a series of transcription factors will be activated, including XBP1, ATF3/4/6 and CHOP, etc. Some researchers found that after ER stress, BAP1 and PRC1 are recruited to the promoters of CHOP and ATF3 to regulate the ubiquitination level of H2A located on the promoters of CHOP and ATF3, thereby inhibiting the transcriptional regulation of CHOP and ATF3, which further leads to changes in the expression levels of a large number of other genes downstream , whose ultimate purpose is to help cells solve the problem of metabolic reprogramming, thereby maintaining cell survival[21].

3. NSCLC treatment ideas triggered by metabolic reprogramming

Based on the above research progress, the role of metabolic reprogramming in tumor microenvironment in tumor development and drug resistance has been gradually elucidated. Therefore, the development of targeted therapy drugs is particularly important. To find therapeutic drugs through the regulation of oxidative stress, ER stress and epigenetic modification in the tumor microenvironment, many new studies have been carried out in the world. We focus on the current main treatment options for NSCLC and some new research directions.

Given the important role of NRF2 in oxidative stress, recent studies suggest that the NRF2/KEAP1 pathway may have emerged as an ideal target to restore tumor sensitivity to drugs[22-25]. Therefore, immunotherapy is used when patients with active tumors carry NRF2, using NRF2 inhibitors, and inhibitor-related therapies for downstream NRF2 effectors are all under investigation[7].

3.1 Immune checkpoint inhibitors have become one of the standard treatment options for NSCLC, but the problem of drug resistance still needs to be solved

Immune Checkpoint Inhibitors (ICIs) have shown significant effects in the treatment of NSCLC, but there are still some patients who do not respond to ICIs therapy. In recent years, people have found the reasons why cancer patients do not respond to ICIs through the study of tumor microenvironment and metabolic reorganization, so more and more combination regimens have begun to be presented in clinical studies, some of these studies have been completed and the results have been published, as shown in Table 1. These studies found that the combination of chemotherapy, targeted therapy and other ICIs therapy can indeed improve the response rate of NSCLC.

Table 1 Completed and fruitful studies of PD-1/PD-L1 in NSCLC

NCT number	Title	Condition or disease	Interventions
03181308	Study of Carotuximab (TRC105) Plus Nivolumab in Patients With Metastatic NSCLC	Carcinoma, Non-Small-Cell Lung	Drug: Carotuximab (TRC105)|Drug: OPDIVO
03308942	Effects of Single Agent Niraparib and Niraparib Plus Programmed Cell Death-1 (PD-1) Inhibitors in NSCLC Participants	Neoplasms	Drug: Niraparib|Biological: Pembrolizumab|Biological: TSR-042 (Dostarlimab)
02007070	Study of Pembrolizumab (MK-3475) in Participants With Advanced NSCLC (MK-3475-025/KEYNOTE-025)	NSCLC	Biological: Pembrolizumab
02434081	NIvolumab COmbination With Standard First-line Chemotherapy and Radiotherapy in Locally Advanced Stage IIIA/B Non-Small Cell Lung Carcinoma	NSCLC Stage III	Drug: Nivolumab
02142738	Study of Pembrolizumab (MK-3475) Compared to Platinum-Based Chemotherapies in Participants With Metastatic NSCLC (MK-3475-024/KEYNOTE-024)	Non-Small Cell Lung Carcinoma	Drug: Pembrolizumab|Drug: Paclitaxel|Drug: Carboplatin|Drug: Pemetrexed|Drug: Cisplatin|Drug: Gemcitabine
01905657	Study of Two Doses of Pembrolizumab (MK-3475) Versus Docetaxel in Previously Treated Participants With NSCLC (MK-3475-010/KEYNOTE-010)	Non Small Cell Lung Cancer (NSCLC)	Biological: Pembrolizumab|Drug: Docetaxel

02404441	Phase I/II Study of PDR001 in Patients With Advanced Malignancies	Melanoma|Non-small Sell Lung Cancer (NSCLC)|Triple Negative Breast Cancer|Anaplastic Thyroid Cancer|Other Solid Tumors	Biological: PDR001
03322540	Pembrolizumab Plus Epacadostat vs Pembrolizumab Plus Placebo in Metastatic NSCLC (KEYNOTE-654-05/ECHO-305-05)	Lung Cancer	Drug: Pembrolizumab|Drug: Epacadostat|Drug: Placebo
01295827	Study of Pembrolizumab (MK-3475) in Participants With Progressive Locally Advanced or Metastatic Carcinoma, Melanoma, or Non-small Cell Lung Carcinoma (P07990/MK-3475-001/KEYNOTE-001)	Cancer, Solid Tumor	Biological: Pembrolizumab
03085914	A Study of Epacadostat in Combination With Pembrolizumab and Chemotherapy in Participants With Advanced or Metastatic Solid Tumors (ECHO-207/KEYNOTE-723)	Solid Tumor	Drug: Epacadostat|Drug: Pembrolizumab|Drug: Oxaliplatin|Drug: Leucovorin|Drug: 5-Fluorouracil|Drug: Gemcitabine|Drug: nab-Paclitaxel|Drug: Carboplatin|Drug: Paclitaxel|Drug: Pemetrexed|Drug: Cyclophosphamide|Drug: Cisplatin|Drug: Investigator's choice of platinum agent

Reactive oxygen species in oxidative stress can present an opportunity for combination drug therapy by activating mitochondrial function in tumor-reactive T cells. In one study, researchers did not observe any antitumor activity when a solution of tert-butyl hydroperoxide (Luperox) was injected into MC38-bearing mice, but when combined with PD-L1 mAb, tumor-bearing. The small molecule antitumor activity and survival were significantly improved in mice, indicating a synergistic effect of ROS generator Luperox and PD-L1 mAb therapy. Small molecule activators of AMPK and mTOR or PGC-1α also synergistically enhanced tumor growth inhibition by PD-1 blockade therapy. These results suggest that if PD-1 blockade therapy is combined with metabolism-based therapy, there will be a synergistic antitumor effect[26].

Breakthrough results from a prospective study, KEYNOTE-042 (NCT02220894) [27], found that STK11/KEAP1 mutations may help identify patients with high pTMB values who do not respond well to pembrolizumab. The researchers used whole-exome sequencing (WES) to assess the gene status of

STK11 and KEAP1 and tumor mutational burden (TMB). Results from KEYNOTE-042 showed that pembrolizumab monotherapy could be a standard first-line treatment option for advanced PD-L1-positive NSCLC regardless of STK11 and KEAP1 mutations.

3.2 Antibody-conjugated drugs enter the golden age of development

Molecular targeted therapy drugs mainly inhibit the important targets in the occurrence and development of tumors and play an anti-tumor effect. This class of drugs has shown its advantages more and more in the continuous update iteration. Not only monotherapy, but also in combination therapy has good efficacy and safety. A phase II clinical study found that standard EGFR-TKI therapy combined with metformin significantly prolonged PFS and OS in patients with EGFR-mutant lung adenocarcinoma[28].

In addition to targeting classical pathways such as EGFR and ALK, some new targets have been discovered in recent years, such as targeting HER2. A recent study of ADC drugs drew attention, with the DESTINY-Lung01 study showing that the trastuzumab deruxtecan showed durable anticancer activity in previously treated HER2-mutant NSCLC patients despite HER2-targeting antibodies, was observed in this international phase 2 study to produce a definitive objective response in 55% of patients tested (95% CI, 44-65) [29].

A promising future for ADC medicine is also possible with another Phase I clinical study showing Telisotuzumab Vedotin (Teliso-V, an ADC targeting c-Met signaling) monotherapy in c-Met+ NSCLC It was well tolerated and demonstrated antitumor activity, with objective responses in 23% of patients and a median duration of response of 8.7 months; median progression-free survival was 5.2 months[30].

3.3 T cell therapy is still a hot research direction

Tumor-infiltrating lymphocytes (TILs), T-cell receptor (TCR)-T cells, and chimeric antigen receptor (CAR)-T cells are all adoptive T-cell immunotherapy, and research on these therapies has been hot in recent years. Among them, CAR-T therapy has been the most popular research direction in recent years, and it has also shown good efficacy in some tumors. However, immunosuppressive factors in TME, such as hypoxia and tumor-associated macrophages (TAMs), inhibit the Antitumor function of CAR-NK cells[31-33]. In solid tumors such as NSCLC, the expression of various target antigens varies greatly, and the tumor itself can down-regulate these target antigens, thereby evading the attack of CAR-T cells, which leads to non-specific treatment and serious adverse effects[34], while CIK cells showed stronger antitumor efficacy and lower adverse reactions in NSCLC patients than CAR-T cells[35].

In animal experiments, researchers have tried a common anti-cholesterol drug to reduce the amount of cholesterol in the membranes of cancer cells. By stiffening cell membranes, the drug makes it easier for T cells to destroy cancer cells. The anti-cholesterol drug was then used in combination with adoptive T-cell immunotherapy, and it was found that giving both treatments at the same time improved survival from 0% to 50% [36].

TCR-T therapy shows initial success in NSCLC. NY-ESO-1 antigen in NSCLC (11.8-21% expression), a small study of 4 NSCLC patients enrolled in a clinical trial (NCT02457650) designed to initially evaluate NY-ESO-1 TCR-T Safety and feasibility of cell therapy in patients with NSCLC, in which a female patient with advanced lung adenocarcinoma showed a partial response (PR, 4 months) with no apparent toxicity.

3.4 Can Cancer Vaccines Bring Hope to NSCLC?

Unlike general vaccines, cancer vaccines often cannot prevent the occurrence of cancer, but are therapeutic vaccines. If they can improve the survival of advanced NSCLC, they can be regarded as effective. This method can make up for some of the deficiencies of immunotherapy, so it is often necessary to combine immunity therapy.

Tumor-associated antigens (TAAs) have been developed for tumor-specific vaccines, administered with immune adjuvants, but such vaccines have certain limitations, as TAAs can only exhibit limited antigenicity.

Human Telomerase Reverse Transcriptase (hTERT) is one of the target antigens for immunotherapy in patients with non-small cell lung cancer (NSCLC), a therapeutic vaccine Hu-rhEGF developed by BIOTECH PHARMA Co., Ltd. and Molecular Immunology Center in Cuba - rP64k/Mont, consisting of antigen and adjuvant, this vaccine has shown strong immunogenicity in clinical experience in advanced (IIIB/IV) NSCLC, reducing EGF concentrations, increasing anti-EGF antibody titers, and being well tolerated Well, these characteristics were also confirmed in a phase I clinical trial in China[37]. UV1 is also a novel hTERT vaccine with robust safety and immunogenicity found in a Phase I clinical study in patients with advanced NSCLC[38].

In another randomized phase 2 clinical trial involving 221 subjects, the first vaccine based on an "optimized cryptopeptide" approach, Vx-001, targeted TERT, although results showed that Vx-001 did not. There were >grade 2 treatment-related toxicities, and Vx-001 did induce a specific CD8+ immune response, but the study still failed to meet its primary endpoint[39].

Although many vaccines have completed phase II or phase II clinical studies, their results are often good and bad, as shown in Table 2. From the research status, the use of appropriate combination therapy may be conducive to produce better results in NSCLC, but the safety is still a problem that needs to be paid attention to.

Table 2 Completed clinical trials of NSCLC vaccines with results

Drug	Indications	Arms	Study stage	Main results	NCT number
1650-G	NSCLC	1650-G (n=12)	phase II	A robust and well-defined immune response occurred in 6/11 of the immunized patients. Relative frequency and kinetics of responses were similar to those with DC vaccine (1650+autologous DC)	00654030
Tecemotide	NSCLC, patients who did not progress following frontline chemoradiotherapy for unresectable stage III non-small-cell lung cancer	Tecemotide (L-BLP25) + Cyclophosphamide (n=1006) Saline + Placebo (n=507)	phase III	Prolonged OS in patients with tecemotide (29.4 months vs 20.8 months; HR 0.81, 95% CI 0.68-0.98, P = 0.026)	00409188
Autologous DC vaccine	Individuals with histologically confirmed stage I-IIIB NSCLC	Therapeutic autologous dendritic cells (n=32)	phase II	As of the 2007 report,the vaccine was well tolerated, with 6/7 surgically resected patients developing an immune response, 3 of whom received adjuvant medical therapy, and 3/7 unresectable stage III patients developing an immune response.	00103116
TG4010	Non Small Cell Lung Cancer Metastatic	TG4010/Chemotherapy/Nivolumab (n=44)	Phase II	Median ORR: 32.5%, Median PFS: 5.7months	03353675

GSK 249553	NSCLC After Tumour Removal by Surgery	GSK 249553 Group (n=122) Placebo Group(n=60)	Phase II	Although all MAGE-A3-treated patients exhibited a humoral immune response to the MAGE-A3 antigen, there were no significant differences in DFI, Disease-free Survival (DFS), and OS between the two groups.Treatment with no apparent toxicity	00290355
Tecemotide	Stage IIIA or Stage IIIB NSCLC That Cannot Be Removed by Surgery	Tecemotide/Bevacizumab After Chemoradiation (n=70)	Phase II	AE incidence：0.03, Median OS：42.7 months	00828009
GSK1572932A	NSCLC	GSK1572932 Group (n=1515) Placebo Group(n=757)	Phase III	Compared to placebo,Person Year Rate (PYAR) as Regards Disease-free Survival (DFS) in the Overall Population: 0.17 vs. 0.168; Person Year Rate (PYAR) as Regards Disease-free Survival (DFS) in the No-CT Population : 0.169 vs. 0.178	00480025
Vaccine + Cytoxan + ATRA as outlined in Detailed Description	Metastatic Lung Cancer	Combination Immunotherapy (n=24)	Phase II	The number of evaluable participants with tumor response was 5 out of 14 over 3 years, Median Time to Progression (TTP): 2.4 months Median Overall Survival (OS): 8 months Number of Participants With Serious Adverse Events (SAEs): 11/24 (45.83%)	00601796
GSK2302032A	NSCLC	GSK2302032A Group (n=86) Placebo Group (n=47)	Phase II	Time to Occurrence of Any Recurrence of Disease: Compared to placebo, person-year rate: 0.217 vs. 0.067 Number of Subjects With Any Unsolicited Adverse Events (AEs): 90.7% vs. 44.7%	01853878
Viagenpumatucel-L (HS-110)	NSCLC	Viagenpumatucel-L Plus Metronomic Cyclophosphamide (n=45) Chemotherapy Alone(n=21)	Phase II	Compared to Chemotherapy Alone, Median Overall Survival (OS): 176 days vs. 372 days (P<0.05) PFS: 70.0 days vs. 190 days The tolerability was comparable to the control group	02117024

4. Discussion

Currently, the focus of metabolic reprogramming research in NSCLC is located on ER stress in the TME and targeting ER stress. Many drugs are under development, mainly chaperone-binding immunoglobulins, including IRE1α inhibitors, PERK inhibitors, eIF2α inhibitors, BiP inhibitors, etc. Among them, BiP inhibitors have shown preliminary effects in the treatment of NSCLC.

The role of exosomes in tumors is also a hot topic. Exosomes play a very important role in the proliferation, metastasis and angiogenesis of NSCLC tumors. They can also mediate tumor immune

escape and lead to radiotherapy tolerance. Current progress on this topic is also beneficial to optimize the treatment of NSCLC in the future.

Issues and challenges in metabolic reprogramming research in NSCLC

Although the metabolic reprogramming of tumors has attracted much attention in recent years, the research in solid tumors is still limited, and due to the genetic polymorphisms in NSCLC itself, it is difficult to conduct clear research through clinical trials. The current situation is comprehensively analyzed, and the main shortcomings are as follows:

(1) Metabolic reprogramming in TME is a huge and complicated system, which involves gigantic cells, proteins, pathways, as well as metabolic phenotypes. There requires further study to make these contents clear.

(2) In the process of metabolic reprogramming, there are intricate relationships among inflammation, oxidative stress, nutrient supply, ER stress, and even epigenetic modification. To find a better solution to NSCLC, it is necessary to clarify the relationship between these contents.

(3) In view of the new research in recent years, the influencing factors of TME have gradually become clear, and more new biomarkers have been proposed for tumor diagnosis and treatment detection, such as: TMB, circulating tumor DNA (ctDNA), etc. The relationship between these biomarkers and metabolic reprogramming still needs to be further clarified in order to better combine them to find better solutions for the metabolic treatment of NSCLC.

Future: Therapeutic Strategies for NSCLC

The current immunotherapy still has certain shortcomings, such as sensitivity only in some populations, large individual differences, and easy to be affected by the environment, diet and even the microorganisms in the body, nevertheless, combination therapy can solve most of the drug resistance problems of tumors. The idea of combination therapy is to start from the current standard treatment of NSCLC and combine it with other treatment options, such as ICIs and other immunotherapies (such as costimulatory cytokines, or receptors, oncolytic viruses, cancer vaccines, etc.).

From the current research status, the combination of immunotherapy has not yet had ideal results, more study is still needed to verify the effectiveness of this combination regimen, but some current studies on combination therapy have given us inspiration, and the following regimens may be a good choice in the treatment of NSCLC:

(1) There is a theoretical basis for the combination of targeted therapy and immunotherapy, and the high tumor mutational burden (TMB) present in most KRAS- and BRAF-mutated NSCLC provides a rationale for the combination of KRAS-G12C and BRAF-V600 inhibitors with ICIs. Recent studies have shown that targeted therapy and immunotherapy in adjuvant and neoadjuvant therapy show high patient benefit, bringing opportunities for early intervention in NSCLC [40].

(2) Combination of anti-vascular drugs and immunotherapy may be effective, and small molecules targeting VEGF receptors may also enhance the activity of ICIs or desensitize tumors. In China, a clinical trial of allogeneic CD8 + CD56 + NKT killer cells combined with EGFR-TKI in NSCLC is has been carried out (trial number: ChiCTR-IIR-17013471), and the results of these studies are on their way.

(3) The combined use of antibody-drug conjugates (ADCs) with molecularly targeted therapy is an attractive area of research. In recent years, the research on ADC is in full swing, and it is believed that the research of this kind of joint scheme will be shown soon.

(4) In combination with cancer vaccines, the further development of NSCLC vaccines that can improve the immune response to tumor-related antigens, combined with existing ICIs or immunomodulatory drugs, may be more beneficial for NSCLC, especially for the advanced stage treatment.

5. Conclusion

In short, the TME has attracted more and more attention. As a cancer with a high incidence rate, NSCLC has always been a hot research direction on its occurrence, metastasis, and deterioration. With the current emphasis on tumor metabolism and the gradual increase in programming research, the insufficiency of NSCLC treatment has become more and more obvious. Some of the combination therapy methods proposed in this paper can be used as future exploration directions, but these methods still need more large-scale clinical trials to verify.

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