Review Article

Immunotherapy for Lung Cancers

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Lung cancer is the leading cause of cancer-related deaths worldwide. Although treatment methods in surgery, irradiation, and chemotherapy have improved, prognosis remains unsatisfactory and developing new therapeutic strategies is still an urgent demand. Immunotherapy is a novel therapeutic approach wherein activated immune cells can specifically kill tumor cells by recognition of tumor-associated antigens without damage to normal cells. Several lung cancer vaccines have demonstrated prolonged survival time in phase II and phase III trials, and several clinical trials are under investigation. However, many clinical trials involving cancer vaccination with defined tumor antigens work in only a small number of patients. Cancer immunotherapy is not completely effective in eradicating tumor cells because tumor cells escape from host immune scrutiny. Understanding of the mechanism of immune evasion regulated by tumor cells is required for the development of more effective immunotherapeutic approaches against lung cancer. This paper discusses the identification of tumor antigens in lung cancer, tumor immune escape mechanisms, and clinical vaccine trials in lung cancer.

1. Introduction

Lung cancer is the most common cause of cancer death worldwide in both men and women, accounting for 1.2 million deaths per year. Despite recent advances in surgery, irradiation, and chemotherapy, the prognosis is poor [1–3]. Therefore, the development of new therapeutic strategies is essential. Immunotherapy is an attractive candidate because the generation of specific antitumor immune responses through the identification of tumor-specific antigens can promote tumor cell death with minimal impact on normal tissue [4]. However, immunotherapy is effective in only a limited subset of patients. Tumor escape mechanisms from host immune surveillance remain a major obstacle, and many tumor cells, including lung cancer, are able to promote immune tolerance and escape host immune surveillance, resulting in the inhibition of anti-tumor immunity [5, 6]. These include a decrease or loss of the expression of tumor antigen, downregulation or loss of expression of human leukocyte antigen (HLA) molecules, expression of immuno-suppressive factors by cancer cells, regulatory T cells, and tolerant dendritic cells. Understanding of the immune-evasion mechanisms regulated by tumor cells is necessary in developing more effective immunotherapeutic approaches to lung cancer.

2. Immune Recognition of Cancer

Tumor regression *in vivo* is mediated by innate and adaptive immune responses involved with tumor-antigen presentation in the patient's lymphoid tissues. Innate mechanisms trigger inflammatory responses in the tumor microenvironment that presents sufficient local cytokines (i.e., IL-2, IL-12, IL-18, and IL-23) and stimulates antigen presenting cells (APCs) and dendritic cells (DCs) against tumor antigens [7, 8]. After DCs capture and digest tumor cells, tumor antigens associated with human leukocyte antigens (HLA I or HLA II) on the DC surface are presented to T-cell receptors (TCRs) of naive CD4$^+$ and CD8$^+$ T cells, resulting in the activation of naive T cells. Subsequently, costimulatory molecules (CD80, CD86) on DCs interact with CD28 on T cells for the full activation of T cells. After

activation and costimulation, CD4$^+$ and CD8$^+$ cells both produce a series of cytokines that differentiate T-Helper (CD4$^+$) lymphocytes into two subpopulations: Th 1 and Th 2 cells [9–11]. Th 1 cells produce IL-2, IFN-γ, TNF-α, and granulocyte macrophage colony stimulating factor (GM-CSF) that increase the activation of macrophages and upregulation of HLA I molecules on the surfaces of CD8$^+$ cells. Th 2 cells secrete IL-4, IL-5, IL-6, and IL-10 that induce naive B cells to produce specific antibodies.

The shifting towards Th2 pattern has recently been associated with increased tumor metastasis and decreased survival in many human and animal neoplasia. IL-4, IL-6 and IL-10 levels, but not IFN-γ and IL-2, were significantly higher in the serum, secreting supernatant or transcripts produced by PBMCs from lung cancer patients [12, 13]. IL-6 and IL-10 secretion derived from lung cancer cells is upregulated by tumor cell-derived prostaglandins and TGF-β. IL-6 induces directly STAT3 signaling of cancer cells to upregulate several genes, such as c-myc, bcl-2 and Mcl-1, resulting in induction of tumorigenesis [14]. IL-10 also possesses several properties that suppress the generation of anti-tumor immunity [12, 13]. IL-10 inhibits a broad array of immune parameters, including proinflammatory cytokine production by macrophages, antigen-presentation function, T lymphocyte proliferation, and Th1 cytokine production. Increased IL-4 by tumor cells repressed the secretion of Th1 cytokines has been found to have inhibitory effects on anti-tumor immune response. IL-4 directs the development of Th2 cells and downregulates IFN-γ production in Th1 cells, inhibits the production of IL-12 and IFN-γ by monocytes [12, 13]. Therefore IL-4 and IL-10 are key cytokines for the inhibition of Th1 cytokine response and the development of the Th2 cytokine response, which reduces the protective cellular immunity and induces tumor progression.

Cytotoxic T cell (CTL) is a major effector of tumor regression. When CD8$^+$ T cells bind to class I antigens on APCs, Th1 cytokines stimulate the generation of antigen-specific CTL, which expresses perforins, granzyme, and Fas ligand that directly eliminate neoplastic cells. CTLs also secrete specific cytokines (IFN-γ, TNF-α, and TNF-β) and activate macrophages against tumor cells directly [10, 11, 15]. Conversely, depending on the tumor microenvironment, these cytokines also stimulate tumor progression [16].

Natural killer (NK) and Natural Killer T (NKT) cells are innate immune cells critical for the first line of defense against tumorigenesis [17]. Different from T cells, NKs and NKT cells inhibit tumor growth in an MHC-nonrestricted manner [18–23]. Natural Killer (NK) cells are a type of cytotoxic lymphocyte that exhibit cytolytic activity against a variety of allogeneic targets in a nonspecific, contact-dependent, nonphagocytic process which does not require prior sensitization to an antigen [18–20]. NK cells share several properties with conventional cytotoxic T cells (CTL) and appear to possess similar mechanisms for cytolysis including secretion of perforin and granzyme. Their cytotoxic activity is positively regulated by IL-2 and IFN-r. Frequently, tumor cells (like stressed cells) express different glycoproteins (MICA and MICB) on their surfaces that function as ligands for NKG2D receptors on NK cells.

Once activated, these receptors stimulate NK cell activity to lyse tumours through the perforin/granzyme pathway or apoptosis-inducing ligands such as tumour-necrosis factor (TNF-) related apoptosis-inducing ligand (TRAIL or FasL). NK cells secrete IFN-γ by IL-12, which inhibits tumour-cell proliferation, enhances tumour-cell apoptosis, improves tumour antigen presentation and inhibits angiogenesis [18–21].

NKT cells are a subset of T cells that coexpress an $\alpha\beta$ T-cell receptor (TCR), but also express a variety of molecular markers that are typically associated with NK cells, such as NK1.1 [22–24]. NKT cells are restricted by the nonpolymorphic CD1d molecule and are activated by lipid and glycolipid antigens presented by CD1d. NKT cells share other features with NK cells as well, such as CD16 and CD56 expression as well as cytolytic perforin and granzyme release. Although NKT cells possess NK-like cytolytic activity, their activation results in rapid production of IFN-γ and expression of CD40L, thus providing help for activation of CD40-expressing APCs and generation of cellular and humoral immune responses [17, 22–24]. Under the existence of tumor cells, NKTs cell recognition of glycolipid antigens of tumor cells presented by CD1d can either lyse tumour cells directly using the perforin/granzyme system or ligands (TRAIL or FasL) for death receptors or stimulate other cytotoxic cells such as NK and CD8$^+$ T cells through IFN-γ secretion [22–24]. NK and NKT cells both produce chemokines that are important for recruiting effector T cells, B cells, neutrophils, and other NK and NKT cells to the disease site. NK- and NKT-derived IFN-r by stimulation of IL-12 is able to up-regulate the expression of the chemokine receptor CXCR3, which mediates subsequent recruitment of CXCR3$^+$ T and NK cells to tumor-infiltrated tissues [17, 22–24].

3. Vaccine Strategies

The capture and presentation of tumor antigen by APCs are key steps for successful active immunotherapy [25, 26]. In comparison to restriction of class I or class II pathways and selective stimulation of either CD4$^+$ Th cell or CD8$^+$ cytotoxic T-cell effectors by peptides, whole recombinant proteins are processed into multiple peptides and presented by APCs via class I and class II pathways to CD4$^+$ and CD8$^+$ T cells, respectively, and have the potential for generating immune effectors and immune memory [25]. Tumor-derived antigen mixtures contain multiple dominant and minor antigenic determinants within whole proteins, permitting the host to select, process, and present on HLA, the most immunogenic epitopes relative to that individual [25].

The most commonly used multivalent formulations employ autologous or allogeneic tumor cells. Autologous tumor vaccine is produced by isolating adequate amounts of tumor cells from an individual and processing these tumor cells into a vaccine formulation in vitro; the vaccine is then administered to the individual from whom the tumor cells were isolated. Autologous tumor vaccines have been shown to have immunologic activity in a number of studies.

An autologous tumor vaccine usually combined with an adjuvant elicits effectively a specific CTL-mediated cytolytic response against tumor cells [25–28].

Allogeneic tumor vaccine composed of tumor cells isolated from the tumor of one patient, killed and processed, and administered to another patient in order to stimulate cytotoxic immune responses to a similar tumor cell type. The cells found in this type of whole-cell vaccine express many cell-surface tumor-associated antigens. This vaccine is frequently administered with an adjuvant immunostimulant. Using allogeneic antigens also generates a uniform preparation, which speeds up the immune assessment and comparability not offered by the use of autologous tumor antigen, thereby allogeneic approaches are attractive during therapeutic development and clinical testing [25–28].

Two additional allogeneic sources of antigen are synthetic peptide and recombinant protein. In contrast to allogeneic tumor, peptides and proteins are applied in monovalent formulations. In spite of being easily synthesized and uniform, providing the simplest and most reproducible immunologic measures of biological efficacy, peptides require patient selection based on HLA tissue typing and also have designated restriction to class I or class II pathways, selectively stimulating either $CD8^+$ cytotoxic T-cell effectors or $CD4^+$ Th cells, responsible for immune memory. By contrast, whole recombinant proteins are processed into multiple peptides and presented by APCs via class I and class II pathways to $CD4^+$ and $CD8^+$ T cells, respectively, and have the potential for generating responses of immune effectors and immune memory [25–28].

By identification of tumor-associated antigens, many tumor vaccines have been established by investigators and effective generate specific immunity against tumor cells and treatment in lung cancer patients. Cancer-associated mucins are a potential target for immunotherapy. These molecules facilitate adhesion of malignant cells to the endothelial cell surface and promote metastases. They are tumor-specific immunogens because they exhibit unique glycosylation patterns [29]. The BLP25 liposome vaccine (L-BLP-25) carries the mucin-1 (MUC-1) protein admixed with monophosphoryl lipid A as an immune adjuvant. Trials of the L-BLP-25 vaccine in stage III and IV NSCLC patients have demonstrated safety but not a statistically significant survival benefit. Nonetheless, a subset of patients ($n = 75$) with IIIB disease has shown a trend towards improved survival ($P = .09$). In 2007, Merck Serono sponsored a multicenter (international) phase III, randomized, double-blind, placebo-controlled trial where 1300 patients with unresectable stage III NSCLC responded to first-line, platinum-based chemoradiotherapy [30].

The C-T antigens (MAGE-1, MAGE-3, BAGE, BAGE, GAGE, KK-LC-1, and NY-ESO-1) are encoded by genes that are completely silent in most normal tissues but are activated in a wide variety of tumors. Although normal cells, placental trophoblasts, and male germ-line cells express C-T antigen, the cells lack HLA I molecules and cannot present the antigens to T cells [31, 32]. Therefore, tumor C-T antigens are considered to be highly promising targets for anticancer vaccine [33]. MAGE-3 is aberrantly expressed in a wide variety of tumors, including NSCLC. Several $CD8^+$ T-cell epitopes of MAGE-3 have been identified *in vitro*. GlaxoSmithKline produced a vaccine that carries recombinant MAGE-3 fusion protein (His-tagged/full-length MAGE-3 protein/influenza protein D) plus immune adjuvant AS02B (monophosphoryl lipid A and QS21) [34]. A recent randomized phase II trial conducted on 182 stage IB or II NSCLC MAGE-3 positive patients (122 vaccine and 60 placebo) has demonstrated a trend towards improved survival in stage II patients receiving the vaccine compared to placebo. The results are enough for a phase III investigation. The study plans to accrue 2270 MAGE-3-positive patients with completely resected stage IB, II, or IIIA NSCLC. Furthermore, epitopes from the CT antigens TTK protein kinase (TTK), lymphocyte antigen 6 complex locus K (LY6 K), and insulin-like growth factor (IGF)-II mRNA-binding protein 3 (IMP-3) have been demonstrated to elicit CD8 responses in 20%–70% of HNSCC patients tested [35], and 50% HNSCC (5/10) patients vaccinated against these peptides have resulted in clinical responses [36].

Epidermal growth factor (EGF), now a well-established target for biologic therapy, is also a potential tumor antigen. Preclinical studies have established the antigenicity and antitumor activity of EGF protein administered to animals [37]. In two randomized phase II studies, recombinant EGF conjugated to Neisseria meningitides P64K protein as carrier protein and emulsified with the adjuvant Monotanide ISA51 was administered to 40 advanced NSCLC patients. Anti-EGF antibody responses were identified with a significant increase in survival for patients who maintained antibody response (9.1 months versus 4.5 months). The same agent was tested in a larger randomized phase II clinical trial that vaccinated 100 patients with stage IIIB or IV NSCLC who had progressed through first-line chemotherapy, and 45% of vaccinated patients developed a strong anti-EGF antibody response and decreased serum EGF concentration. Compared to controls (best supportive care), those who received the treatment had significantly longer overall survival (8.5 versus 4.3 months) [38, 39].

Xenogeneic anti-idiotype antibodies are quite unique antigen-mimic preparations, generated as antibodies to tumor antigen-binding sites on other antibodies (that generates a template of the antigen). The xenogeneic nature of these preparations makes them inherently immunogenic, and the similarity of the antiidiotype antibody to the tumor antigen allows cross recognition of the parent/native protein. Antiidiotypic vaccines are used to elicit tumor-specific antibodies as the dominant effectors for therapeutic activity; these have been the most widely tested immunotherapy approaches in SCLC [25–28].

Tumor antigens like the ganglioside, GD-3, have been identified as targeted active immunotherapy strategies become more feasible. In SCLC patients after chemotherapy or combined chemotherapy and radiotherapy, vaccination with an anti-idiotype GD3 monoclonal antibody (BEC2) and BCG induces antiganglioside GD3 antibodies and prolong survival compared to control subjects. However, this agent provides no survival benefit in a large randomized international phase III trial by Merck. BEC plus BCG vaccine

induces humoral response in only one-third of 213 patients and the investigators suggest that a multivalent rather than a monovalent approach may be better in the treatment of lung cancer patients [40].

Other tumor-associated antigens, hyaluronic acid-mediated motility (RHAMM) and carboanhydrase IX (G250/CAIX), are overexpressed in HNSCC and served as immunogens *in vivo* in 4 of 8 HLA-A2+ patients, while 0.06%–0.13% of CD8+ effector T cells recognized tetramers for RHAMM or G250 and secreted IFN-r and granzyme B in ELISPOT assays [41]. Otherwise, NKG2D ligands MHC class I-related chain molecules A (MICA) and UL16-binding proteins (ULBPs) are over-expressed in the primary HNSCC as compared to nontumor tissues of vocal cord polyps. The ligands reportedly activate NK cells and generate adaptive immunity through binding to NKG2D receptor. However, other studies demonstrate significant variability of expression [42, 43].

4. Promotion of Antigen Recognition

In order to initiate or promote antigen-specific responses, tumor antigens have to incorporate adjuvants that lead to increases in various arms of the immune cascade, antigen recognition, uptake, presentation, and/or antigen-specific cellular reactivity [25, 26]. Some biologic adjuvants [25, 26, 44] (bacillus Calmette-Guerin (BCG), diphtheria toxoid, and tetanus toxoid and chemical adjuvants (aluminum hydroxide, montanide ISA 51, and incomplete Freund's adjuvant) induce an inflammatory response at the site of delivery, which accelerates the migration of APCs to the site of delivery and enhance the capture and processing of tumor antigens by APCs in the inflammatory environment. Moreover, DC precursors are harvested from patients and cultured with antigen to activate DCs *ex vitro* [45]. The activate DCs are subsequently delivered back to the individual, where they expectedly migrate to the lymph node and come to the desired antigen-specific immune response.

Small molecules like Toll-like receptor-9 (TLR9) agonists [46, 47] can stimulate Toll-like receptors and initiate the innate and adaptive immune responses and have been under investigation for treating cancer. TLR9 is expressed in endosomes of dendritic cells, plasmocytoid dendritic cells, and T and B lymphocytes and regulates innate antigen-specific immunity via the recognition of pathogen-associated molecular pattern. Activation of TLR9 signalling pathway by TLR9 agonists leads to increased production of proinflammatory cytokines and chemokines and stimulation of an immune response with antitumor effects. Several new immunomodulatory oligonucleotides have been evaluated in models of human cancer [46, 47]. Among these, PF-3512676 (ProMune) is particularly promising. It contains unmethylated cytosine and guanine (CpG) motifs and a nuclease-resistant phosphorothioate backbone. The anticancer activity of PF-3512676 is related to direct and indirect immunomodulation of both innate and adaptive immune responses. Plasmocytoid dendritic cells stimulated by PF-3512676 express increased levels of MHC I and II and costimulatory molecules (leading to improved antigen presentation) secrete cytokines and chemokines that enhance

natural killer (NK) cell activity directed toward tumor cells, present tumor-specific antigens and costimulatory molecules to B and T cells and generate long-living antigen specific cytotoxic T-lymphocytes, and antibody responses. A good indicator of activation and maturation of dendritic cells by PF-3512676 is the production of IFN-α and the subsequent induction of interferon-inducible protein 10 (IP-10), an antiangiogenic cytokine [46]. In NSCLC, a phase II study enrolling 112 chemonaive patients with NSCLC was conducted. The patients received PF3512676 in combination with platinum, and taxane doublet chemotherapy. Twenty-eight (37%) patients had a partial or complete response with the combination of chemotherapy and PF-3512676 and 7 (19%) with chemotherapy alone. Based on these preliminary data, two phase III trials were conducted to test the efficacy of PF-3512676 in combination with platinum based chemotherapy in advanced NSCLC patients [46, 47].

Cytokine can be used at the site of tumor or combined with exogenous tumor antigen to promote APC maturation and activation and HLA class I molecule expression on tumor cells, which generates effective CTL responses againt tumor cells [25, 48]. *In vivo* cytokine gene transfer can also target normal cells in the tumor environment, thereby achieving high local concentrations of cytokine that avoid toxicities associated with systemic administration. Gene therapy has been applied in clinical trials for over a decade. Gene transfer of cytokines or costimulatory molecules directly to tumor cells *ex vivo* and *in vivo* are attractive ways of making nonimmunogenic cells more immuno-stimulatory [25, 49]. The cytokine granulocyte-monocyte colony stimulating factor (GM-CSF), a significant mediator of proliferation, maturation, and migration of dendritic cells can enhance the generation of potent, durable anti-tumor immunity [50, 51]. GM-CSF and IL-2 combined with tumor antigen causes high local concentrations of stimulatory cytokines at the site of antigen delivery and stimulates APC and T cell activation. Fas ligand (FasL) and GM-CSF coexpressed in tumor cells administrate in to mice, which accelerate the recruitment of innate immune cells, activation of dendritic cells, and the generation of specific and memorial anti-tumor immunity against tumor cells *in vivo* [52]. The benefit of incorporating GM-CSF into anti-tumor vaccines is well established. In a multi-center phase I/II trial, Nemunaitis et al. produced a vaccine (GVAX) that contains autologous, irradiated lung tumor (NSCLC) cells engineered to secrete GM-CSF. Among 33 patients with advanced NSCLC, three (2 with bronchoalve-olar carcinoma) achieved complete response and prolonged remission. Longer median survival was observed in patients whose vaccines secreted more GM-CSF (17 months versus 7 months), suggesting a cytokine dose-response relationship. Eight of ten patients with early-stage lung cancer remained disease-free with a medium followup of 12 months. However, establishing GVAX required much time. In the beginning, 83 tumors had to be harvested. Vaccines could not be successfully produced in 16 patients and 11 others died before vaccine was delivered. The medium generation time was 49 days. There were only 43 patients immunized with the vaccine [50, 51].

Most immunomodulatory drugs, including cyclooxygenase-2 (COX-2) inhibitors and thalidomide-like agents (Lenalidomide), have immunologic properties that promote a favorable immune environment [53, 54]. Furthermore, antisuppressive agents like cyclophosphamide and fludarabine abrogate the activity of immunosuppressive cells-regulatory T cells (T-reg). Therefore, these agents have therapeutic potential that can synergize with cancer vaccines and other active immunotherapy strategies [25, 55–60].

COX-2 is an enzyme that catalyzes the synthesis of prostaglandins (PGs), including prostaglandin E2 (PGE2) [61]. COX-2 and PGE2 overexpression are seen in many malignancies including lung cancer. In nonsmall cell lung cancer (NSCLC), COX-2 is overexpressed in most adenocarcinomas and squamous cell carcinomas. Elevated tumor COX-2 and PGE2 levels have been implicated in angiogenesis, tumor invasion, resistance to apoptosis, and suppression of antitumor immunity. PGE2 secretion mediated by COX-2 can negatively regulate T-lymphocyte proliferation and cytotoxicity, and mediate the imbalance between IL-10 and IL-12 in favor of IL-10 production. The tumor microenvironment is predominantly polarized toward Th2-like or immunosuppressive immune responses. The overexpression of phosphoglycerate kinase (PGK-1) [62, 63] or Interlukin-27 (IL-27) [64] in lung cancer cells both downregulate COX-2 and PGE2, which not only directly suppress tumorigenesis but also enhance the activation of immune cells and generation of specific Th1 anti-tumor immune response *in vivo*. Preclinical animal model studies show tumor reduction when animals are treated with either nonspecific or specific inhibitors of COX-2. Based on these observations, celecoxib, a selective COX-2 inhibitor, has been evaluated in combination with chemotherapy for the management of metastatic NSCLC in patients who have failed prior chemotherapy. Ongoing clinical trials are also evaluating the combination of celecoxib with chemotherapy (paclitaxel and carboplatin) and/or radiation or celecoxib in combination with epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI; gefitinib) of NSCLC [53, 61].

Lenalidomide [47, 54] was synthesized based on the structural backbone of thalidomide, by adding an amino group at position 4 of the phthaloyl ring and removal of the carbonyl group of the 4-amino-substituted phthaloyl ring. Such structural changes were designed to enhance its immunomodulatory and antitumor activity. Lenalidomide induces increase in IL-2 and IFNγ secretion and upregulation of CD40L expression on anti-CD3-stimulated T cells, resulting in activation of natural killer cells, and thus improving host immunity against tumor cells. Compared to thalidomide, lenalidomide is 50 to 2000 times more potent in stimulating T-cell proliferation and activation and 50–100 times more potent in augmenting IL-2 and IFNγ production. In addition, lenalidomide has been shown to inhibit endothelial cell migration and adhesion, perhaps by downregulating endothelial cell integrins. Lenalidomide is reported to downregulate key cytokines such as TNF-α, IL-6, IL-8, and VEGF, that is, cytokines which favour tumor cell survival, proliferation and possibly resistance to therapy, mainly by affecting the tumor vasculature. In solid tumors, lenalidomide proved to have a good safety profile both in monotherapy and in combination with chemotherapy showing results in terms of antitumor activity in several tumor types and also in NSCLC. In fact, Miller et al. tested the feasibility of lenalidomide at a dose escalated from 5 to 10 to 25 mg/day in 20 patients with solid tumors refractory to standard treatment. One partial response and three stable diseases were documented; of these patients, three had NSCLC diagnosis. This study recommended 25 mg/day, orally, of lenalidomide as single agent for 4 weeks followed by 2-week rest period. Similarly, Kalmadi et al. explored safety and tolerability of lenalidomide in association with docetaxel and carboplatin in 14 patients with advanced solid tumors.

5. Challenges in Immunotherapy: Tumor Escape Mechanism

By identifying tumor-specific antigens recognized by CTL, several clinical trials of therapeutic vaccine bearing with these antigens have promoted tumor-specific immunity. However, only 2%–4% of patients have observed tumor regression [65]. There is a number of escape mechanisms from the host's immunosurveillance regulated by cancer cells, including loss of tumor antigen, downregulation of HLA molecule expression, and secretion of immunosuppressive soluble factors ligands [65].

During tumor progression, tumor cells often display loss or down-regulation of HLA I antigen. In surgically resected specimens, 25%–94% of NSCLCs have down-regulated HLA I expression. Thereafter, one possible mechanism of the escape host immuno-surveillance immune escape is tumor cells with abnormal HLA I antigen expression, leading to develop clinical cancer [66]. A haplotype loss of HLA I antigen is a common cause of abnormal HLA expression in various types of tumors, as mentioned above [67–70]. Moreover, β2-microglobulin gene (β2-m) abnormality is common in abnormal expressions of HLA I [67]. Transduction of the wild-type β2-m gene renders them positive for HLA class I expression. An autologous CTL clone is induced by stimulating the wild-type β2m-transduced lung cancer cell line with the genetic abnormality of β2m. HLA class I-deficient cancer cells can escape from an attack by CTLs, and a reformation of HLA class I expression in cancer cells restores CTL recognition against cancer cells.

Cancer cells often secrete immuno-suppressive cytokines, including transforming growth factor-β (TGF-β), interleukin-10 (IL-10), and indoleamine 2,3-dioxygenase (IDO) [71, 72]. IDO is a tryptophancatabolism enzyme that is overexpressed in various tumors. It leads to T-cell dysfunction and apoptosis through the depletion of tryptophan. Arginase, an amino acid-catabolizing enzyme, is expressed in tumor cells to decrease CD3z expression of T-cell clones [73] and inhibit antigen-specific recognition. The infiltrating T cells in the patients possess a high level of arginase activity (arginase I) and decreased CD3ξ levels. Soluble MHC class I chain-related molecule A (MICA) derived from tumor cells is able to systemically downregulate NKG2D expression on the surface of CD8 T cells and natural killer (NK) cells [74], thereby impairing activity of effector

cells against tumor cells. Thus, tumor-derived soluble factors assist tumor cells in the evasion of immune attack, allowing tumor progression and metastasis.

Many cancers express immuno-suppressive costimulatory molecules such as programmed death ligand-1 (PD-L1) [75, 76] PD-L1 has been shown to suppress immune responses through PD-1 receptor on activated T cells and B cells, which decreases immune responses. PD-L1 on lung cancer cells demonstrates that it is able to increase apoptosis of antigen-specific T cells and to inhibit CD4 and CD8 T cell activation, resulting in reduced anti-tumor immunity and evasion of host immune surveillance [75, 76]. Fas system is one of the killing pathways by CTLs and NK cells to tumor cells in human body. However, reducing Fas expression and the over-expression of Fas ligands are observed in lung cancer, contributing to tumor immune privilege by inducing FasL-mediated apoptosis of host CTL and NK cells and destructing infiltrating Fas-bearing lymphocytes [77].

6. Immunosuppressive Immune Cells (MDSC, TAM, Treg)

Solid tumors consist of both malignant cells and a number of nonmalignant stromal cell types, including endothelial cells, fibroblasts, and various immune cells. Complex interactions occur between these within the tumor microenvironment and impact on immunosurveillence and tumor progression [78]. It has been reported that anti-tumor immune responses are downregulated by immuno-suppressive immune cells, which include myeloid-derived suppressor cells (MDSCs), M2 macrophages, and regulatory T cells (Tregs). VEGF, GM-CSF, M-CSF, IL-6, and IL-10 secreted by growing tumors and stromal cells cause abnormal myelopoiesis that ultimately leads to the suppression of immune responses. The success of immune therapy for cancer will depend on integrating strategies that down-regulate immune suppression [79, 80].

Studies provide evidence that MDSCs are directly involved in the suppression of immune responses in cancer. An increase in the number of MDSCs has strong natural suppressive activity in cancer patients or tumor-bearing mice [81, 82]. In murine tumor models, the number of MDSCs in spleen increase by 5- to 20-fold, depending on the tumor model, and is easily detected in the lymph node and tumor site. Recent findings demonstrate that ROS and peroxynitrite derived from MDSCs can induce antigen-specific CD8^{+} T cell tolerance through a posttranscription mechanism that involves the modification of CD8 and TCR itself on the T cell surface [83–85]. CD8^{+} T cells from MDSC-treated mice are unable to produce IFN-r and interleukin-2 in response to specific peptides and do not kill peptide-load target cells. MDSCs, in addition to inducing tumor-specific T-cell tolerance, also cause the development of Tregs. MDSCs in tumor-bearing hosts also reduce the number and activation of T-cells through the production of nitric oxide (NO) and arginase-1 [86, 87]. NO inhibits T cells through the blockade of activity in the JAK3 and STAT5, inhibition of HLA II gene expression, and induction of T cell apoptosis, while arginase 1 causes the depletion of arginine and translational blockade of the ξ-chain of CD3. Combination of high

arginase activity and increased NO production by MDSCs also leads to increased ROS production. This increase is able to suppress T cells by cell-to-cell contact. Depleting of MDSCs by using anti-Gr1 antibodies has been shown to significantly improve CD8^{+} T cell immune response and allow for eradication of the variant tumor cell lines [81, 82]. In addition, elimination of MDSCs with All transretinoic acid (ATRA) has also been found to promote CD4- and CD8-mediated tumor-specific immune responses, and may open an opportunity to improve the effect of cancer vaccine [81, 82].

In some cases, macrophages can represent 50% of the cellularity within a tumor. The increased number of M2 macrophages in the tumor stroma is associated with poor prognosis in NSCLC [88–92]. M2 macrophages are derived from circulating monocytes that are recruited to tumors by chemotactic factors such as CCL2, VEGF and M-CSF [88–90]. M2 macrophages are able to secrete IL-10 and TGF-β and inhibit Th1 immune response, leading to enhanced wound healing and tissue remodeling as well as promotion of tumor formation. Differentiation of M2 macrophages is induced by IL-4, IL-10, IL-13, IL-21, activin A, immune complexes, and glucocorticoids. M2 macrophages also express high levels of IL-1 receptor antagonist, CC ligand 22 (CCL22), scavenger, mannose receptor, galactose receptor, arginase I, and CD163 antigen. In tumor angiogenesis, M2 macrophages play an important role of secreting proangiogenic factors and enzymes, including vascular endothelial growth factor (VEGF) and matrix metalloproteinase 9 (MMP9) [91, 92]. Several studies have shown that the activation of TLRs, such as TLR9, decreases the development and activity of M2 macrophage [88–90, 93], and activation of TLR9 by synthetic CpG oligodendronucleotides demonstrated anti-tumor effects and survival increased significantly in many preclinical models. Knock-down of a crucial phosphatase, SHIP1, has been showed to suppress development of M2 macrophages in mice, and thus, pharmacological modulators of this phosphatase are under investigation currently [88–90, 93].

The accumulation of regulatory T cells (Tregs) in tumor is reportedly associated with unfavorable prognosis in NSCLC patients [94]. The number of Tregs exist in high proportions in the TIL of patients with lung cancer and play a role in suppressing anti-tumor immune responses. Tregs can be recruited to tumor sites by secretion of CCL22 derived from tumor cells and TAMs [95]. Tregs isolated from tumors mediate the potent inhibition of proliferation of autologous peripheral blood T cells stimulated by anti-CD3 or anti-CD3/anti-CD28 [96]. These Tregs play a role in inducing or maintaining tolerance to tumor in patients with lung cancer. Tregs are known to suppress DC function via TGF-β and IL-10 [97]. Recent clinical studies indicate that high levels of tumor infiltration by activated CD8^{+} T cells combined with a low number of Tregs is a significant positive prognostic factor for survival in cancer patients [98, 99]. Thus, reducing the number or activity of Tregs in tumor-bearing hosts may induce effective tumor immunity by activating tumor-specific as well as nonspecific effector cells. Removal of Tregs by anti-CD25 antibody can augment effector T cell-mediated

tumor immunity that strongly inhibits tumor growth in cancer patients [100, 101]. Activation of GITR signaling by agonist anti-GITR antibody or GITR ligand can inhibit the suppressive activity of Tregs and enhance tumor-specific CD4$^+$ and CD8$^+$ T cell responses. CTLA-4 blockade by anti-CTLA-antibody also augments tumor inhibition by attenuating Treg suppression and augmenting effector T-cell activity. The combination therapy of anti-CTLA-4-blocking antibody and anti-GITR agonist antibody has demonstrated that there have synergistic antitumor effects causing rejection of advanced stage tumors compared with either antibody therapy alone [100, 101].

7. Conclusion

Immunotherapy for lung cancer is potentially effective treatment in terms of high specificity, low toxicity, and prolonged activity. Nonetheless, it is necessary to integrate novel approaches with traditional therapeutic methods to offer more appropriate therapy, including representation of antigen epitopes, restoration of APC immune-stimulating activity, expansion of tumor-reactive T cells, and down-regulation of suppressor pathways. In the future, using combinations of multiple immunologically active agents, conventional treatment modalities, and novel targeted therapies will overcome limitations of any single approach and lead to significant improvements in therapeutic outcomes of lung cancer.

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