CCL21 Programs Immune Activity in Tumor Microenvironment

7

Sherven Sharma, Pournima Kadam, and Steven Dubinett

Abstract

CCL21 promotes immune activity in the tumor microenvironment (TME) by colocalizing dendritic cells (DC) and T cells programing ectopic lymph node architectural structures that correlate with cancer prognosis. Innovative strategies to deliver CCL21 in cancer patients will reactivate the downregulated immune activity in the TME. Immune escape mechanisms are upregulated in the TME that promote tumor immune evasion. CCL21 combined with inhibition of dominant pathways of immune evasion will aid in the development of effective immunotherapy for cancer.

Keywords

CCL21 · Tumor microenvironment · T cells · Dendritic cells · Antigen-presenting cells · Activated T cells · Immune activity · Programmed cell death protein 1 (PD-1) · Immune checkpoint blockade · Immune suppression · Immunotherapy

7.1 Introduction

Lung cancer remains a challenging health problem with more than 1.1 million deaths worldwide annually. With current therapy, the long-term survival for the majority of lung cancer patients remains low, and thus new therapeutic strategies are needed. One such strategy would be to develop immune therapy for lung cancer. Immune approaches remain attractive because although surgery, chemotherapy, and radiotherapy alone or in combination produce response rates in all histological types of lung cancer, relapse is frequent. Strategies that harness the immune system to react against tumors can be integrated with existing forms of therapy for optimal responses toward this devastating disease. Antigen-presenting cell (APC) and T cell activities are reduced in the lung tumor microenvironment (TME). In this review we discuss our experience with efforts to restore host APC and T cell activities in lung cancer microenvironment by intratumoral administration

S. Sharma (✉) · S. Dubinett
Department of Medicine, UCLA Lung Cancer Research Program, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

Molecular Gene Medicine Laboratory, Veterans Affairs Greater Los Angeles Healthcare System, Los Angeles, CA, USA

Jonsson Comprehensive Cancer Center, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA
e-mail: SSharma@mednet.ucla.edu

P. Kadam
Molecular Gene Medicine Laboratory, Veterans Affairs Greater Los Angeles Healthcare System, Los Angeles, CA, USA

A. Birbrair (ed.), *Tumor Microenvironment*, Advances in Experimental Medicine and Biology 1231, https://doi.org/10.1007/978-3-030-36667-4_7

of dendritic cells (DC) expressing the CCR7 receptor ligand CCL21 (secondary lymphoid chemokine, SLC). Based on the results demonstrating that CCL21 is an effective anticancer agent in the preclinical lung tumor model systems, a phase I clinical trial was initiated using intratumoral injection of CCL21 gene-modified autologous DC in lung cancer. Results from the trial reveal tolerability, immune enhancement, and tumor shrinkage via this approach. Although CCL21 induces immune responses, an immune-tolerant TME promotes immune evasion. Combining CCL21 and downregulating immune evasion pathways will program durable antitumor immune responses. One immune escape mechanism is dependent on immune inhibitory molecules that are upregulated on T cells in tumors causing a downregulation of antitumor activity. The programmed cell death protein 1 (PD-1, also known as CD279) is an inhibitory receptor that regulates immune responses. The PD-1 receptor interaction with the PD-L1 and PD-L2 ligands deliver inhibitory signals that regulate the balance between T cell activation and tolerance. We recently completed the first-in-man phase I clinical trial with CCL21 in which patients with advanced stage NSCLC received intratumoral administration of autologous DC overexpressing CCL21 (CCL21-DC). We observed CD8 T cell infiltration of the tumor and induction of systemic immune responses as evidenced by PBL IFNγ production in response to autologous tumor antigens [1]. However, our observation of T cell immune regulatory checkpoint expression in the TME of these patients suggests that tumor-mediated impairment of T cell function may be forestalling a more robust CCL21-mediated antitumor response. Combining CCL21 with PD-1 inhibition can augment host antitumor immunity and capture more NSCLC patients to be responsive to PD-1 checkpoint blockade therapy.

7.2 Requirements for Effective Antitumor Immune Activity

One of the challenges in developing immunotherapy for cancer is enlisting the host response to recognize poorly immunogenic tumors. Effective antitumor responses require antigen-presenting cells (APC), lymphocytes, and natural killer (NK) effectors. Although lung cancer cells express tumor antigens, limited expression of MHC antigens, defective transporters associated with antigen processing, and lack of costimulatory molecules make them ineffective APC. Both APC and T cell activities are reduced in lung cancer [2, 3], and intratumoral infiltration by relatively high numbers of activated T lymphocytes [4, 5] and APC [6] leads to better prognosis in lung cancer patients. Utilizing preclinical models of lung cancer, we are evaluating intratumoral delivery of immune-potentiating CCL21 chemokine via DC and stromal cell-based approaches for effective recruitment and activation of APC and T cells for the promotion of antitumor activity in lung cancer. The preclinical findings demonstrate that effective anticancer immunity can be achieved by CCL21-mediated recruitment of professional host APC for tumor antigen presentation to promote specific T cell activation [7–10]. Results from a phase I trial of intratumoral administration of autologous DC expressing CCL21 to lung cancer patients meets the objectives of the study in terms of safety and the induction of antitumor immune responses [1].

7.3 Rationale for CCL21 Chemokine in Cancer Therapy

Chemokines, a group of homologous, yet functionally divergent proteins, directly mediate leukocyte migration and activation and play a role in regulating angiogenesis. They also function in maintaining immune homeostasis and secondary lymphoid organ architecture. CCL21 has been identified as a lymphoid chemokine that is predominantly and constitutively expressed by high endothelial venules in lymph nodes and Peyer's patches, lymphatic vessels and stromal cells in spleen and appendix [11]. CCL21 binds to the chemokine receptor CCR7 and is a chemoattractant for mature DC and naive and memory T cells. Acting through the G-protein-coupled CCR7 transmembrane receptor, CCL21 mediates

the recruitment and colocalization of naive lymphocytes and antigen stimulated DC into T cell zones of secondary lymphoid organs, facilitating T cell activation. T cell activation in vivo occurs in a lymphoid milieu that presents chemotactic and T cell receptor signals concurrently. The T cell zone chemokines such as CCL21 are bound to the surface of lymph node DC. Contact with antigen-presenting cells bearing CCL21 chemokine co-stimulates T cells by a two-step contact mechanism. T cells initially form an antigen-independent "tethered" adhesion on CCL21-bearing antigen-presenting cells. The formation of these tethers supersedes T cell receptor signaling and immunological synapse formation. However, chemokine-tethered T cells are hyperresponsive to subsequent contacts with antigen-presenting cells. Thus, T cells are co-stimulated "in trans" and sequentially after initial engagement with their CCL21-rich environment [12]. This chemokine, along with CCL19, is required for normal lymphoid tissue organization that is ultimately essential for effective T cell-DC interactions. DC are uniquely potent APCs involved in the initiation of immune responses. Serving as immune system sentinels, DC are responsible for Ag acquisition in the periphery and subsequent transport to T cell areas in lymphoid organs where they prime specific immune responses. Thus, chemokines that attract both DC and lymphocyte effectors into the tumor can serve as potent agents in immunotherapy. In addition to inducing chemotactic migration, CCL21 co-stimulates expansion of CD4+ and CD8+ T cells and induces Th1 polarization. The immune suppressor cell population, CD4+CD25+ regulatory T cells are hyporesponsive to CCL21-induced migration and unresponsive to CCL21 co-stimulation [13]. These functions of CCL21 to both attract naïve T cells and co-stimulate their proliferation, differentiation, and activation suggest that CCL21 is a pivotal molecule for priming T cell responses and has therapeutic implications for local delivery of CCL21. The antitumor effectors NK and NKT cell subsets also express the CCR7 receptor and are chemoattracted by CCL21. The recruitment of NK and NKT cells is advantageous because these effectors can recognize tumor targets in the absence of MHC expression. The use of chemokines to attract DC, lymphocyte, NK, and NKT effectors into tumors can serve as an effective antitumor strategy. In addition, CCL21 has potent angiostatic effects, thus adding further support for its use in cancer therapy. CCL21 priority ranking is 13 among the list of 20 National Cancer Institute ranked biological agents with high potential for use in cancer therapy. Generation of an antitumor immune response requires the coordinate interaction of NK, T, and DC effectors. There is a paucity of these effectors in the tumor. One regimen to initiate antitumor responses is through the use of chemokines that induce both efficient recruitment and strong activation of effector cells in the tumor mass. The rationale for the use of CCL21 for immune therapy against solid tumors is that CCL21 sculpts host immune responses by recruiting and colocalizing NK, DC, and T cell effectors to mediate potent antitumor activity.

7.4 CCL21 Induction of Antitumor Immune Responses

The development of intratumoral therapies to effectively augment local and systemic antitumor immunity in lung cancer can lead to a paradigm shift in the current forms of therapy. In preclinical model systems, intratumoral administration of DC led to both local and systemic antitumor responses [14]. This form of therapy can be augmented by utilizing intratumoral administration of genetically modified DC overexpressing certain cytokine genes [15]. Congruent with this overall concept, the intratumoral administration of recombinant CCL21 mediated T cell-dependent antitumor responses [7]. In immune competent mice, intratumoral CCL21 injection led to a significant increase in CD4 and CD8 T lymphocytes and DC infiltrating both the tumor and draining lymph nodes. Studies performed in CD4 and CD8 T cell knockout mice revealed a direct therapeutic requirement for both CD4 and CD8 T cell subsets for CCL21-mediated tumor regression. These findings were the first demon-

stration of effective antitumor responses mediated by CCL21 [7]. CCL21-mediated antitumor responses exhibited an increased influx of CD4 and CD8 T cell subsets as well as DC at the tumor sites. Accompanying this cell infiltrates were increases in IFNγ, MIG/CXCL9, IP-10/CXCL10, GM-CSF, and IL-12, but a concomitant decrease in the immunosuppressive molecules PGE-2 and TGFβ. Lymphocytes from CCL21-treated tumor-bearing mice demonstrated enhanced specific responses against autologous tumors suggesting the generation of systemic immune responses [7, 9]. The importance of IFNγ, MIG/CXCL9, and IP-10/CXCL10 in CCL21 therapy was assessed. In vivo depletion of IP-10/CXCL10, MIG/CXCL9, or IFNγ indicates that the full potency of CCL19 or CCL21-mediated antitumor responses requires the induction of IFNγ, MIG/CXCL9, and IP-10/CXCL10 in concert in this model. Neutralization of any one of these cytokines led to a decrease in the frequency of CXCR3+ve T cells and CD11c+ve DC in the tumor [9, 16]. Based on these results, experiments were performed to evaluate the tumorigenicity of CCL21 gene-modified murine lung cancer cells. In all three tumor models, subcutaneous implantation of retroviral-mediated CCL21 gene-modified lung cancer cells led to T cell-mediated tumor eradication. Following our initial description of the antitumor activities of CCL21, several groups have reported that CCL21 has potent antitumor properties in a variety of model systems [17–21]. In all models, CCL21 demonstrated potent regression of tumors, which was shown to be dependent on host T cell immunity. All these studies reaffirmed the antitumor efficacy of CCL21, further supporting the rationale to proceed with clinical investigations of this chemokine.

7.5 CCL21 Gene-Modified DC Therapy for Lung Cancer

Our studies demonstrate that intratumoral administration of recombinant CCL21 reduced tumor burden in murine lung cancer models [7]. However the antitumor activity induced by recombinant CCL21 required high and frequent dosing because proteins administered intratumorally are not retained locally for prolonged periods. Although these studies delineated the role of CCL21 as an effective antitumor agent, frequent high dose intratumoral administration in the lung is invasive and clinically limiting with the potential of unnecessary systemic toxicity. Based on the limitations of this approach, we examined the use of DC for intratumoral CCL21 delivery [9, 10]. The intratumoral approach utilizes in situ tumor as a source of antigen. In contrast to immunization with purified peptide Ag, autologous tumor has the capacity to provide the DC administered in the tumor site access to the entire repertoire of available antigens in situ. This increases the likelihood of a response and reduces the potential for tumor resistance due to phenotypic modulation. To achieve in situ tumor antigen uptake and presentation, intratumoral administration of *ex vivo*-generated CCL21 gene-modified murine bone marrow-derived DC was utilized in a subcutaneous murine lung cancer model [9]. To determine if a cell type other than DC expressing CCL21 could also induce tumor reduction in this model, fibroblast cells were also evaluated as a delivery vehicle. In addition to fibroblast cells' ability to process and present antigens, the use of fibroblasts represents a promising treatment approach for lung cancer. These cells contribute to the formation of tumor-associated stroma [22], and the tumor microenvironment preferentially promotes their engraftment as compared with other tissues [23], making them an ideal system for tumor-selective delivery. Our data also support that reprogramming the TME with fibroblasts modified to express CCL21 alters the inflammatory infiltrates in the TME and promotes antitumor activity. The advantages of using transduced fibroblast cells for paracrine secretion of CCL21 are that fibroblasts (1) produce physiologically relevant levels of CCL21 after transduction, (2) are readily available for culture and expansion, (3) provide a platform for the development of CCL21-based antitumor strategies, (4) can process and present antigens to T cells, and (5) potentiate the activities of immune and innate

effectors in the TME. For translation to lung cancer patients we have the option of utilizing bone marrow-derived MHC-matched GMP-grade genetically modified donor stromal cells from a tissue bank that will circumvent autologous DC preparation, minimize batch-to-batch variability, and allow for comparability and standardization. DC or fibroblasts were transduced with an adenoviral vector expressing secondary lymphoid tissue chemokine (CCL21/SLC) to attract mature host DC and activated T cells in the tumor site. Established palpable tumors were treated with intratumoral DC-AdCCL21, Fib-AdCCL21, or controls. Intratumoral therapy with 10^6 DC-AdCCL21 (7–10 ng/ml/10^6 cells/24 h of CCL21) at weekly intervals for 3 weeks showed tumor eradication in 60% of the mice whereas therapy with 10^6 fib-AdCCL21 had complete resolution of tumors in 25% of mice because an optimum dose of Fib-AdCCL21 may not have been utilized for these studies [9]. Further investigation is required to determine the dose of fib-AdCCL21 that will perform as effectively as CCL21 gene-modified DC. In contrast, only 12% of the mice treated with unmodified or control vector modified DC (DCAdCV) showed complete tumor eradication. Intratumoral injection of AdCCL21 also led to tumor reduction, though at the dose tested it was not as effective as DC-AdCCL21 [9]. In addition, circulating neutralizing antibodies against adenovirus in patients will be a limiting factor for the use of adenovirus-based vectors to deliver CCL21. In the tumor model tested, intratumoral DC-AdCCL21 administration led to increases in the CD4+, CD8+, and CD3+CXCR3+ T cells as well as DC expressing CD11c+ and DEC205+ but decreases in CD4+CD25+ T regulatory cells infiltrating the tumors. Accompanying the tumor cellular infiltrates were enhanced elaboration of GM-CSF, IFNγ, MIG/CXCL9, IP-10/CXCL10, and IL-12 but decreases in the immunosuppressive mediators TGFβ and PGE2. DC-AdCCL21-treated tumor-bearing mice showed enhanced frequency of tumor-specific T lymphocytes secreting IFNγ and induced protective immunity [9]. The reduction in tumor growth may be explained by an increase in the frequency of activated T effector cell-mediated tumor apoptosis and/or T IFNγ-mediated anti-angiogenesis. In vivo depletion of IP-10/CXCL10, MIG/CXCL9, or IFNγ significantly reduced the antitumor efficacy of DC-AdCCL21 [9]. Based on these observations, we determined the antitumor effects of DC-AdCCL21 in a clinically relevant model of lung cancer. We utilized transgenic mice in which the adenocarcinomas develop in an organ-specific manner and have an average life span of 4 months. DC AdCCL21 (10^6 cells) or controls (diluent, DC (10^6 cells) and DC–AdCV (10^6 cells), AdCV (10^6 pfu) and AdCCL21 (10^6 pfu)) were administered once into the lungs of 3-month-old transgenic mice. When evaluated at 4 months of age, there was reduced tumor burden in DC-AdCCL21-treated CC-10 mice compared with the control groups. Median survival was 18 ± 2 weeks for all control-treated mice. In contrast, mice treated with DC-AdCCL21 had a median survival of 24 ± 1 weeks ($p < 0.01$ for DC-AdCCL21 compared to controls). In addition to marked tumor reduction, histological examination revealed areas of distinct mononuclear infiltration in remaining tumor [10].

7.6 Clinical Translation of DC-AdCCL21 Therapy to Lung Cancer Patients

Based on the results in the preclinical model systems, a clinical trial was initiated using intratumoral injection of CCL21 gene-modified autologous DC in lung cancer. The intratumoral route of DC administration is used to activate specific immune responses within the TME and, in addition, to generate systemic immunity. Several studies reveal [14, 24] that intratumoral DC administration may be particularly effective as an antitumor strategy. Lung cancer patients have decreased numbers of circulating competent DC; thus, injecting DC within the lung tumor site may be a particularly effective approach. A correlation exists between the number of tumor-infiltrating DC and survival in cancer patients. In fact, there is a relationship between tumor-infiltrating DC aggregation and

apoptosis in situ in human NSCLC. This is consistent with recent studies indicating that attraction and activation of DC at the site of tumor elicits potent antitumor immunity [25]. Dieu-Nosjean et al. [6] have identified ectopic lymph node or tertiary lymphoid structures within human NSCLC specimens and demonstrated a correlation of their cellular content with clinical outcome. These structures have been referred to as tumor-induced bronchus-associated lymphoid tissue, which are follicle-like and contain germinal centers, similar to those in secondary lymphoid follicles of lymph nodes. The density of DC-Lamp, mature DC within these structures is a predictor of long-term survival in lung cancer patients [6]. These findings reveal that tumor-induced bronchus-associated lymphoid tissues have clinical relevance and participate in the host's antitumor immune response, and they are consistent with previously reported preclinical and clinical data [26, 27]. For example, in murine tumor models, Mulé reported that DC genetically modified to secrete CCL21 can produce lymphoid cell aggregates and, importantly, prime naive T cells extranodally within a tumor mass, resulting in the generation of tumor-specific T cells and subsequent tumor regression [17, 27]. Thus, the intratumoral approach may achieve tumor antigen presentation by using the tumor as an in vivo source of antigens for DC. In contrast to immunization with purified peptide antigen(s), autologous tumor has the capacity to provide the activated DC administered at the tumor site access to the entire repertoire of available antigens in situ. This increases the likelihood of a response and reduces the potential for tumor resistance because of phenotypic modulation. CCL21 is distinctly advantageous because of its capacity to elicit a type 1 cytokine response in vivo that promotes antitumor activity. Intratumoral infiltration of T lymphocytes and DC in lung cancer has been shown to be associated with a better patient outcome. In accord with this observation in lung cancer, a recent study demonstrates that the presence of lymph node (LN)-like vasculature in tumors, characterized by expression of peripheral node addressin and chemokine CCL21, is correlated with T cell infiltration and positive prognosis in breast cancer and melanoma patients [28]. The authors further demonstrated that LN-like vasculature is present in murine models of melanoma and lung carcinoma. LN-like vasculature enables infiltration by naive T cells that significantly delay tumor outgrowth after intratumoral activation. The mechanisms contributing to the development of this vasculature is attributed to effector CD8 T cells and NK cells that secrete LTα3 and IFNγ. LN-like vasculature is also associated with organized aggregates of B lymphocytes and gp38(+) fibroblasts, which resemble tertiary lymphoid organs that develop in models of chronic inflammation. The results of this study establish that LN-like vasculature as both a consequence of and key contributor to antitumor immunity [28]. On the basis of preclinical results, a phase I clinical evaluation was initiated at University of California Los Angeles (in collaboration with the National Cancer Institute—Rapid Access to Intervention Development program now NCI Experimental Therapeutics Program) in patients with advanced stage NSCLC. The safety and clinical activities of the intratumoral administration of autologous DC transduced with a replication deficient adenoviral vector to express CCL21 in patients with pathologically confirmed and radiographically measurable NSCLC (Stage IIIB/IV) who have tumor accessible by CT-guided or bronchoscopic intervention and are refractory to standard therapy were selected. A GMP-grade AdCCL21 replication-deficient virus [29] was made available through the RAID program to conduct the Phase I clinical trial. Human DCs transduced with advenovirus-CCL21 produce CCL21 to attract T cells and DCs. Results demonstrate tumor-specific systemic immune responses as assessed by the IFNγ T cell ELISPOT. Multiplex assessment of plasma cytokines before and after therapy in these patients revealed induction of IL-2, γ, IFN IL-12, and CXCL10. Immunohistochemistry of post-tumor biopsies revealed an influx of CD4-expressing tumor-infiltrating lymphocytes.

Results indicate that vaccination is safe with no associated adverse reactions at the DC-AdCCL21 (1×10^6, 5×10^6, 1×10^7, or 3×10^7 DC-AdCCL21 cells/injection) doses administered (days 0 and 7) and antitumor immune responses can be elicited particularly in higher doses.

7.7 Polymer-Based CCL21 Delivery

The science of biomaterial engineering for drug delivery has evolved considerably for the past 30 years. Novel technology allows to design functional, biocompatible, and biodegradable polymer vehicles, such as poly-ε-caprolactone (PCL), poly (lactide-co-glycolide) (PLG), as well as alginate and fibrin hydrogel, for molecular and cellular delivery in cancer immunotherapy [30]. Three-dimensional porous polymer scaffolds exhibit great ability to deliver cytokine molecules and immune cells with spatiotemporal specificity, to promote cell-cell interaction in matrix, and to direct cell function [30]. This ability forms the rationale for polymer-based CCL21 cancer immunotherapy for programming host immune cells in vivo. These materials can be further integrated with other anticancer treatments in the design of next-generation therapy against cancer [31]. PCL/PLCL copolymer loaded with DC-CCL21 or chemotherapy drug cisplatin has been tested in an animal model of head and neck squamous cell carcinoma (HNSCC) to prevent cancer recurrence [32, 33]. HNSCC is difficult to resect completely by surgery due to complicated context and therefore exhibits high recurrence rate in the patients [34]. A drug delivery platform with spatiotemporal specificity is in demand for anti-recurrence therapy. In order to accomplish these requirements, a polymer platform was made from a mixture of a ratio of 70:30 of PCL to PLCL with relevant amount of CCL21 and/or cisplatin and was spread on a glass to form a thin sheet. The final product is a flexible sheet that exhibits nice drug release kinetics and can adhere to the surgical resected tissue contours [32]. In the initial animal study, cisplatin-loaded PCL/PLCL polymer was applied intraoperatively to the surgical bed after partial tumor resection, replicating the difficult situation seen in patients. The cisplatin-secreting polymer effectively reduced tumors by over 16-fold as compared to control plain polymer and intratumoral cisplatin injection groups. When combined with radiation, polymer therapy led to a statistically significant lower tumor weight compared to the radiation alone group and the control group [32]. Based on the above data, the PCL/PLCL scaffold was later tested for antitumor efficacy of DC-CCL21 therapy. In order to improve DC culture condition for immunotherapy, a thin layer of fibrin hydrogel with 10^6 DCs seated inside was added to the surface of PCL/PLCL polymer [33]. The component of hydrogel and polymer was optimized for the maximum production of bioactive CCL21. After implantation to the partially resected tumor, the gradient of local CCL21 that resulted from its sustained and localized release led to the recruitment of CD4+ T cells and CD11c+ DCs into the tumor, while tumor-infiltrating Treg cells were decreased. Overall, DC-CCL21 polymer treatment significantly reduced tumor burden, compared to control DC group or recombinant CCL21 injection group [33]. Currently, antitumor efficacy of polymer loaded with recombinant CCL21 is being further evaluated with combination of cisplatin chemotherapy, immune checkpoint blockade, and radiation therapy. In addition to cytokines and immune cells, tumor-associated antigen can also be loaded in polymer to activate DCs. Subcutaneous implantation of PLG polymer loaded with cytokine GM-SCF, TLR agonist CpG, and tumor lysate as antigen led to host DC recruitment, activation, and subsequent homing to lymph nodes [35]. This vaccine induced 90% prophylactic tumor protection and therapeutic protection. The polymer scaffold also displayed long-term activity for months postimplantation, which is superior to all soluble administration methods to date [35]. Other biomaterials, such as vault nanoparticles, were investigated for intratumoral CCL21 delivery. In a well-characterized Lewis lung cancer model, CCL21-vault nanoparticle system showed effective antitumor efficacy.

A single intratumoral injection of CCL21-vault nanoparticles was able to recruit antitumor effectors that induced potent antitumor activity and inhibit tumor growth [36]. The nanoparticle system can be further designed for target delivery and specific payloads to prime the immune system.

7.8 Diagnostics and Prognostic Monitoring of CCL21 and Effects

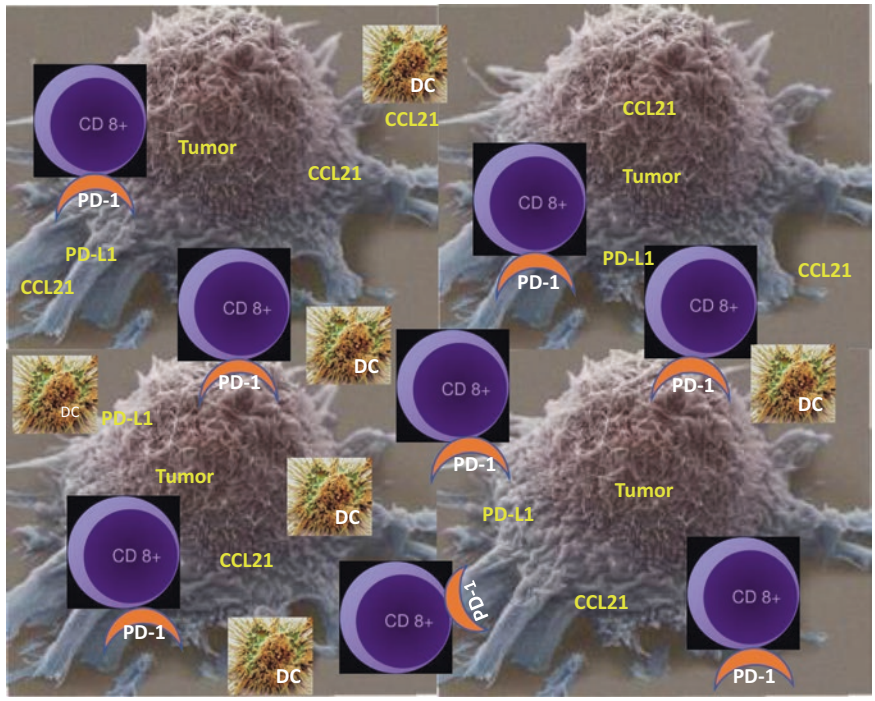
Diagnostic tests for CCL21 expression and protein concentrations in samples are performed by RT-PCR and ELISA. Tissue expression of CCL21 is assessed by immunocytochemistry and flow cytometry. Based on the preclinical data, high levels of CCL21 expression in tumors may be indicative of immune reactivity and serve as a prognostic marker for patient survival. Immune effects of CCL21 is monitored by antigen-specific IFN-γ T lymphocyte ELISPOTS, ELISA, or RT-PCR for Th1 cytokines and immunocytochemistry for T lymphocytes, NK and DC effector cell infiltrates. CCL21-mediated T cell activation can be monitored by immunocytochemistry for perforin and granzyme B-secreted activated T cells (Fig. 7.1).

7.9 Therapeutics

CCL21 is being developed as an anticancer therapeutic agent. The phase I clinical trials were in lung cancer and melanoma, but as the preclinical data warrants in other tumor models, this form of therapy may be extended to include other solid cancers. There is a strong rationale to combine CCL21 with immune checkpoint blockade therapy to increase T cell infiltrates in the tumor of patients who have minimal response to immune checkpoint blockade therapy. Recent groundbreaking studies in lung cancer immunotherapy reveal robust antitumor activity and durable responses in previously treated patients with progressive locally advanced or metastatic NSCLC. Immune inhibitory molecules are upregulated on T cells in tumors causing a downregulation of antitumor activity. The programmed cell death protein 1 (PD-1; also known as CD279) is an inhibitory receptor that regulates immune responses. The PD-1 receptor interaction with the PD-L1 and PD-L2 ligands delivers inhibitory signals that regulate the balance between T cell activation and tolerance. Recent studies reveal responses in approximately 20% of NSCLC patients treated with inhibitors of the PD-1 checkpoint. This includes robust and durable responses in previously treated patients with progressive locally advanced or metastatic NSCLC [37–42]. Studies in NSCLC and melanoma patient-derived tumor specimens reveal that responses to checkpoint blockade rely on tumor infiltration of activated T effector cells [38–41, 43]. It has been suggested that among patients who are nonresponsive or respond poorly to checkpoint blockade immunotherapies, there will be individuals who lack preexisting antitumor T cell responses [44]. This group appears to be comprised of patients who have absent or very limited immune responsiveness prior to initiation of therapy, and thus have limited CD8 T cell infiltration of the tumor and/or PD-L1 expression by tumor or TME. This situation appears to occur in approximately 50–60% of NSCLC cases [45]. Thus, it has been suggested that this deficit could be addressed with regimens that increase T cell infiltration combined with checkpoint inhibitors. Congruent with this concept, in a recent study, CCL21 enhanced the antitumor activity of PD-1 in a murine model of lung cancer [46].

7.10 Future Prospects

The results of the phase I studies in lung cancer and melanoma are promising. CCL21 is important in the formation of tertiary lymphoid structures and their presence in tumors is associated with favorable immune responses. Immunogenic tumors, with immune response positive gene signatures and/or increased TIL, have a better prognosis. Based on the findings on CCL21, it is



Control **CCL21-DC Peptide Vaccine + Anti-PD-1**

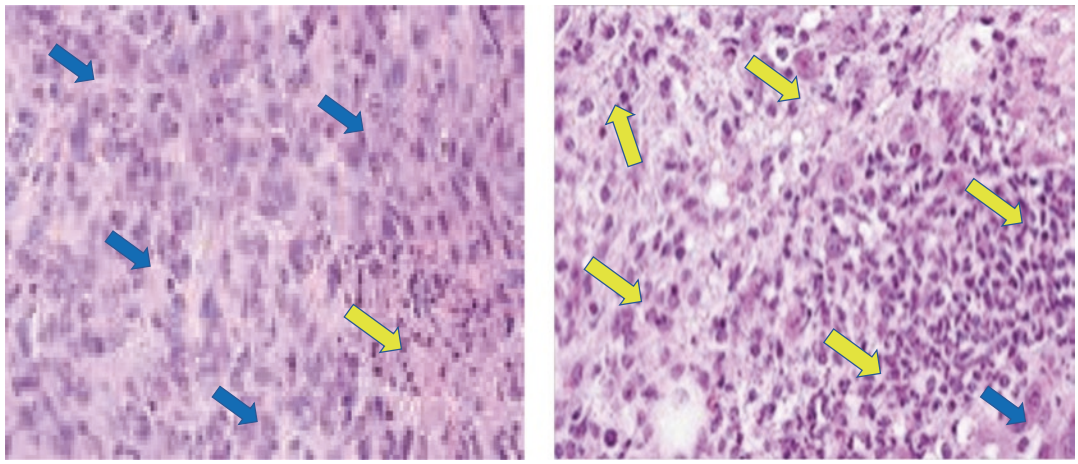


Fig. 7.1 (**a**–**d**) CCL21 programs PD-1 blockade antitumor activity. (**a**) CCL21-based strategies increase CD8 T and dendritic cells (DC) in the TME. PD-1 expression is increased on recruited T cells that makes them tolerant to the tumor. CCL21 combined with PD-1 blockade induces durable antitumor immunity leading to tumor regression/eradication. (**b**) CCL21-DC peptide vaccine combined with PD-1 blockade enhances activated T cells in the TME resulting in lung tumor destruction. Blue arrows indicate tumor and yellow immune infiltrates. *Magnification 60× objective.* (**c**) CCL21-DC peptide vaccine combined with PD-1 blockade increases CD3 T cells in the TME. Brown indicates CD3 T cells staining by immunohistochemistry. *Magnification 60× objective.* (**d**) CCL21-DC peptide vaccine combined with PD-1 blockade enhances activated T cells that secrete granzyme B in the TME resulting in lung tumor destruction. Brown indicates granzyme B staining by immunohistochemistry. *Magnification 60× objective*

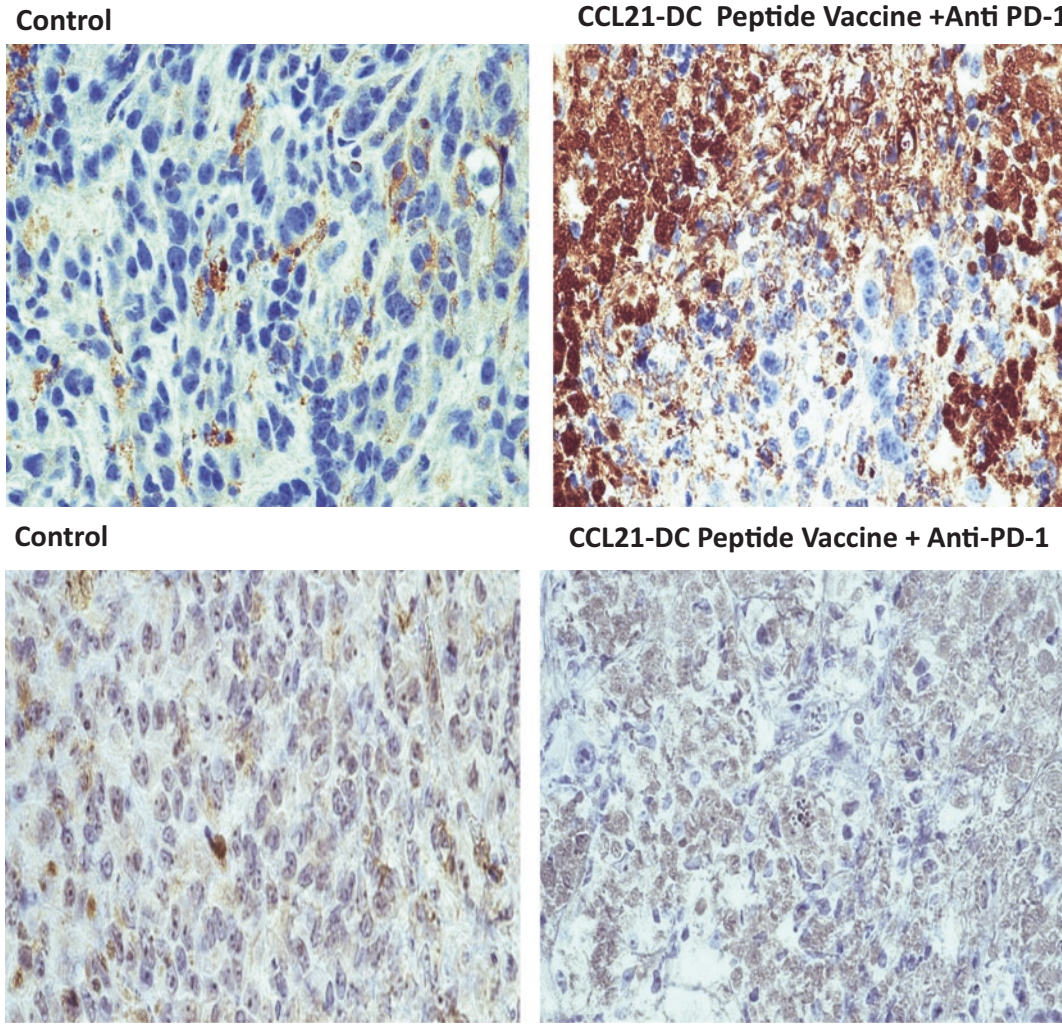


Fig. 7.1 (continued)

anticipated that the rational combination with immune checkpoint blockade therapy will improve the antitumor benefit of this chemokine in a broad range of solid tumors with low TIL frequency. Future studies could assess the combined efficacy of CCL21-based regimens with immune checkpoint blockade therapy in various solid tumors. CCL21-based therapeutic vaccination approaches will prove beneficial for tumors that are not accessible to intratumoral administration of CCL21 or that require multiple dosing. CCL21 in combination with PD-1 inhibition will capture more NSCLC patients that are not responsive to PD-1 blockade monotherapy. Furthermore, material and nanoparticle engineering provide several attractive strategies to design more potent CCL21 immunotherapy for cancer.

References

1. Lee JM, Lee MH, Garon E, Goldman JW, Salehi-Rad R, Baratelli FE et al (2017) Phase I trial of intratumoral injection of CCL21 gene-modified dendritic cells in lung cancer elicits tumor-specific immune responses and CD8(+) T-cell infiltration. Clin Cancer Res 23(16):4556–4568
2. Zou W (2005) Immunosuppressive networks in the tumour environment and their therapeutic relevance. Nat Rev Cancer 5:263–274

3. Almand B, Resser JR, Lindman B, Nadaf S, Clark JI, Kwon ED et al (2000) Clinical significance of defective dendritic cell differentiation in cancer. Clin Cancer Res 6:1755–1766
4. Johnson SK, Kerr KM, Chapman AD, Kennedy MM, King G, Cockburn JS et al (2000) Immune cell infiltrates and prognosis in primary carcinoma of the lung. Lung Cancer 27:27–35
5. Hiraoka K, Miyamoto M, Cho Y, Suzuoki M, Oshikiri T, Nakakubo Y et al (2006) Concurrent infiltration by CD8+ T cells and CD4+ T cells is a favourable prognostic factor in non-small-cell lung carcinoma. Br J Cancer 94:275–280
6. Dieu-Nosjean MC, Antoine M, Danel C, Heudes D, Wislez M, Poulot V et al (2008) Long-term survival for patients with non-small-cell lung cancer with intratumoral lymphoid structures. J Clin Oncol 26:4410–4417
7. Sharma S, Stolina M, Luo J, Strieter RM, Burdick M, Zhu LX et al (2000) Secondary lymphoid tissue chemokine mediates T cell-dependent antitumor responses in vivo. J Immunol 164:4558–4563
8. Sharma S, Stolina M, Zhu L, Lin Y, Batra R, Huang M et al (2001) Secondary lymphoid organ chemokine reduces pulmonary tumor burden in spontaneous murine bronchoalveolar cell carcinoma. Cancer Res 61:6406–6412
9. Yang SC, Hillinger S, Riedl K, Zhang L, Zhu L, Huang M et al (2004) Intratumoral administration of dendritic cells overexpressing CCL21 generates systemic antitumor responses and confers tumor immunity. Clin Cancer Res 10:2891–2901
10. Yang SC, Batra RK, Hillinger S, Reckamp KL, Strieter RM, Dubinett SM et al (2006) Intrapulmonary administration of CCL21 gene-modified dendritic cells reduces tumor burden in spontaneous murine bronchoalveolar cell carcinoma. Cancer Res 66:3205–3213
11. Gunn MD, Tangemann K, Tam C, Cyster JG, Rosen SD (1998) Williams LT. A chemokine expressed in lymphoid high endothelial venules promotes the adhesion and chemotaxis of naive T lymphocytes. Proc Natl Acad Sci U S A 95:258–263
12. Friedman RS, Jacobelli J, Krummel MF (2006) Surface-bound chemokines capture and prime T cells for synapse formation. Nat Immunol 7:1101–1108. Epub 2006 Sept 10
13. Flanagan K, Moroziewicz D, Kwak H, Horig H, Kaufman HL (2004) The lymphoid chemokine CCL21 costimulates naive T cell expansion and Th1 polarization of non-regulatory CD4+ T cells. Cell Immunol 231:75–84. Epub 2005 Jan 21
14. Sharma S, Miller P, Stolina M, Zhu L, Huang M, Paul R et al (1997) Multi-component gene therapy vaccines for lung cancer: effective eradication of established murine tumors in vivo with Interleukin 7/Herpes Simplex Thymidine Kinase-transduced autologous tumor and ex vivo-activated dendritic cells. Gene Therapy 4:1361–1370
15. Miller PW, Sharma S, Stolina M, Butterfield LH, Luo J, Lin Y et al (2000) Intratumoral administration of adenoviral interleukin 7 gene-modified dendritic cells augments specific antitumor immunity and achieves tumor eradication. Hum Gene Ther 11:53–65
16. Sharma S, Stolina M, Yang SC, Baratelli F, Lin JF, Atianzar K et al (2003) Tumor cyclooxygenase 2-dependent suppression of dendritic cell function. Clin Cancer Res 9:961–968
17. Kirk CJ, Hartigan-O'Connor D, Mule JJ (2001) The dynamics of the T-cell antitumor response: chemokine-secreting dendritic cells can prime tumor-reactive T cells extranodally. Cancer Res 61:8794–8802
18. Novak L, Igoucheva O, Cho S, Alexeev V (2007) Characterization of the CCL21-mediated melanoma-specific immune responses and in situ melanoma eradication. Mol Cancer Ther 6:1755–1764
19. Liang CM, Zhong CP, Sun RX, Liu BB, Huang C, Qin J et al (2007) Local expression of secondary lymphoid tissue chemokine delivered by adeno-associated virus within the tumor bed stimulates strong anti-liver tumor immunity. J Virol 81:9502–9511. Epub 2007 Jun 13
20. Wu S, Xing W, Peng J, Yuan X, Zhao X, Lei P et al (2008) Tumor transfected with CCL21 enhanced reactivity and apoptosis resistance of human monocyte-derived dendritic cells. Immunobiology 213:417–426. Epub 2007 Nov 28
21. Yousefieh N, Hahto SM, Stephens AL, Ciavarra RP (2009) Regulated expression of CCL21 in the prostate tumor microenvironment inhibits tumor growth and metastasis in an orthotopic model of prostate cancer. Cancer Microenviron 2(1):59–67
22. Hall B, Andreeff M, Marini F (2007) The participation of mesenchymal stem cells in tumor stroma formation and their application as targeted-gene delivery vehicles. Handb Exp Pharmacol 180:263–283
23. Studeny M, Marini FC, Champlin RE, Zompetta C, Fidler IJ, Andreeff M (2002) Bone marrow-derived mesenchymal stem cells as vehicles for interferon-beta delivery into tumors. Cancer Res 62:3603–3608
24. Tatsumi T, Huang J, Gooding WE, Gambotto A, Robbins PD, Vujanovic NL et al (2003) Intratumoral delivery of dendritic cells engineered to secrete both interleukin (IL)-12 and IL-18 effectively treats local and distant disease in association with broadly reactive Tc1-type immunity. Cancer Res 63:6378–6386
25. Lapteva N, Aldrich M, Rollins L, Ren W, Goltsova T, Chen SY et al (2009) Attraction and activation of dendritic cells at the site of tumor elicits potent antitumor immunity. Mol Ther 17:1626–1636. Epub 2009 Jun 16
26. Coppola D, Mule JJ (2008) Ectopic lymph nodes within human solid tumors. J Clin Oncol 26:4369–4370
27. Kirk CJ, Hartigan-O'Connor D, Nickoloff BJ, Chamberlain JS, Giedlin M, Aukerman L et al (2001) T cell-dependent antitumor immunity mediated by

secondary lymphoid tissue chemokine: augmentation of dendritic cell-based immunotherapy. Cancer Res 61:2062–2070
28. Peske JD, Thompson ED, Gemta L, Baylis RA, Fu YX, Engelhard VH (2015) Effector lymphocyte-induced lymph node-like vasculature enables naive T-cell entry into tumours and enhanced anti-tumour immunity. Nature Commun 6:7114
29. Baratelli F, Takedatsu H, Hazra S, Peebles K, Luo J, Kurimoto PS et al (2008) Pre-clinical characterization of GMP grade CCL21-gene modified dendritic cells for application in a phase I trial in non-small cell lung cancer. J Transl Med 6:38
30. Huebsch N, Mooney DJ (2009) Inspiration and application in the evolution of biomaterials. Nature 462:426–432
31. Li WA, Mooney DJ (2013) Materials based tumor immunotherapy vaccines. Curr Opin Immunol 25: 238–245
32. Hu D, Lau OD, Wang L, Wang G, Schaue D, Zhu L et al (2012) A novel modular polymer platform for the treatment of head and neck squamous cell carcinoma in an animal model. Arch Otolaryngol Head Neck Surg 138:412–417
33. Lin Y, Luo J, Zhu WE, Srivastava M, Schaue D, Elashoff DA et al (2014) A cytokine-delivering polymer is effective in reducing tumor burden in a head and neck squamous cell carcinoma murine model. Otolaryngol Head Neck Surg 151:447–453
34. Ross DA, Hundal JS, Son YH, Ariyan S, Shin J, Lowlicht R et al (2004) Microsurgical free flap reconstruction outcomes in head and neck cancer patients after surgical extirpation and intraoperative brachytherapy. Laryngoscope 114:1170–1176
35. Ali OA, Huebsch N, Cao L, Dranoff G, Mooney DJ (2009) Infection-mimicking materials to program dendritic cells in situ. Nat Mater 8:151–158
36. Kar UK, Srivastava MK, Andersson A, Baratelli F, Huang M, Kickhoefer VA et al (2011) Novel CCL21-vault nanocapsule intratumoral delivery inhibits lung cancer growth. PLoS One 6:e18758
37. Carreno BM, Magrini V, Becker-Hapak M, Kaabinejadian S, Hundal J, Petti AA et al (2015) Cancer immunotherapy. A dendritic cell vaccine increases the breadth and diversity of melanoma neoantigen-specific T cells. Science 348:803–808
38. Herbst RS, Soria JC, Kowanetz M, Fine GD, Hamid O, Gordon MS et al (2014) Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. Nature 515:563–567
39. Brahmer JR, Tykodi SS, Chow LQ, Hwu WJ, Topalian SL, Hwu P et al (2012) Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. N Engl J Med 366:2455–2465
40. Gettinger SN, Horn L, Gandhi L, Spigel DR, Antonia SJ, Rizvi NA et al (2015) Overall survival and long-term safety of nivolumab (Anti-Programmed Death 1 Antibody, BMS-936558, ONO-4538) in patients with previously treated advanced non-small-cell lung cancer. J Clin Oncol 33(18):2004–2012
41. Soria JC, Marabelle A, Brahmer JR, Gettinger S (2015) Immune checkpoint modulation for non-small cell lung cancer. Clin Cancer Res 21:2256–2262
42. Garon EB, Rizvi NA, Hui R, Leighl N, Balmanoukian AS, Eder JP et al (2015) Pembrolizumab for the treatment of non-small-cell lung cancer. N Engl J Med 372:2018–2028
43. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L et al (2014) PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature 515:568–571
44. Delamarre L, Mellman I, Yadav M (2015) Cancer immunotherapy. Neo approaches to cancer vaccines. Science 348:760–761
45. Velcheti V, Schalper KA, Carvajal DE, Anagnostou VK, Syrigos KN, Sznol M et al (2014) Programmed death ligand-1 expression in non-small cell lung cancer. Lab Invest 94:107–116
46. Salehi-Rad R, Walser T, So S, Park S, Sharma L, Jay SD (2017) CCL21 combined with PD-1 blockade cooperatively inhibits tumor growth in KRAS murine model of NSCLC. J Thorac Oncol 12:S1537