Oxaliplatin facilitates tumor-infiltration of T cells and natural-killer cells for enhanced tumor immunotherapy in lung cancer model

Min Xin[a], Dan Lin[a], Nahan Yan[a], Huiling Li[a], Jieping Li[b] and Zongming Huang[c]

Platinum is reported to have adjuvant immune properties, whether oxaliplatin (OXA) could be utilized to synergize with anti-programmed cell death-1 (PD-1) antibody or anti-NKG2D (natural-killer group 2, member D) antibody is investigated. Subcutaneous A549 lung cancer and murine Lewis lung carcinoma (LLC) models were constructed, which were further intravenously injected with platinum-based drugs or concomitant administrated with anti-PD-1 antibody and or anti-NKG2D antibody. The tumor volume and the proportion of myeloid cells ($CD45^+CD11b^+$), $CD3^+$T cells and NK ($NK1.1^+$) cells were detected. The relative expression of chemokine (C-X-C motif) ligand 9 (CXCL9), CXCL10 and CXCL11 and C-X-C motif chemokine receptor 3 (CXCR3) was detected with the ELISA, western blot and flow cytometry. The three platinum drugs (cisplatin, DDP; carboplatin, CBP; OXA) showed similar effects to inhibit A549 tumor growth in immune-deficient mice. While OXA exhibited better antitumor efficacy in wild-type mice bearing LLC with downregulated myeloid cells proportion, upregulated concentration of CXCL9, CXCL10 and CXCL11, and upregulated proportion and CXCR3 expression on T cells and NK cells. OXA combined with anti-PD1 or anti-NKG2D synergistically improved tumor growth inhibition and survival. The combination of OXA to anti-PD1 and anti-NKG2D antibodies will provide the most appropriate treatment benefit. Oxaliplatin promotes T cells and NK cells infiltration through the CXCL9/10/11-CXCR3 axis to enhance anti-PD1 or anti-NKG2D immunotherapy in lung cancer. *Anti-Cancer Drugs* 33: 117–123 Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc.

Anti-Cancer Drugs 2022, **33**:117–123

Keywords: anti-PD1, lung cancer, NK cells, oxaliplatin, T cells

[a]Department of Oncology, [b]Department of Clinical Laboratory, and [c]Department of Pathology Hospital of People's Armed Police of Fujian, Fuzhou, Fujian, China

Correspondence to Dr. MinXin, Department of Oncology, Hospital of People's Armed Police of Fujian, 159 Northwest Road, Fuzhou, Fujian 350003, China
Tel: +86 13600885170; e-mail: wjxinmin@sina.com

Received 17 July 2021 **Revised form accepted** 4 August 2021

Introduction

Due to the intrinsic aggressive and rapidly growing characteristics, lung cancer is the leading cause of cancer death among men and women [1,2]. Despite extensive efforts to improve survival outcomes, platinum chemotherapy (cisplatin, DDP; oxaliplatin, OXA; carboplatin, CBP) is still recommended as the first-line treatment option for advanced non–small cell lung cancer (NSCLC) for decades, which is mainly attributed to the promotion of cancer cell apoptosis by the covalent binding to DNA [3,4]. Unfortunately, most patients with lung cancer will relapse and become resistant to platinum-based therapy with unsatisfied efficacy.

In recent years, immunotherapy has emerged as an essential treatment option in lung cancer [5]. Immune checkpoint blocking antibodies, such as anti-programmed cell death-1 (PD-1) antibody (pembrolizumab and nivolumab), anti-programmed death-ligand 1 (PD-L1) antibody (atezolizumab and avelumab), have been approved for the treatment of NSCLC. Although PD-1/PD-L1 antibody shows the tremendous commercial value and broad clinical application prospects, the effective rate of PD-1/PD-L1 antibody in the treatment of advanced lung cancer is only about 20% [6,7], which seriously limits the range of patients who can benefit from it [8,9]. The main reason for the limited effect of PD1/PDL1 antibody therapy is the lack of infiltrating T cells in tumor tissues [10,11]; aside from T cells, preclinical and clinical trials have shown that other immune cells infiltration, such as natural-killer (NK) cells, also have significant antitumor effects [12,13].

It is worth noting that recent basic and clinical investigations demonstrate that at least part of the antitumor efficacy of platinum chemotherapy may be due to potentiating immunogenic effects, such as signal transducer and activator of transcription (STAT) regulation, immunogenic type of cancer cell death induction, PD-L1 modulation [14]. At the same time, there is no relevant investigation to deciphering the immunogenic effect of platinum in lung cancer.

In this study, we find that oxaliplatin can promote the infiltration of T cells and NK cells into tumor tissue, and combining with anti-PD1 and or anti-NKG2D (natural-killer group 2, member D) antibody may have a more significant antitumor effect.

0959-4973 Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc. DOI: 10.1097/CAD.0000000000001248

Methods and materials

Tumorigenesis mice model

Athymic male C57BL/6 nude Foxn1nu mice (6–8 weeks old) and C57BL/6 mice were ordered from Peking Vital River Laboratory Animal Ltd. (Beijing, China), and both A549 human lung adenocarcinoma cells and murine Lewis Lung Carcinoma Cell Line (LL/2) were purchased from China Center for Type Culture Collection. A549 cells or LLC cells (2×10^5 cells in Hank's Buffered Salt Solution containing 1.35 mg/mL Matrigel) were subcutaneously injected into the right flank of C57BL/6 nude mice or C57BL/6 wide type mice. After 4 days after inoculation, the length and width of the tumor mass were measured and calculated as V=length.width^2/2, and the platinum-based chemotherapy (3.0 mg/kg, every 4 days for three times) and or anti-PD1 or anti-NKG2D treatment (2.5 mg/kg, every 4 days for three times) was initiated when tumor volume reached about 50 mm^3. The study protocol was approved by the institutional animal care and use committee of Hospital of People's Armed Police of Fujian.

ELISA

The relative content of C-X-C Motif Chemokine Ligand 9 (CXCL9), CXCL10 and CXCL11, transforming growth factor (TGF)-β, interleukin (IL)-12p70 and interferon (IFN)-γ were detected with relevant ELISA kit (eBioscience, San Diego, California, USA) according to the manufacturer's instructions. All standards and samples were measured with a SpectraMax M5 microplate (Molecular Devices) at a wavelength of 450 nm.

Flow cytometry

The tumor tissues were dissociated with gentle MACS, and the cells (1×10^6) were suspended with 100 µl PBS, which were then incubated with labeled primary antibodies (4°C, for 30 min) and analyzed with FCSalyzer. The antibodies utilized in this investigation included anti-mice PE-CD45 antibody, anti-mice PerCP-Cy5.5-CD11b antibody, anti-mice BV510-CD3 antibody, anti-mice APC-NK1.1 antibody and anti-mice PE-Cy7-CXCR3 antibody (Biolegend, San Diego, California, USA).

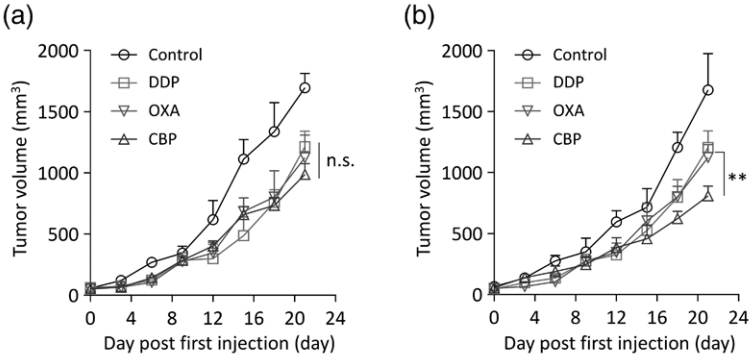
Quantitative real-time RT-PCR

TRIzol reagent (Invitrogen, Waltham, Massachusetts, USA) was used to extract total RNA according to the manufacturer's instructions. cDNA was reverse-transcribed from 1 µg RNA with a high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, California, USA). SYBR Green master mix (Roche, Penzberg, Upper Bavaria, Germany) was utilized to detect the amplification of interest genes. The reaction procedures were as follows: 95°C for 10 min, 40 cycles of 95°C for 15 s and 60°C for 1 min. Expression data were normalized to β-actin mRNA expression. Primer sequences were listed: β-actin, forward primer 5′-CTAAGGCCAACCGTGAAAAG-3′, reverse primer 5′-TACATGGCTGGGGTGTTGA-3′; CXCL9, 5′-CCTAGTGATAAGGAATGCACGATG-3′, reverse primer 5′-CTAGGCAGGTTTGATCTCCGTTC-3′; CXCL10, forward primer 5′- ATCATCCCTGCGAGCCTATCCT-3′, reverse primer 5′- GACCTTTTTTTGGCTAAACGCTTTC-3′; CXCL11, forward primer 5′- CCGAGTAACGGCTGCGACAAAG-3′, reverse primer 5′-CCTGCATTATGAGGCGAGCTTG-3′.

Western blotting

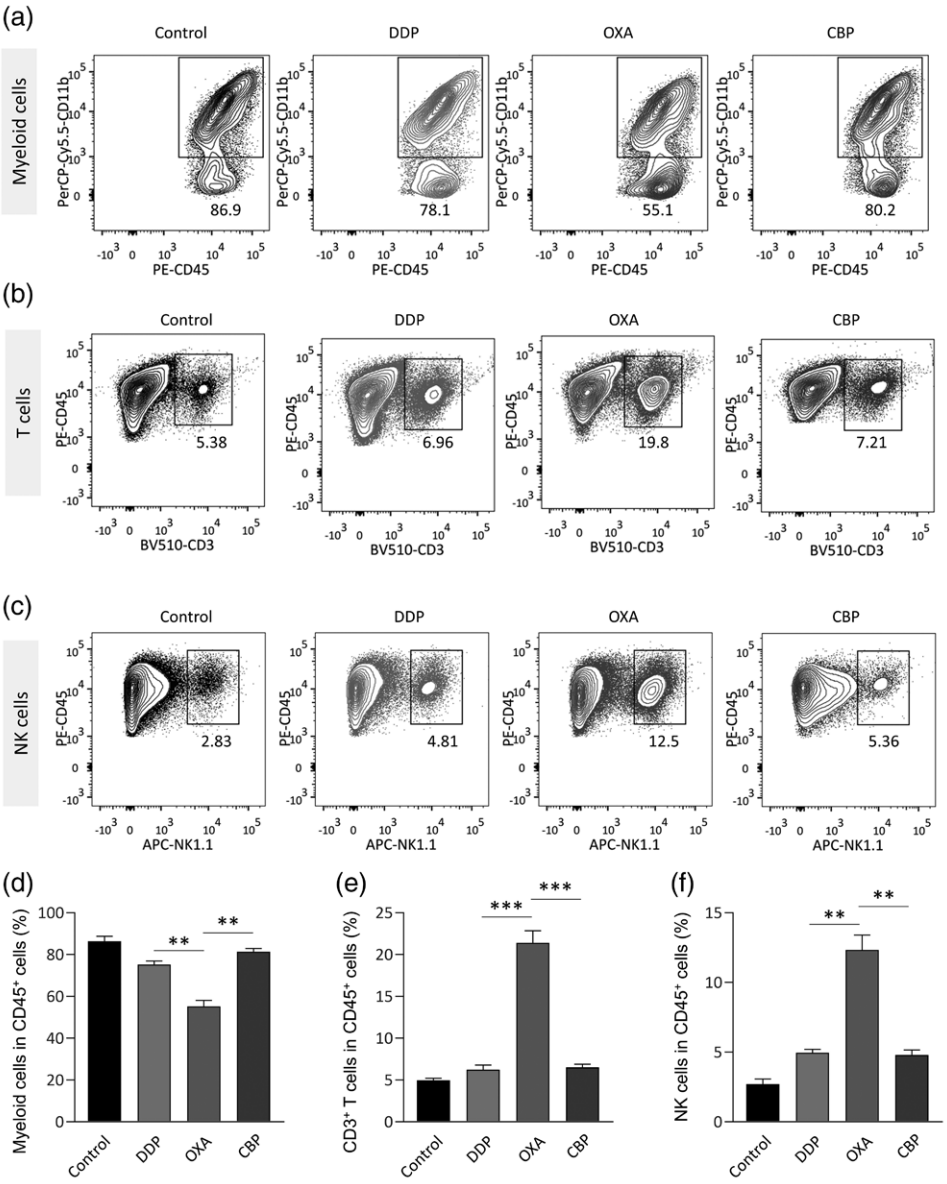
Cultured cancer cells were isolated and lysed with RIPA lysis and extraction buffer, which were further separated by 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene fluoride

Fig. 1



Oxaliplatin (OXA) exhibits better antitumor efficacy in wild-type mice bearing subcutaneous murine Lewis lung carcinoma (LLC). (a) Therapeutic efficacy of cisplatin (DDP), oxaliplatin (OXA) and carboplatin (CBP) in nude mice bearing human non–small cell lung cancer cell A549. A549 lung carcinoma model was established in nude mice, and mice were intravenously injected with DDP, OXA, CBP every 3 days for three times when tumor volumes reached 50 mm^3, the injection dose was 3.0 mg/kg. (b) Therapeutic efficacy of DDP, OXA and CBP in C57BL/6 mice bearing murine Lewis lung carcinoma (LLC). Tumor-bearing mice were intravenously injected with DDP, OXA, CBP every 3 days for three times when tumor volumes reached 50 mm^3; the injection dose was 3.0 mg/kg. Data are presents as mean ± SD. $^{**}P < 0.01$.

Fig. 2



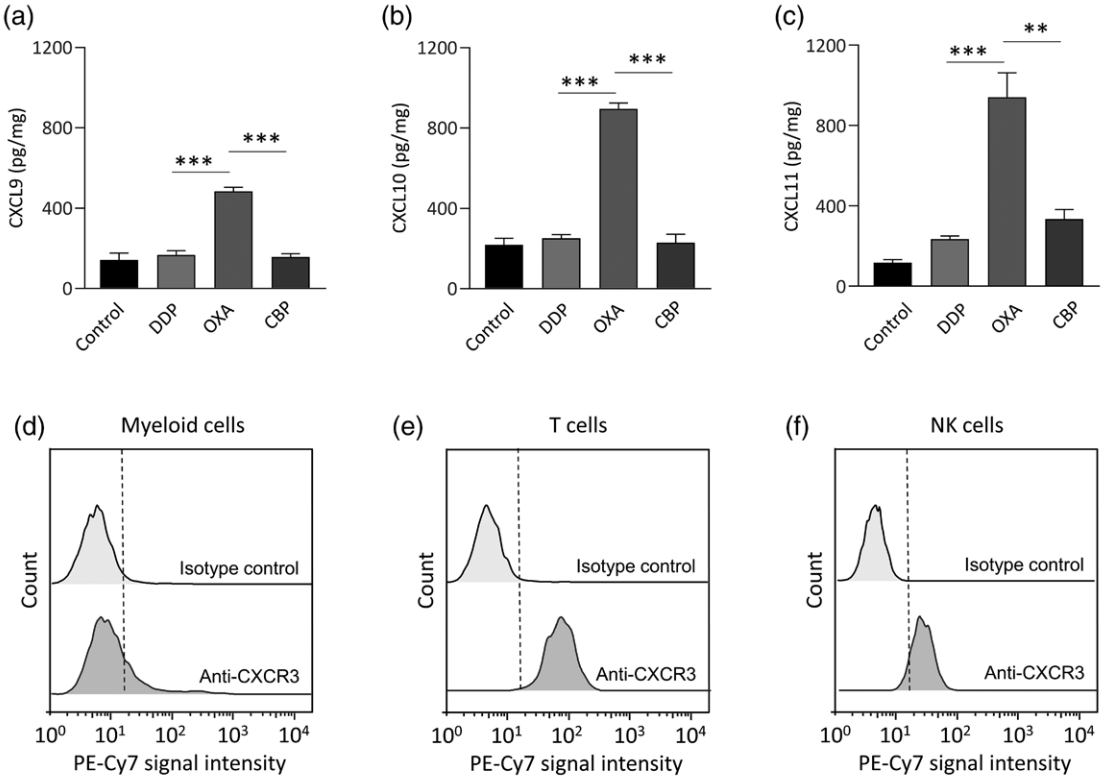
Oxaliplatin treatment efficiently promotes tumor-infiltration of T cells and natural-killer (NK) cells in C57BL/6 mice bearing LLC. Murine Lewis lung carcinoma (LLC) was established and treated as indicated above. (a) Representative flow cytometry images showed the abundance of $CD45^+CD11b^+$ myeloid cells in tumor tissues at the end of antitumor treatment. (b) Representative flow cytometry images showed the percentages of $CD45^+CD3^+$ T cells in tumor-infiltrating immune cells. (c) Representative flow cytometry images showed the percentages of $CD45^+NK1.1^+$ NK cells in tumor-infiltrating immune cells. (d) Statistic analysis of the percentages of myeloid cells in tumor tissues. (e) Statistic analysis of the percentages of T cells in tumor tissues. (f) Statistic analysis of the percentages of NK cells in tumor tissues. Data represent means ± SD. $n = 6$ mice. $^{**}P < 0.01$, $^{***}P < 0.001$.

(PVDF) membranes. After blocking with 5% nonfat milk, the membranes were incubated with the primary antibodies specific for CXCL9, CXCL10 and CXCL11 (Santa Cruz, 1:1000 dilution, 4°C overnight). The membranes were then incubated with peroxidase-conjugated secondary antibody (Sigma-Aldrich, St. Louis, Missouri, USA, 1:1000 dilution, 2 h, at room temperature) and developed with an ECL system (GE Healthcare Life Sciences, Little Chalfont, Buckinghamshire, United Kingdom). The relative intensity of the interest bands was normalized with β-actin intensity in the same sample with NIH-Image J1.51p 22 (National Institutes of Health).

Statistical analysis

Differences of quantitative parameters between groups were assessed using *t* test, one-way or two-way ANOVA analysis with a post hoc test. The significance level was set as *P* value <0.05. All statistical analyses were

Fig. 3



Oxaliplatin treatment induces the concentrations of chemokines in tumor tissues. Murine Lewis lung carcinoma (LLC) was established and treated as indicated above. Concentrations of chemokines including CXCL9 (a), CXCL10 (b), and CXCL11 (c) in the treated tumor tissues were examined using ELISA. The expressions of CXCR3 (the receptor of CXCL9/10/11) on myeloid cell (d), T cells (e), and NK cells (f) were examined using flow cytometry. Data represent means ± SD. $n = 6$ mice. $^{**}P < 0.01$, $^{***}P < 0.001$.

performed using GraphPad Prism (GraphPad Software, Inc).

Results

Oxaliplatin exhibits better antitumor efficacy in immune-competent mice

DDP, OXA and CBP could inhibit the growth of A549 cells in nude mice when compared with the vehicle control group, while the three platinum drugs did not show any difference in the inhibition of tumor growth (Fig. 1a). To our surprise, OXA showed a more effective antitumor growth effect than the other two platinum drugs in the LLC model (Fig. 1b, $P < 0.01$). When considering the immune-competent nature of LLC model, the treatment benefit may be attributed to the immune microenvironment.

Oxaliplatin treatment promotes tumor-infiltration of T cells and NK cells

Twenty-four hours after the end of the treatment, the proportion of myeloid cells ($CD45^+CD11b^+$) was significantly lower in the oxaliplatin treatment group than the other two platinum treatment groups (Fig. 2a,d). In contrast, the proportion of T cells (Fig. 2b,d) and NK cells (Fig. 2c,d) that played a role in tumor killing were significantly increased, indicating that oxaliplatin could effectively regulate the tumor immune microenvironment in the LLC model.

Oxaliplatin treatment induces chemokines expression in tumor tissues

Cisplatin and carboplatin treatment did not alter the concentrations of CXCL9 (Fig. 3a), CXCL10 (Fig. 3b) and CXCL11 (Fig. 3c) in the LLC tumor tissues when compared with the vehicle control group. As expected, oxaliplatin treatment significantly increased the concentrations of CXCL9 (Fig. 3a), CXCL10 (Fig. 3b) and CXCL11 (Fig. 3c) in the LLC tumor tissues when compared with the cisplatin and carboplatin monotherapy group. It was worth noting that CXCR3 (the receptor of CXCL9/10/11) was highly expressed on T cells (Fig. 3e) and NK cells (Fig. 3f), while there was no significant change in myeloid cells (Fig. 3d) after the administration of oxaliplatin in LLC model. All of these indicated

CXCL9/10/11-CXCR3 axis may contribute to the chemotaxis of T cells and NK cells.

The combination of oxaliplatin, anti-PD1 antibody and anti-NKG2D antibody exhibit remarkably antitumor effect

As our previous results showed that oxaliplatin treatment could significantly increase the infiltration of T cells and NK cells in tumor tissue. We further tested whether the combination of oxaliplatin with anti-PD1 and anti-NKG2D antibodies can achieve a better therapeutic effect. The results showed that the combination of the three drugs could effectively inhibit the growth of LLC tumor (Fig. 4a) and prolong the survival time of tumor-bearing mice (Fig. 4b) with the downregulated concentration of TGF-β (Fig. 4c) and upregulation of IL-12p70 (Fig. 4d) and IFN-γ (Fig. 4e) when compared with oxaliplatin combined with anti-PD1 antibody or oxaliplatin combined with anti-NKG2D antibody group. All of these indicated that oxaliplatin synergized with anti-PD1 or anti-NKG2D therapy, concomitant administration of oxaliplatin to anti-PD1 and anti-NKG2D antibody enhanced the antitumor efficacy of oxaliplatin and anti-PD1 or anti-NKG2D chemotherapy.

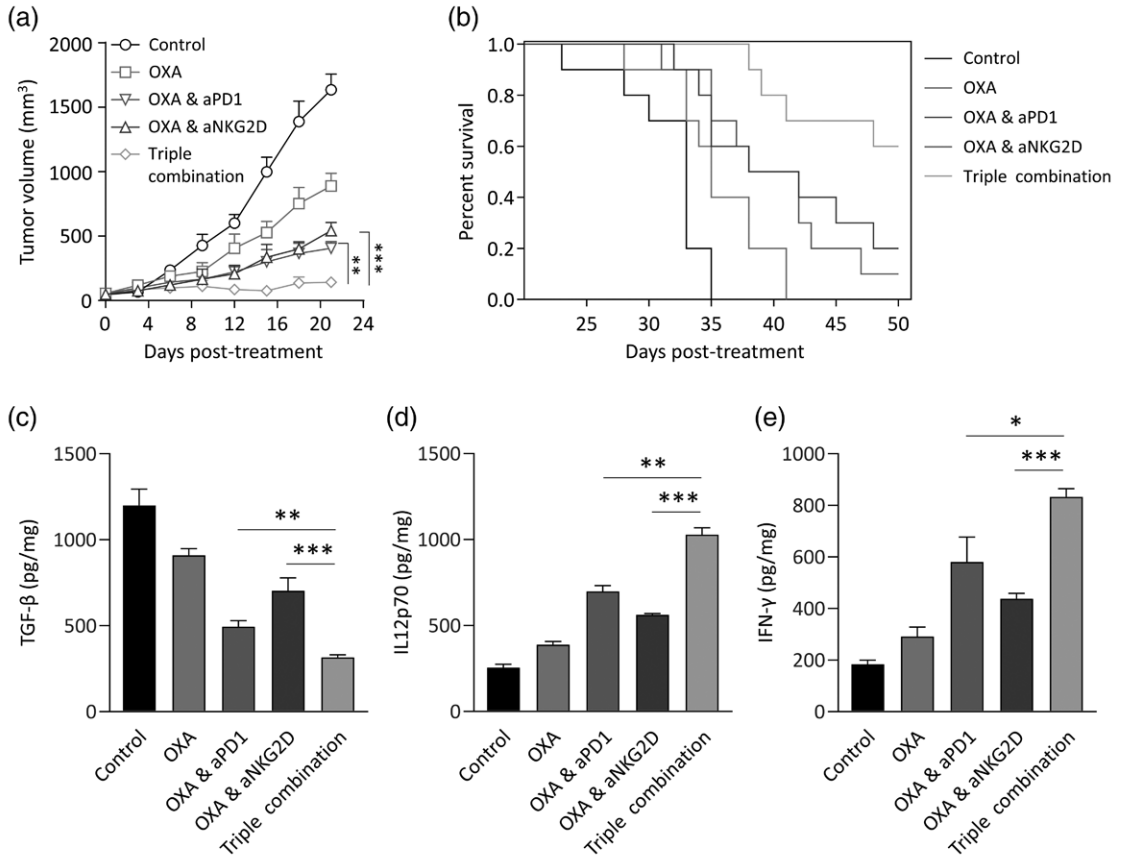
Oxaliplatin treatment induces chemokines expression in human lung cancer cells

Two types of human lung cancer cells A549 (Fig. 5a) and H460 (Fig. 5b) were treated with oxaliplatin (2.5 μM) for 48 h, and the relative mRNA and protein expressions of CXCL9, CXCL10 and CXCL11 were examined *via* RT-PCR and western blot, which indicated that oxaliplatin-induced upregulated chemokines expression in tumor tissue might be attributed to tumor cells.

Discussion

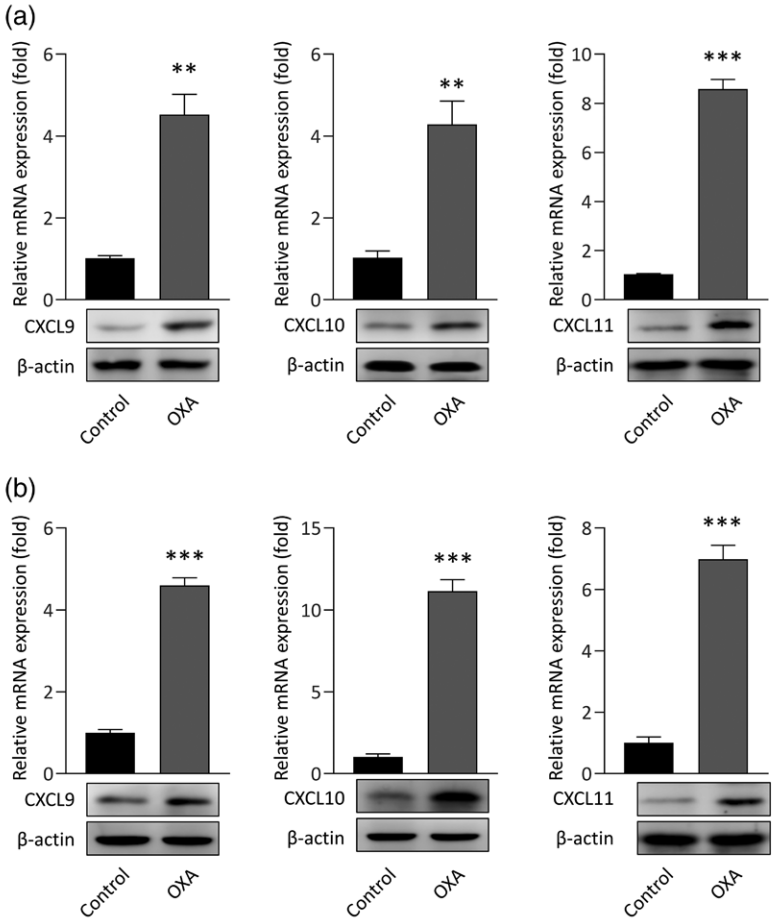
The PD-1/PD-L1 signaling pathway is an essential component of tumor immunosuppression to

Fig. 4



The combination of OXA, anti-PD1 antibody and anti-NKG2D antibody exhibit remarkably antitumor effect in the LLC model. The subcutaneous LLC tumors were established by injecting 1×10^5 LLC cells into the right flank of C57BL/6 mice, and the antitumor study started when tumor volume reached about 50 mm^3. Mice were divided into five groups: control group, OXA group, OXA & aPD1, OXA & aNKG2D and the triple combinations. OXA and antibodies were injected every 4 days three times, and the injection doses of OXA and antibodies were 3.0 mg/kg and 2.5 mg/kg, respectively. (a) Tumor growth curve. (b) Survival curve of tumor-bearing mice. ELISA results of cytokines production in the tumors from mice receiving indicated treatments (c: TGF-β; d: IL12p70; e: IFN-γ). Data represent means ± SD. $n = 6$ mice, $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Fig. 5



Oxaliplatin treatment induces the expressions of chemokines in human lung cancer cells. Two types of human lung cancer cells A549 (a) and H460 (b) were treated with OXA (2.5 μM) for 48 h, and then the expressions of CXCL9, CXCL10 and CXCL11 in mRNA and protein levels were examined via real-time PCR and western blot. Data represent means ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

inhibit T-cell activation and result in immune tolerance. Subcutaneous A549 and LLC models have been constructed in this investigation to mimics the tumor microenvironment in human malignancies. Our results indicate that the therapeutic effectiveness of platinum is determined not only by the malignant behavior of tumors but also by the immune microenvironment. Cisplatin and oxaliplatin are reported to induce similar immunogenic changes in head and neck cancer [15]. In our study, we find that oxaliplatin has a better effect on promoting immunogenic change than cisplatin and carboplatin, which indicates that oxaliplatin may be the appropriate chemical to be utilized to induce immunogenic changes in lung cancer. Mechanistically, we demonstrate that oxaliplatin induces multiple recruiting chemokines, such as CXCL9, CXCL10 and CXCL11 in the tumor microenvironment, to facilitate the recruitment of T cells and NK cells.

Lung tumor cells typically reside in the nonlymphoid tissues, where T cells may not traffic efficiently without appropriate inflammatory signals. Oxaliplatin can activate tumor macrophages to express T-cell-recruiting chemokines to remodel the tumor microenvironment, resulting in improved chimeric antigen receptor (CAR) T-cell infiltration and increased sensitivity to anti-PD1/anti-PD-L1 treatment [16,17]. Moreover, tumor cells can recruit myeloid cells to suppress T-cell activation, which could be diminished by the administration of oxaliplatin, as testified in our study. All of these indicate that, as immunogenic chemotherapy, oxaliplatin activates tumor macrophages to produce chemokines that facilitate the recruitment of T cells and NK cells to lung tumors and diminishes the enrichment of myeloid cells.

Although NK cells are less frequently detected, the increased proportion of intratumoral NK cells is associated with better survival [18–20]. Thus, anti-NKG2D

antibody would be a plausible strategy to increase infiltration of NK cells to elicit NK cell-based antibody-dependent cell-mediated cytotoxicity (ADCC) effect. However, the presence of NK cells may be controversial, as NK cells also have the ability to suppress $CD8^+$ T-cell responses during chronic infections [21,22]. The precise mechanism mediated by the anti-NKG2D antibody should be deciphered in further study.

There are some limitation should be indicated here. The CXCL9, -10, -11/CXCR3 axis not only induces immune cell activation, differentiation and migration to suppress tumor through paracrine mechanism but also promotes tumor growth and metastasis through autocrine mechanism [23,24]. A deep understanding of the CXCL9, -10, -11/CXCR3 axis and the treatment benefit of oxaliplatin is necessary. Pretreatment differences in immune cell composition in NSCLC are associated with survival and dependent on smoking status and histological subtype [25,26]. Thus, stratified human research is urgent to verify the observation in our research.

All in all, our investigation indicates that oxaliplatin can synergize with anti-PD1 or anti-NKG2D monotherapy, concomitant administration of oxaliplatin to anti-PD1 and anti-NKG2D antibody enhance the antitumor efficacy. Oxaliplatin administration could be considered as a strategy to improve inbound leukocyte traffic to tumors.

Conclusion

Oxaliplatin can be utilized in lung cancer to facilitate the infiltration of T cells and NK cells to enhance tumor immunotherapy.

Acknowledgements

This research was supported by the Natural Science Foundation of Fujian Province of China [2018J01128].

Conflicts of interest

There are no conflicts of interest.

References

1 Ortega-Franco A, Ackermann C, Paz-Ares L, Califano R. First-line immune checkpoint inhibitors for extensive stage small-cell lung cancer: clinical developments and future directions. *ESMO Open* 2021; **6**:100003.
2 Rudin CM, Brambilla E, Faivre-Finn C, Sage J. Small-cell lung cancer. *Nat Rev Dis Primers* 2021; **7**:3.
3 Johnstone TC, Suntharalingam K, Lippard SJ. The next generation of platinum drugs: targeted Pt(II) agents, nanoparticle delivery, and Pt(IV) prodrugs. *Chem Rev* 2016; **116**:3436–3486.
4 Rottenberg S, Disler C, Perego P. The rediscovery of platinum-based cancer therapy. *Nat Rev Cancer* 2021; **21**:37–50.
5 Vanneste BGL, Van Limbergen EJ, Reynders K, De Ruysscher D. An overview of the published and running randomized phase 3 clinical results of radiotherapy in combination with immunotherapy. *Transl Lung Cancer Res* 2021; **10**:2048–2058.
6 Di Federico A, De Giglio A, Parisi C, Gelsomino F, Ardizzoni A. PD-1/PD-L1 inhibitor monotherapy or in combination with chemotherapy as upfront treatment for advanced NSCLC with PD-L1 expression ≥ 50%: selecting the best strategy. *Crit Rev Oncol Hematol* 2021; **160**:103302.
7 Passiglia F, Reale ML, Cetoretta V, Novello S. Immune-checkpoint inhibitors combinations in metastatic NSCLC: new options on the horizon? *Immunotargets Ther* 2021; **10**:9–26.
8 Lee YJ, Lee JB, Ha SJ, Kim HR. Clinical perspectives to overcome acquired resistance to anti-programmed death-1 and anti-programmed death ligand-1 therapy in non-small cell lung cancer. *Mol Cells* 2021; **44**:363–373.
9 Passiglia F, Leone G, Olmetto E, Delcuratolo MD, Tabbò F, Reale ML, *et al*. Immune-checkpoint inhibition in stage III unresectable NSCLC: challenges and opportunities in the post-PACIFIC era. *Lung Cancer* 2021; **157**:85–91.
10 Goleva E, Lyubchenko T, Kraehenbuehl L, Lacouture ME, Leung DYM, Kern JA. Our current understanding of checkpoint inhibitor therapy in cancer immunotherapy. *Ann Allergy Asthma Immunol* 2021; **126**:630–638.
11 Balasubramanian A, Onggo J, Gunjur A, John T, Parakh S. Immune checkpoint inhibition with chemoradiotherapy in stage III non-small-cell lung cancer: a systematic review and meta-analysis of safety results. *Clin Lung Cancer* 2021; **22**:74–82.
12 Suresh K, Naidoo J, Lin CT, Danoff S. Immune checkpoint immunotherapy for non-small cell lung cancer: benefits and pulmonary toxicities. *Chest* 2018; **154**:1416–1423.
13 Baldessari C, Guaitoli G, Valoriani F, Bonacini R, Marcheselli R, Reverberi L, *et al*. Impact of body composition, nutritional and inflammatory status on outcome of non-small cell lung cancer patients treated with immunotherapy. *Clin Nutr ESPEN* 2021; **43**:64–75.
14 Hato SV, Khong A, de Vries IJ, Lesterhuis WJ. Molecular pathways: the immunogenic effects of platinum-based chemotherapeutics. *Clin Cancer Res* 2014;**20**:2831–2837.
15 Park SJ, Ye W, Xiao R, Silvin C, Padget M, Hodge JW, *et al*. Cisplatin and oxaliplatin induce similar immunogenic changes in preclinical models of head and neck cancer. *Oral Oncol* 2019;**95**:127–135.
16 Srivastava S, Furlan SN, Jaeger-Ruckstuhl CA, Sarvothama M, Berger C, Smythe KS, *et al*. Immunogenic chemotherapy enhances recruitment of CAR-T cells to lung tumors and improves antitumor efficacy when combined with checkpoint blockade. *Cancer Cell* 2021; **39**:193–208.e10.
17 Stojanovska V, Prakash M, McQuade R, Fraser S, Apostolopoulos V, Sakkal S, *et al*. Oxaliplatin treatment alters systemic immune responses. *Biomed Res Int* 2019; **2019**:4650695.
18 Coca S, Perez-Piqueras J, Martinez D, Colmenarejo A, Saez MA, Vallejo C, *et al*. The prognostic significance of intratumoral natural killer cells in patients with colorectal carcinoma. *Cancer* 1997; **79**:2320–2328.
19 Choi C, Finlay DK. Optimising NK cell metabolism to increase the efficacy of cancer immunotherapy. *Stem Cell Res Ther* 2021; **12**:320.
20 Nersesian S, Schwartz SL, Grantham SR, MacLean LK, Lee SN, Pugh-Toole M, *et al*. NK cell infiltration is associated with improved overall survival in solid cancers: a systematic review and meta-analysis. *Transl Oncol* 2021; **14**:100930.
21 Lang PA, Lang KS, Xu HC, Grusdat M, Parish IA, Recher M, *et al*. Natural killer cell activation enhances immune pathology and promotes chronic infection by limiting CD8+ T-cell immunity. *Proc Natl Acad Sci U S A* 2012; **109**:1210–1215.
22 Cook KD, Whitmire JK. The depletion of NK cells prevents T cell exhaustion to efficiently control disseminating virus infection. *J Immunol* 2013; **190**:641–649.
23 Prajapati K, Perez C, Rojas LBP, Burke B, Guevara-Patino JA. Functions of NKG2D in CD8+ T cells: an opportunity for immunotherapy. *Cell Mol Immunol* 2018; **15**:470–479.
24 Tokunaga R, Zhang W, Naseem M, Puccini A, Berger MD, Soni S, *et al*. CXCL9, CXCL10, CXCL11/CXCR3 axis for immune activation - A target for novel cancer therapy. *Cancer Treat Rev* 2018; **63**:40–47.
25 Tamminga M, Hiltermann TJN, Schuuring E, Timens W, Fehrmann RS, Groen HJ. Immune microenvironment composition in non-small cell lung cancer and its association with survival. *Clin Transl Immunology* 2020; **9**:e1142.
26 Giannone G, Ghisoni E, Genta S, Scotto G, Tuninetti V, Turinetto M, *et al*. Immuno-metabolism and microenvironment in cancer: key players for immunotherapy. *Int J Mol Sci* 2020; **21**:E4414.