Macrophage polarization synergizes with oxaliplatin in lung cancer immunotherapy via enhanced tumor cell phagocytosis

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ABSTRACT
Calreticulin (CALR) exposure is required for most immunogenic cell death (ICD) in the anti-tumor immunity induced by chemotherapeutic agents. The present study aimed to explore the anti-tumor efficacy of the combined administration of oxaliplatin (OXA) and R848 (an agent for macrophage polarization) in lung cancer cells. Flow cytometry and immunostaining assays were performed to evaluate CALR exposure induced by OXA in the murine Lewis lung carcinoma (LLC) cells. The phagocytosis of macrophages was determined using flow cytometry and western blotting assays. The anti-tumor efficacy of the OXA and R848 combination was evaluated using flow cytometry and western blotting in vitro and in vivo. OXA induced CALR exposure on the surface of LLC cells after low dose and short duration of treatment (20 μM OXA for 24 h). LLC cells pretreated with OXA were more prone to be phagocytized by M1 than M2 macrophages. M2 macrophages repolarized to M1 by R848 in vitro showed enhanced phagocytic ability to OXA-treated LLC cells. Finally, combined administration of OXA and R848 exhibited a synergistic anti-tumor effect than single agent applied in vitro and in vivo. Macrophage polarization from pro-tumor M2 to anti-tumor M1 synergizes with OXA in lung cancer immunotherapy via enhanced tumor cell phagocytosis.

Introduction
Among all diagnosed cancer cases, lung cancer is the most common one (11.6%) and remains the leading cause of cancer-related death worldwide (18.4%), with over 2.1 million newly diagnosed cases and 1.8 million deaths predicted in 2018 [1]. Non-small cell lung cancer (NSCLC) consists about 85% of all lung cancer cases, and when diagnosed more than a half have been already at the advanced stage with metastasis [2]. For the advanced NSCLC, chemotherapy is the main component of systemic therapy aimed at reducing cancer-related symptomatic burdens, as well as increasing survival time and improving quality of life. Platinum-based therapies have been widely applied in a broad spectrum of cancers, including lung cancer. Platinum drugs usually exert their anti-tumor efficacy by targeting nuclear DNA and inducing cytotoxic effects in tumor cells [3,4]. However, the increased drug resistance and uncontrolled cytotoxic effect during platinum-based therapy have hindered its wide application in advanced NSCLC [5,6].

In the recent decade, the tumor microenvironment (TME) has caused considerable attentions for its critical role in the modulation of tumor cell survival and pathological development [7,8]. During this process, tumor-associated macrophages (TAMs) serve as the main component of tumor-associated stromal cells that contribute to tumor-related inflammation [9]. TAMs are heterogeneous in TME and can be polarized into tumor-suppressive M1 or tumor-promoting M2 phenotypes depending on different stimuli. An M2-to-M1 switch by specifically inhibiting the nuclear factor κB (NF-κB) signaling pathway in TAMs is reported to result in tumor regression in ovarian cancer cells [10,11]. To repolarize M2 into M1 phenotype is supposed to be therapeutic potential and will add up to the existing cancer therapies [10,12].

Immunogenic cell death (ICD) is defined by chronic exposure of damage-associated molecular patterns (DAMPs) in the TME and has become a novel target for cancer therapy. ICD-related DAMPs, serving as the anti-cancer vaccine of ICD [13], mainly include surface calreticulin (CALR), surface heat shock protein 90 (HSP90) and secreted ATP [14]. Surface CALR acts as an “eat me” signal to promote the phagocytosis of cells by dendritic cells. CALR exposure on the surface of cancer cells can also induce tumor antigen presentation and tumor cytotoxic T lymphocyte responses [15,16].

Oxaliplatin (OXA) is one of the classic ICD inducers that promotes...
CALR translocation from the endoplasmic reticulum (ER) to cell surface [17]. OXA is now the front-line drug in lung cancer chemotherapy [18, 19]. However, as one of the platinum-containing chemotherapeutic drugs, OXA inevitably induces drug resistance in lung cancer cells which remains a major challenge to its long-term usage [20].

The present study aimed to explore whether the polarization of macrophages in TME could affect the anti-tumor ability of OXA in lung cancer, in order to find a more effective therapy against lung cancer.

Methods and materials

Cell lines

The murine Lewis lung carcinoma (LLC) cell line, the I.929 fibroblast cell line and the KLN 205 cells were purchased from American Type Culture Collection (Manassas, VA). Human lung cancer cell lines, A549 and NCI-H460, were obtained from Cell bank of Chinese Academy of Sciences. LLC cells were cultured in DMEM (Gibco, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS, Gibco, Billings, MT), and 1% penicillin/streptomycin (P/S, Gibco). I.929 cells were cultured in murine bone-marrow-derived macrophages (BMDM) medium, which was DMEM medium with 10% FBS, 1% Glutamax (Gibco), and 1% P/S. KLN 205 cells shared the same culture condition as that of LLC cells. A549 and NCI-H460 cells were cultured in RPMI-1640 (Gibco) with 10% FBS. All cell lines were maintained at 37 °C, 5% CO₂.

L-cell conditioned medium (LCCM)

I.929 cells were cultured in BMDM medium until confluent. Then supernatants were collected, centrifuged and filtered using 0.22-µm filters, followed by dilution with BMDM (1:5) to prepare the LCCM. The latter was placed at 4 °C until use.

Mice

Female C57BL/6 mice at 6 to 8 weeks of age were purchased from GemPharmatech (Nanjing, China). Mice were fed under specific pathogen-free conditions with free access to water and food. All animal-involved procedures have been approved by the Ethic Committee of Cangzhou Central Hospital (2020-j32).

BMDM isolation and polarization

BMDMs were isolated and cultured according to previously described protocols [21,22]. Briefly, after mice were euthanized, femurs were quickly removed and flushed with ice cold DMEM. Then the cell suspension was centrifuged at 300 g and the resulting cells were incubated at 37 °C for 24 h in BMDM medium. Afterwards, non-adherent cells were reseeded and cultured in 10-cm dishes in LCCM for 10 to 20 days. The purity of BMDMs was verified by flow cytometry using F4/80, and 1% purity was considered to be qualified (data not shown).

For polarization, BMDMs were seeded at the density of 5 × 10⁵ cells/cm² in 6-well plates in the LCCM for 24 h, followed by 24-hour incubation in BMDM medium. Then BMDMs were cultured in serum-free DMEM supplemented with vehicle, a mix of 100 ng/ml lipopolysaccharides (LPS, Sigma, St. Louis, MO) and 20 ng/ml interferon γ (IFN γ, Peprotech, Rocky Hill, NJ) for M1 polarization, and 10 ng/ml interleukin 4 (IL-4, Peprotech) for M2 polarization, respectively. 18 hours later, polarized BMDMs were ready for further analysis or application in other experiments.

CALR exposure on cell surface

CALR exposure on the surface of live LLC cells was determined by flow cytometry according to previously described protocol [23]. LLC cells were seeded at the density of 10⁵ cells/well in 12-well plates and cultured for 24 h before treatment with 20 µM OXA. Another 24-hour incubation later, cells were stained with propidium iodine (PI, Sigma) and Phycoerythrin (PE) labeled anti-CALR antibody (anti-CALR-PE, Abcam) diluted in phosphate buffer containing 2% FBS at 4 °C for 30 min. Surface CALR in viable LLC cells (PI negative) was detected using cyttofluorometric analysis on a FACS Vantage. CALR exposure was also analyzed by immunofluorescence staining. Cell coverslips from each group were fixed with 4% paraformaldehyde (PFA) at room temperature for 30 min. After 1-h block with 5% BSA in PBS at room temperature, samples were incubated with rabbit anti-CALR monoclonal antibody (Abcam, Cambridge, UK) at 4 °C overnight. After 3 washes with PBS, samples were incubated with DAPI (Invitrogen, Carlsbad, CA, USA), Alexa Fluor™ 488 Phalloidin (Invitrogen) and the fluorescence-conjugated secondary antibody for 1 h at room temperature. Images were acquired using a Zeiss LSM 780 confocal microscope.

In vitro phagocytosis detection

LLC cells were pre-treated with 20 µM OXA for 24 h and labeled with CFSE before co-cultured with polarized BMDMs, M1 and M2 phenotypes, respectively. Phagocytosis of tumor cells was detected by examining the percentage of CFSE positive macrophages (F4/80⁺ CFSE⁺ cells) as well as the number of CD91-PE positive M1 or M2-like macrophages (F4/80⁺ CD91⁻) through flow cytometry. The phagocytosis ability was also assessed by detecting the expression of CD91 (the main receptor of CALR) in M1 or M2 macrophages using western blot analysis.

Western blotting

Collected LLC cells or tumor tissues were lysed and homogenized in RIPA lysis supplemented with protein inhibitor. Total proteins were quantified using a BCA assay kit (Beyotime, Shanghai, China). Then equal amounts of protein from each group were loaded and separated by SDS-PAGE followed by transferring to PVDF membranes (EMD Millipore Corp., Burlington, MA). After blocking with 5% bovine serum albumin (BSA, Sigma) for 1 h at room temperature, membranes were incubated with primary antibodies overnight at 4 °C. The next day, membranes were incubated with the corresponding HRP-conjugated secondary antibodies (1:2000, Sigma) for 2 h at room temperature. Finally, bands were visualized using the ECL reagent (Thermo Fisher Scientific, Wal-tham, MA, USA). Primary antibodies used were rabbit anti-CALR (1:2000), mouse anti-GAPDH (1:1000), rabbit anti-CD206 (1:2500) and rat anti-MHC II (1:1000), all of which were purchased from Thermo Fisher Scientific. GAPDH served as the internal control.

RT-qPCR

BMDMs were first treated with IL-4 (10 ng/ml) for 24 h, and then treated with 100 nM R848 for another 48 h before further analysis. Total RNA was extracted from R848-treated M2-like macrophages using TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. 1 µg of total RNA from each group was reverse transcribed using the cDNA Synthesis Kit (Thermo scientific). qPCR was performed using SYBR Select Master Mix (Thermo scientific) to detect the mRNA expression of Arg1, Mrcl, IL12 and Nos2 in M2 macrophages after R848 treatment. Primer used were as follows (5’ to 3’): Arg1, CTCAAGGC- CAAAGCTCTTAGAG (Forward) and AGGAGCTGTCTATAGGGCATC (Reverse); Mrcl, CTATGGCGTGCCTATACAG (Forward) and AAA-GAAATGCGAGGCAAGAG (Reverse); IL12, ACCCTGACCATCAGTCT- CAA (Forward) and GTGGAGCAGCAGATGTGAGT (Reverse); Nos2, GGAGTGCAGGCCAACATGACT (Forward) and TGATCTGCA- CACCTGGTGTAAC (Reverse); GAPDH, ACAACCTTTGCGGATTGGAA (Forward) and GATGCAGGATGATGTTCTG (Reverse). The reactions were performed using the following qPCR parameters: 95 °C for 10 min, 40 cycles of 95 °C for 15 s, and 60 °C for 1 min. The relative expression of target gene was calculated using the 2⁻ΔΔCT method. All values were
normalized to GAPDH.

**In vivo efficacy assay**

Female C57BL/6 mice (at the age of 6 weeks) were inoculated $1 \times 10^5$ GFP-LLC cells subcutaneously in the flanks. When the GFP-LLC tumors reached the size of about 50 mm$^3$, inoculated mice were randomly divided into 4 groups, the PBS, OXA (1.5 mg/kg \(\cdot\) weight), R848 (3.0 mg/kg \(\cdot\) weight), and OXA\&R848 groups. Reagents were administered intravenously every 3 days for 4 times. Tumor growth was detected by assessing tumor volume using the following formula at 0, 6, 12, 18 and 24 days post-treatment: tumor volume $= L \times B^2/2$, where $L$ stands for the longest diameter and $B$ for the shortest one. At 24 days after different treatments, tumor tissues were dissected to explore the expression of MHC II (M1 marker) and CD206 (M2 marker) through western blotting. Tumor tissues were also used to detect the number of F4/80$^+$ CFSE$^+$ macrophages as well as the percentage of CD8$^+$ in CD45$^+$ cells through flow cytometry, in order to explore the tumor phagocytosis of macrophages and anti-tumor immunity in vivo.

![Figure 1](image-url)

**Figure 1.** Oxaliplatin (OXA) treatment induces CALR exposure on the surface of Lewis lung carcinoma (LLC) cells. (A) The surface exposure of calreticulin (CALR) was determined by flow cytometry among viable (propidium iodine negative) cells after treated with Oxaliplatin (OXA) for 24 h. (B) The percentages of CALR$^+$ cells were quantified. Data represent means $\pm$ SD. Statistical significance was calculated via one-way ANOVA with the Tukey post-hoc test. **$p$<0.01. The concentration of OXA was 20 $\mu$M. Treated LLC cells were stained with PI and PE labeled anti-CALR antibodies according to the manufacturer’s instructions. (C) Fluorescent imaging of CALR exposure on the surface of LLC cells. LLC cells were treated with OXA, and then stained with FITC labeled anti-CALR antibodies, DAPI and Alexa Fluor™ 488 Phalloidin, followed by confocal observation. Scale bar = 10 $\mu$m.
Statistical analysis

Each experiment was conducted at least in triplicate. Data were presented as mean ± SD. Statistical analyses were conducted using SPSS 17.0 (IBM Corp., Armonk, NY, USA). Student’s t test or ANOVA analysis (one- or two-way) with post hoc tests was applied to compare means between two different groups. *P* < 0.05 was considered to be statistically significant.

Results

**OXA treatment induces CALR exposure on the surface of LLC cells**

We first investigated whether OXA treatment could influence the expression pattern of CALR in tumor cells. After 24-h treatment of OXA, the percentage of CALR+ cells among viable LLC cells was significantly higher than that in PBS-treated group (Figure 1A and 1B). Immunofluorescent staining showed that more CALR were expressed on the surface of LLC cells in OXA-treated group than the PBS-treated group (Figure 1C). These results suggested that OXA facilitated CALR exposure.

![Diagram showing M1 macrophages exhibit stronger phagocytic ability against OXA-treated LLC cells.](image-url)

**Figure 2.** M1 macrophages exhibit stronger phagocytic ability against OXA-treated LLC cells. (A) Schematic overview. Murine bone-marrow-derived macrophages (BMDMs) were treated with IL-4 or LPS/IFN-γ for phenotype polarization, and the co-cultures with CFSE-labeled LLC cells. Phagocytosis of tumor cells was detected by examining the percentages of CFSE positive macrophages. LLC cells were treated with OXA for 24 h before co-cultured with macrophages. Flow cytometry gating (B) and histogram analysis (C) of the percentages of F4/80+ CFSE+ cells were detected. OXA-treated LLC cells and M2- or M1-like macrophages were co-cultured and the phagocytosis was examined by flow cytometry. Data represent means ± SD. Statistical significance was calculated via one-way ANOVA with the Tukey post-hoc test. **p < 0.01, ***p < 0.001. Expressions of CD91 on M1- or M2-like macrophage were examined by flow cytometry (D) and western blot (E). CD91 was the main receptor of CALR.
on the surface of LLC cells, demonstrating its anti-tumor capability in vitro.

**M1 macrophages exhibit stronger phagocytic ability against OXA-treated LLC cells**

As OXA-treated LLC cells showed enhanced CALR exposure, we next investigated whether the phagocytosis of macrophages against these cells were affected. M1 and M2-like macrophages were polarized using IL-4 and LPS/IFN-γ, respectively. Then they were co-cultured with LLC cells pre-treated with PBS or OXA to evaluate their phagocytic ability to tumor cells (Figure 2A). Compared with the intra-class control, M2- and M1-like macrophages engulfed more LLC cells (F4/80^+^ CFSE^+^), and the latter engulfed even more than the former (Figure 2B and 2C). More CD91-PE^+^ cells were detected in M1-like macrophages compared with that in the M2-like group (Figure 2D). Western blot analysis also illustrated the enhanced expression of CD91 in M1-like macrophages (Figure 2E). As CD91 is one of the main receptors of CALR, we speculated that M1 macrophages exhibited stronger phagocytic ability against OXA-treated LLC cells at least partially due to the enhanced expression of CD91 on M1 macrophages.

**R848 repolarizes M2 macrophage towards M1 subtype in vitro and promotes phagocytic ability**

R848 is the TLR7/8 agonist capable of polarizing M2 macrophages towards M1 subtype, and we assessed whether R848 could reprogram the phenotype of M2-like macrophages in the present study. Compared with the control group, R848-treated M2 macrophages expressed lower Arg1 and Mrc1 (M2 phenotype), but higher IL-12 and Nos2 (M1 phenotype) at the mRNA levels, illustrating that R848 indeed conversed IL-4-polarized M2 macrophages into M1 subtype (Figure 3A). The expression of CD91 was increased obviously after R848 repolarization of M2 macrophages, to comparable level of that in M1 macrophages (Figure 3B). Phagocytosis analysis showed that R848-treated M2 macrophages engulfed more LLC cells than the untreated M2 macrophages,

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**Figure 3.** R848 repolarizes macrophage towards M1 subtype in vitro and promotes phagocytic ability. (A) BMDMs were treated with IL-4 (10 ng/mL) for 24 h, and treated with 100 nM R848 for 48 h. Expression of Arg1, Mrc1, IL12 and Nos2 at the mRNA level in M2 macrophages after R848 treatment was analyzed through RT-qPCR. Data are shown as means ± SD, **p < 0.01 (versus control group). (B) CD91 expression on M2 macrophage post R848 treatment. M1-like macrophage was set as control. (C) R848-treated M2-like macrophages were co-cultured with OXA-treated LLC cells for 24 h, and the phagocytosis was examined by flow cytometry. Data are shown as means ± SD. Statistical significance was calculated via one-way ANOVA with the Tukey post-hoc test. **p < 0.01, n.s., no significant difference.
and there was no significance difference between R848-treated M2 group and the M1 group, suggesting that R848 promoted the phagocytic ability of M2 macrophages (Figure 3C).

In vivo anti-tumor effect of OXA and R848

The above in vitro results showed that OXA treatment promoted the phagocytic ability of M1-like macrophages, and R848 enhanced that of M2-like macrophages. We wanted to further clarify their function in vivo and the effect of their combination. We explored the individual and combined efficacy of OXA and R848 in two murine lung carcinoma models, LLC and KLN 205. C57BL/6 mice bearing GFP-LPC tumors of ~50 mm³ received PBS, OXA, R848 or OXA & R848 every three days for 4 times, the administration dosage of OXA and R848 were 1.5 mg/kg and 3.0 mg/kg, respectively. As shown in Figure 4A, mice in both OXA and R848 group showed decreased tumor growth compared with the untreated control group, and the combination of OXA and R848 brought an accumulative tumor suppression efficacy, evidenced by the lowest tumor growth curve among all the groups (Figure 4A). At the end of the anti-tumor therapy, tumor tissues were collected for further analyses. The expression of MHC II (M1 marker) in the tumor tissues was significantly increased in OXA, R848 and OXA&R848 groups compared with the untreated control group, while the expression pattern of CD206 (M2 marker) exhibited an opposite trend (Figure 4B and 4C), meaning there were more M1 and less M2 macrophages in tumor tissues after drug treatment. Fluorescent imaging of CD206 and MHC II (Figure 4D and 4E) were in consistent with the western blotting results, further confirming the profile of more M1 and less M2 macrophages in drug-treated cancer cells. Results of flow cytometry showed that there were more F4/80 and GFP double positive macrophages in the tumor tissues of the OXA&R848 group than that in OXA or R848 group (Figure 4F), suggesting that the combined application induced obvious phagocytosis of tumor cells. Also, in the tumor tissues, there were more CD8+ T cells in CD45+ leukocytes in the OXA&R848 group than the individual application groups, meaning that the combination of two drugs exerted synergistic synergetic cytotoxic effect on tumor cells (Figure 4G). To explore the application of the combined application, we introduced another lung tumor model, KLN 205. Tumor growth curves exhibited a similar pattern as those in LLC tumor model, where both drugs individually suppressed tumor growth, and the combined application showed synergetic anti-tumor efficacy (Figure 5). We also verified our finding using other NSCLC cell lines, A549 and NCI-H460. R848-treated M2-like macrophages (THP-1 cells incubated with IL4 for 24 h) were cocultured with OXA-treated A549 and NCI-H460 for 24 h, and the phagocytosis was examined by flow cytometry. Similar to LLC cells, R848 treatment significantly promoted the phagocytic ability of M2-like macrophages against tumor cells (Figure S1).

Discussion

In the present study, we found that OXA induced CALR exposure on the surface of LLC cells after low dose and short duration of treatment (20 μM OXA for 24 h). LLC cells pretreated with OXA were more prone to be phagocytized by M1 than M2 macrophages. M2 macrophages polarized to M1 by R848 in vitro showed enhanced phagocytic ability to OXA-treated LLC cells. Finally, combined administration of OXA and R848 exhibited a synergistic anti-tumor effect than single agent applied in vivo in two different lung cancer animal models.

The activation of tumor immune responses including ICD has been reported to be an important process in the anti-tumor efficacies of chemotherapeutic agents [24]. ICD opens a new path for cancer therapy to enhance the effectiveness of chemotherapeutic drugs. Dying tumor cells release DAMPs to elicit an effective anti-tumor immune response. ICD inducers [24] are mostly reported to induce ICD in intestinal cancers such as colorectal cancer and liver metastasis [26], as well as rectal cancer [27]. Certain chemotherapeutic drugs including OXA are recognized as ICD inducers [24]. OXA is mostly reported to induce ICD in intestinal cancers such as colorectal cancer and liver metastasis [26], as well as rectal cancer [27]. Recently, evidences indicate the OXA-induced ICD in lung cancer cells [28,29]. Studies illustrate that OXA induces anti-tumor immunity through the pre-apoptotic exposure of CALR in colon cancer cells [17]. In the present study, we found that surface CALR was significantly upregulated after the treatment of low dose OXA for 24 h in LLC cells, suggesting OXA possesses potent ICD induction capability in lung cancer cells, consistent with previous study [28].

Reagents inducing M2 macrophage polarization lead to the reduction of immunosuppressive cells in TME, and their incorporation in chemotherapy exerts enhanced local and/or systemic anti-tumor immune responses in many cancers, including glial brain tumors, colorectal cancer and osteosarcoma [32-34]. Recently Bahmani B et al. emphasized that the local delivery of R848 loaded in nanoparticles resulted in total tumor regression via activating local immune responses in a colorectal tumor model [35]. Koh J et al. reported that R848-loaded nanoemulsion activated tumor-specific T cells and exhausted migrating T cells of murine lung cancer models [36]. Considering M2 is the main subtype of macrophages in tumor tissues [37], and OXA treatment could induce CALR exposure on the surface of LLC cancer cells, we designed the following ideal scenario. On the one hand, measures were taken to induce or re-polarize M2 macrophages into M1 subtype in tumor tissues; on the other hand, CALR-inducing drugs were taken, such as OXA. We next explored the effects of combining these two measures together on lung cancer cells. Results of in vitro experiments showed that R848-repolarized M2 macrophages exhibited increased phagocytic ability to OXA-treated cancer cells. In vivo analysis further confirmed the above findings, that the combination of OXA and R848 exerted synergistic anti-tumor efficacy. Besides, we obtained similar data using other two typical NSCLC lines, suggesting the role of R848 in lung cancer cells was not cell line-dependent. However, there is still a long way before the clinical application of R848 in cancer therapy, as all the findings nowadays are obtained from in vitro or animal experiments. There are still some short comings in the present study. We only explored the CALR exposure after OXA treatment at low dose, and in our future work a gradient dose of OXA would be applied. In the in vivo model, OXA and R848 were injected simultaneously, and the effect of injecting order on the anti-tumor efficacy will be investigated in our future study. Also, we didn’t clarify the potential molecular mechanisms and associated upstream and downstream factors involved. Additionally, we only investigated short term changes. In our future work, corresponding experiments would be performed.

Conclusion

In the present study, LLC cells pretreated with OXA were more prone to be phagocytized by M1 than M2 macrophages. M2 repolarized to M1 by R848 in vitro showed enhanced phagocytic ability to OXA-treated LLC cells. Finally, combined administration of OXA and R848
Figure 4. In vivo anti-tumor effect of OXA and R848 in LLC tumor model. C57BL/6 mice bearing GFP-LLC tumors of ~50 mm$^3$ received PBS, OXA, R848 or OXA & R848 every three days for 4 times, the administration dosage of OXA and R848 were 1.5 mg/kg and 3.0 mg/kg, respectively. (A) Average tumor growth curves of LLC tumors in control and treated groups. Data represent means ± SD, n = 6. Statistical significance was calculated via one-way ANOVA with the Tukey post-hoc test. *p < 0.05, **p < 0.01. At the end of antitumor therapy, tumor tissues were collected, the expression of CD206 (B) and MHC II (C) in tumor tissues were examined by western blot. CD206 and MHC II were the markers of M1- and M2-like macrophages, respectively. Tumor tissues from different groups were sectioned and stained with FITC-labeled anti-CD206 (D) or MHC II (E) antibodies, followed by confocal imaging. Scale bar = 50 μm. (F) The GFP signal in F4/80 positive macrophages were detected by flow cytometry. (G) Percentage of CD8$^+$ T cells in CD45$^+$ cells in the tumor tissues at the end of treatment. Data represent means ± SD, n = 6. Statistical significance was calculated via one-way ANOVA with the Tukey post-hoc test. *p < 0.05, **p < 0.01.
exhibited a synergistic anti-tumor effect than single agent applied in vitro and in vivo. In short, the present study indicates that OXA potently induces CARL exposure in lung cancer cells. Macrophage polarization from pro-tumor M2 to anti-tumor M1 synergizes with OXA for lung cancer therapy via enhanced tumor cell phagocytosis. This study demonstrates the orchestrating and therapeutic potentials of macrophage polarization in the chemotherapy of lung cancer.

Author contribution

Study Design; Manuscript Preparation: Fei Li, Xue Zheng, Xue Wang, Jinhua Xu and Qianyun Zhang

Literature Search; Data Collection; Statistical Analysis; Data Interpretation: Fei Li and Qianyun Zhang

Declaration of Competing Interest

The authors declare that there is no conflict of interests.

Ethics approval

All animal-involved procedures have been approved by the Ethics Committee of Cangzhou Central Hospital.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tranon.2021.101202.

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