PRPS2 Enhances Resistance to Cisplatin via Facilitating Exosomes-mediated Macrophage M2 Polarization in Non-small Cell Lung Cancer

Gaohua Liu*, Yang Luo*, and Peifeng Houa,b,c

*Department of Oncology, Fujian Medical University Union Hospital, Fuzhou, Fujian, China; bFujian Medical University Stem Cell Research Institute, Fuzhou, Fujian, China; cFujian Key Laboratory of Translational Cancer Medicine, Fuzhou, Fujian, China

ABSTRACT

Background: Phosphoribosyl pyrophosphate synthetases 2 (PRPS2) is reported as an oncogene in various cancers. However, the role of PRPS2 in cisplatin (DDP) resistance of non-small cell lung cancer (NSCLC) remains unclear. The present study aimed to explore the effect of PRPS2 in DDP resistance of NSCLC.

Methods: mRNA expression levels of genes were detected by RT-PCR. Enzyme-linked immunosorbent assay (ELISA) and Western blot were used to detect protein expression levels. Cell viability was determined by the MTT assay and colony formation assay. Cell apoptosis was detected using nucleosome ELISA assay and caspase-3 activity assay. PRPS2 silencing was achieved using siRNA transfection. Exosomes of cultured cells were isolated through ultracentrifugation.

Results: Elevated PRPS2 was correlated with DDP resistance and poor prognosis in NSCLC patients. PRPS2 silencing enhanced sensitivity of DDP-resistant cells to DDP treatment. NSCLC cell-derived exosome induced M2 macrophage polarization. PRPS2 was enriched in the exosomes of NSCLC cells. Exosomal PRPS2 mediated M2 macrophage polarization to promote DDP resistance of NSCLC cells.

Conclusions: In conclusion, PRPS2 potentiates resistance to DDP by promoting exosome-mediated macrophage M2 polarization in NSCLC.

KEYWORDS

Cisplatin resistance; exosome; M2 polarization; NSCLC; PRPS2

Instruction

Lung cancer is one of the most common cancers worldwide and a major cause of cancer-related death (Bray et al. 2018; Siegel et al. 2017). The 5-year overall survival of lung cancer patients is less than 20% (Zappa and Mousa 2016). Therefore, lung cancer has become a threat to public health. Approximately 85% of lung cancer cases are non-small cell lung cancer (NSCLC), while small cell lung cancer (SCLC) only represents for around 15% of all cases (Leung et al. 2016). Due to limited early-stage detection, many NSCLC patients are already at late stage when diagnosed (Kogita et al. 2014). Currently, NSCLC treatments in the clinic include surgery, radiation and chemotherapy. For advanced NSCLC, cisplatin

CONTACT Peifeng Hou hpeifeng76@163.com Department of Oncology, Fujian Medical University Union Hospital, Fujian Medical University Stem Cell Research Institute, Fujian Key Laboratory of Translational Cancer Medicine, Fuzhou, Fujian 350000, China

*These authors contributed equally to this work.

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(DDP) is currently the most commonly used chemotherapy drug (Kelland 2007; Schiller et al. 2002). However, the efficacy of DDP treatment is relatively low because most NSCLC cases develop drug resistance eventually (Galluzzi et al. 2012). NSCLC with DDP resistance is more malignant and metastatic, which leads to failure of DDP treatment (D’Antonio et al. 2014). Accordingly, understanding the mechanism responsible for DDP resistance in NSCLC is crucial for improving the efficiency of DDP treatment for NSCLC patients.

The tumor microenvironment (TME) includes cytokines, chemokines, fibroblasts and various types of immune cells, which are crucial for immune regulation during tumor progression (Whiteside 2008; Yu et al. 2020). Macrophage is one of the major types of immune cells in TME. Upon stimulation, macrophages differentiate into either M1 or M2 subtype. M1 macrophages secrete pro-inflammatory cytokines, promote inflammation and participate in a positive immune response. M2 macrophages secrete anti-inflammatory cytokines, suppress inflammation and contribute to immune suppression and tumor growth (Martinez et al. 2008; Murray 2017). Tumor-associated macrophages (TAMs) exhibit the M2 phenotype and promote tumor progression (Noy and Pollard 2014). Higher TAM densities are correlated with worse prognosis of NSCLC patients (Becker et al. 2014), indicating the crucial role of TAMs in NSCLC progression.

In the purine biosynthesis pathway, phosphoribosyl pyrophosphate synthetases 2 (PRPS2) serves as the rate-limiting enzyme. Previous studies have demonstrated that PRPS2 functions as an oncogene in prostate cancer, colorectal cancer and neuroblastoma (Miao and Wang 2019; Qiao et al. 2020; Xue et al. 2016). However, the role of PRPS2 in NSCLC remains unclear. This study aimed to explore the effect of PRPS2 in DDP resistance of NSCLC and explore the mechanism responsible for PRPS2 potentiated DDP resistance. This study provides evidence supporting that PRPS2 may serve as a potential therapeutic target in NSCLC treatment.

Methods

NSCLC samples

A total of 27 DDP-sensitive and 33 DDP-resistant NSCLC tissues were collected in Fujian Medical University Union Hospital. All NSCLC tissues were collected and immediately frozen in liquid nitrogen for further analysis. All NSCLC patients signed the written consents. This study was performed under the guidelines of the Ethics Committee of Fujian Medical University Union Hospital. The study was conducted in accordance with the Basic & Clinical Pharmacology & Toxicology policy for experimental and clinical studies [6].

Cell culture

Human NSCLC cell lines A549 and H1299, human acute monocyte leukemia cells THP-1 were purchased from Shanghai cell bank (Shanghai, China). All cells were cultured in RPMI-1640 medium supplied with 10% fetal bovine serum (FBS, Invitrogen) and 1% penicillin/streptomycin at 37°C in a humidified incubator with 5% CO₂.

DDP-resistant NSCLC cell construction was induced by progressively increasing the concentration of DDP (Sigma-Aldrich, USA). Briefly, after 24-h culture, A549 and H1299
cells were treated using 0.5 μM DDP for 48 h, followed by withdrawing DDP until cells recovered. The procedure was repeated until these cells were resistant to the concentration of DDP. DDP concentration was gradually enhanced to 6 μM. The cells were considered to be DDP-resistant after recovering under 6 μM DDP treatment for 2 months with normal activity. To obtain the THP-1-derived macrophages, $3 \times 10^6$ THP-1 cells were cultured in RPMI-1640 medium with 350 nM PMA (Sigma, USA) for 48 h. Then, the medium replaced with fresh PMA-containing medium, and the cells were differentiated for further experiments.

**RT-PCR**

Total RNA from cells or NSCLC tissues was isolated using TRIzol reagent (Thermo Fisher, USA). A total of 500 ng RNA was used for cDNA synthesis, which was performed using the PrimeScript™ RT-PCR Kit (TaKaRa, Japan). The primers used in this study are PRPS2, F: 5’-AGC TCG CAT CAG GAC CTG T-3’, R: 5’-ACG CTT TCA CCA ATC TCC ACG-3’; IL-10, F: 5’-GAC TTT AAG GGT TAC CTG GGT TG-3’, R: 5’-TCA CAT GCG CCT TGA TGT CTG-3’; Arg-1: F: 5’-ACG GAA GAA TCA GCC TGG TG-3’, R: 5’-GTC CAC GTC TCT CAA GCC AA-3’; CD163: F: 5’-CCA GTC CCA AAG ACT GTC CT-3’, R: 5’-ATG CCA GTG AGC TTC CCG TTC AGC-3’; CD206: F: 5’-ATC CAC TCT ATC CAC CTT CA-3’, R: 5’-TGC TTT TTC ATA TCT GTC TTC A-3’; GAPDH: F: 5’-CCA GGT GGT CTC CTC TGA CTT C-3’, R: 5’-GTT GTC GTT GAG GGC AAT G-3’. The relative gene expression was determined by the $2^{-ΔΔCt}$ method.

**Western blot**

NSCLC cell lines, exosomes or cultured macrophages were lysed using lysis buffer with protease inhibitors (Roche, Switzerland). A total of 20 μg protein was loaded into 10% SDS-PAGE, followed by transferring to a PVDF membrane (Millipore, USA). Then, the membranes were incubated with primary antibodies overnight at 4°C after blocking with 5% milk. The primary antibodies used are anti-PRPS2 (Novus, USA), anti-CD63 (Abcam, USA), anti-TSG101 (tumor susceptibility gene 101) (Abcam, USA), anti-IL-10 (CST, USA), anti-Arg-1 (CST, USA), anti-CD163 (CST, USA), anti-206 (CST, USA), anti-β-actin (CST, USA). Then, the membranes were incubated with HRP-conjugated secondary antibody. The signals were visualized using the electrochemiluminescence kit (Pierce Biotechnology, USA).

**siRNA transfection**

Two siRNAs targeting PRPS2 and the negative control siRNA were designed by RiboBio Company (Guangzhou, China). The sequences of these siRNAs are si-PRPS2#1: CTG CAA GAT TGC GTC ATC A, si-PRPS2#2: CCA CCA AAG TGT ATG CT AT. The siRNA transfection was performed using Lipofectamine 3000 (Invitrogen, USA) following the manufacturer’s instructions. Forty-eight hours after transfection, RT-PCR and Western blot were performed to confirm PRPS2 silencing efficiency.
**MTT assay**

The MTT assay was used to detect viability of NSCLC cells. Briefly, a total of $5 \times 10^3$ cells/well were seeded into 96-well plates and were cultured for 24 h. Then, different concentrations of DDP were added into wells and incubated for 48 h. The cell viability was detected using the MTT kit (Thermo Fisher, USA) according to the manufacturer’s protocol.

**Colony formation assay**

A total of 100 cells/well were seeded into 12-well plates. After 2 weeks of incubation, the wells were stained using 0.05% crystal violet (Beyotime, China). Colonies with more than 50 cells were calculated.

**Caspase-3 activity assay**

The caspase-3 activity assay in different groups of cells was performed using the Caspase-3 Colorimetric Assay kit (Promega, USA) following the manufacturer’s protocol. Briefly, NSCLC cells with different treatments were seeded onto 6-well plates at a density of $1 \times 10^6$ cells/well and treated with 5 μg/ml DDP for 24 h. Ac-DEVD-pNA was added to each well, followed by incubation for 1 h in a humidified atmosphere of 5% CO$_2$ and 95% O$_2$ at 37°C. The OD value was detected at a wavelength of 405 nm using a POLARstar OPTIMA multi-detection microplate reader (Bio-Rad Laboratories, Inc.).

**Nucleosome ELISA assay**

Nucleosome ELISA assay was performed in different groups of cells using the Cell Death Detection ELISAPLUS kit (Roche, Germany) following the manufacturer’s instructions. Color alteration of samples in the cytoplasm was considered as DNA fragment enrichment factor, indicating cell apoptosis.

**Exosome isolation**

Cells were cultured using the irradiated medium containing no bovine exosomes. Exosomes were isolated from cell supernatants (Wang et al. 2020). Briefly, supernatants were first centrifuged at 300 g for 10 min, then 2000 g for 20 min to exclude cell contamination, and then centrifuged at 10,000 g for 30 min. Ultracentrifugation at 100,000 g (Beckman 32Ti rotor) for 70 min at 4°C was performed to collect the pellets, followed by washing using PBS. Then, the collected pellets were centrifuged at 100,000 g (Beckman 60Ti rotor) for 1 h at 4°C. After resuspending in PBS, the pellets were filtered through a 0.22-μm filter (Millipore, USA). The distribution of exosome size was determined using the tunable resistive pulse sensing technology (qNano, New Zealand).

**Co-culture system**

The 0.4-μm pore size six-well transwell apparatus was used for the co-culture of THP-1-derived macrophages and NSCLC cells.
For Figure 3d, a total of $1 \times 10^6$ THP-1 cells were seeded into the lower insert and were cultured in RPMI-1640 medium with 350 nM PMA (Sigma, USA). A total of $5 \times 10^5$ NSCLC cells were seeded into the upper insert and were cultured in RPMI-1640 medium. After 48 h, the THP-1-derived macrophages and NSCLC cells were harvested for further experiments.

For Figure 5a, a total of $1 \times 10^6$ THP-1-derived macrophages were treated with exosomes derived from si-NC-A549 cells, si-PRPS2-A549 cells or PBS and were seeded into the upper insert in RPMI-1640 medium with 350 nM PMA (Sigma, USA). A total of $5 \times 10^5$ NSCLC cells were seeded into the lower insert and were cultured in RPMI-1640 medium. After 48 h, the THP-1-derived macrophages and NSCLC cells were harvested for further experiments.

**ELISA**

IL-10 and Arg-1 concentrations in supernatants were determined using commercial ELISA kits (MyBioSource) following the manufacturer’s instructions.

**Statistical analysis**

Data were presented as mean ± SD. Kaplan–Meier curve was used for analyzing overall survival of NSCLC patients. Student’s t-test and ANOVA with an appropriate post hoc test were used to analyze the data. $P < .05$ was considered as statistically significant.

**Results**

**Elevated PRPS2 is associated with DDP resistance and poor prognosis in NSCLC**

To investigate the role of PRPS2 in DDP resistance of NSCLC, PRPS2 expression levels were determined in A549 and H1299, and DDP-resistant A549/DDP and H1299/DDP cell lines. As shown in Figure 1a,b, PRPS2 mRNA levels in A549/DDP or H1299/DDP cells were significantly higher than A549 or H1299 cells, respectively. Consistently, in terms of protein levels, PRPS2 expression in A549/DDP or H1299/DDP was obviously increased compared with A549 or H1299, respectively (Figure 1c).

PRPS2 expression was further detected in 27 DDP-sensitive and 33 DDP-resistant clinical NSCLC samples. The PRPS2 mRNA levels in DDP-resistant samples were much higher than in DDP-sensitive samples (Figure 1d). Moreover, NSCLC patients with high PRPS2 levels showed worse overall survival than those with low PRPS2 levels (Figure 1e). These results demonstrated that high PRPS2 expression was correlated with DDP resistance and poor prognosis in NSCLC.

**PRPS2 silencing enhanced sensitivity of DDP-resistant NSCLC cells to DDP**

As PRPS2 expression was higher in DDP-resistant NSCLC cells than NSCLC cells, we further silenced PRPS2 expression using siRNA in A549/DDP or H1299/DDP cells to assess the role of PRPS2 in DDP resistance. PRPS2 silencing in A549/DDP or H1299/DDP cells (si-PRPS2#1 and si-PRPS2#2) was confirmed using RT-PCR and Western blot (Figure 2a,b). MTT assay demonstrated that, under the treatment of increasing concentrations of DDP (1 to 160 μg/
ml), viability of A549/DDP cells transfected with PRPS2 siRNAs (si-PRPS2#1 and si-PRPS2 #2) significantly decreased as compared with those transfected with control siRNAs (si-NC) (Figure 2c). A similar phenotype was observed in H1299/DDP cells as well (Figure 2d), and the concentration of 20 μg/ml presented a significant difference among different treatments.

Colony formation assay was further performed. A549/DDP or H1299/DDP cells transfected with si-PRPS2#1 and si-PRPS2#2 exhibited dramatically fewer colony numbers than those transfected with si-NC (Figure 2e). Cell apoptosis in A549/DDP or H1299/DDP cells was further determined by caspase-3 activity assay and nucleosome ELISA assay under the exposure of 5 μg/ml DDP. The relative caspase-3 activity in A549/DDP or H1299/DDP cells transfected with si-PRPS2#1 and si-PRPS2#2 was significantly increased compared with those transfected with si-NC (Figure 2f). Consistently, the nucleosome ELISA assay showed that the enrichment factor in A549/DDP or H1299/DDP cells transfected with si-PRPS2#1 and si-PRPS2#2 was significantly enhanced compared with those transfected with si-NC (Figure 2g). These data indicated that PRPS2 silencing enhanced the sensitivity of DDP-resistant NSCLC cells to DDP.
PRPS2 is enriched in exosomes of NSCLC cells and can be transferred to THP-1-derived macrophages

The mechanism underlying PRPS2 mediated DDP resistance in NSCLC was further explored. Exosomes are crucial for the communication between tumor cells and the TEM (Maia et al. 2018). In this study, exosomes were isolated from the supernatants of A549 and H1299 cells and were detected by transmission electron microscopy. The illustrated
Exosomes from both cell lines were observed as spherical structures (Figure 3a) with the size distribution of 40–250 nm (Figure 3b). The well-known exosomes markers (CD9, Alix, CD63 and TSG101) and PRPS2 were enriched in the isolated exosomes (Figure 3c).

The effect of tumor cell-derived exosomes on PRPS2 expression in macrophages was further explored. Co-cultured tumor cells and THP-1-derived macrophages were illustrated in Figure 3d. After co-culturing with A549 or H1299 cells for 48 h, PRPS2 expression in THP-1-derived macrophages was much higher than co-culturing with PBS control (Figure 3e). Moreover, A549 or H1299-derived exosomes significantly promoted PRPS2 expression in THP-1-derived macrophages (Figure 3f). These results demonstrated that PRPS2 was enriched in the exosomes of NSCLC cells and can be transferred to THP-1-derived macrophages.

To confirm if the increased PRPS2 in macrophages was from cancer-derived exosomes, NSCLC cells were treated with GW4869 and co-cultured with THP-1-derived macrophages, it was found GW4869 significantly decreased the increased protein level of PRPS2 in macrophages due to co-culture with NSCLC cells (Fig. S1), which indicated increased PRPS2 in macrophages was from cancer-derived exosomes.
NSCLC cell-derived exosomes induces macrophage M2 polarization

Next, the effect of NSCLC cell-derived exosomes on macrophage polarization was detected. Co-culturing with A549 or H1299 cells for 48 h significantly enhanced the expression of M2 macrophage markers IL-10, Arg-1, CD163 and CD206 both at mRNA and protein levels in THP-1-derived macrophages (Figure 4a,b). However, there was no difference in terms of M1 macrophage markers TNF-α, IL-1β and HLA-DR (Fig. S2A). Moreover, ELISA assay demonstrated that co-culturing with A549 or H1299 cells for 48 h significantly enhanced the levels of IL-10 and Arg-1 in THP-1-derived macrophages (Figure 4c).

Similarly, A549 or H1299-derived exosome treatment significantly enhanced the expression of IL-10, Arg-1, CD163 and CD206 both at mRNA and protein levels in THP-1-derived macrophages as compared with PBS control (Figure 4d,e). Meanwhile, A549 or H1299-derived exosomes showed no influence on M1 macrophage markers TNF-
α, IL-1β and HLA-DR (Fig. S2B). A549 or H1299-derived exosomes also increased the expression levels of IL-10 and Arg-1 in THP-1-derived macrophages (Figure 4f). These results demonstrated that NSCLC cell-derived exosome induced macrophage M2 polarization.

Figure 5. Exosomal PRPS2 mediates macrophages M2 polarization to promote cisplatin resistance of NSCLC cells. (a) Schematic diagram of co-culture system. THP-1-derived macrophages treated with exosomes derived from si-NC-A549 cells, si-PRPS2-A549 cells or PBS in the upper chambers indirectly co-cultured with A549 or H1299 cells planted in the lower six-well plates for 48 h. (b) mRNA levels of M2 macrophage markers (IL-10, Arg-1, CD163 and CD206) in THP-1-derived macrophages of upper chambers were detected by qRT-PCR. (c) Protein levels of PRPS2 and M2 macrophage markers (IL-10, Arg-1, CD163 and CD206) in THP-1-derived macrophages of upper chambers were detected by Western blot. (d) Protein levels of M2 macrophage markers (IL-10, Arg-1) in supernatants of THP-1-derived macrophages of upper chambers were measured by ELISA. (e and f) Viability of A549 and H1299 cells in the lower six-well plates was evaluated by MTT assay with the treatment of various concentrations of DDP. (g) Colony formation of A549 and H1299 cells in the lower six-well plates was determined with the exposure of 2 μg/ml DDP. (h and i) Cell apoptosis of A549 and H1299 cells in the lower six-well plates was determined by caspase-3 activity assay and nucleosome ELISA assay the exposure of 2 μg/ml DDP. The data represent the mean ± SD from three independent experiments. *P < .05; **P < .01; ***P < .001.
**Exosomal PRPS2-mediated M2 polarization promotes DDP resistance of NSCLC cells**

The role of exosomal PRPS2-mediated M2 polarization in DDP resistance was further determined. As illustrated in Figure 5a, A549 or H1299 cells were co-cultured with THP-1-derived macrophages, which were treated with exosomes derived from si-NC-A549 cells, si-PRPS2-A549 cells or PBS control. Exosomes from si-NC-A549 cells (A549 exo+si-NC) significantly enhanced the expression levels of IL-10, Arg-1, CD163 and CD206 both at mRNA and protein levels in THP-1-derived macrophages, while exosomes from si-PRPS2-A549 cells (A549 exo+si-PRPS2) significantly decreased the expression levels of IL-10, Arg-1, CD163 and CD206 (Figure 5b,c). As expected, the enhanced IL-10 and Arg-1 protein levels by A549 exo+si-NC treatment in the supernatants of THP-1-derived macrophages were significantly decreased by A549 exo+si-PRPS2 (Figure 5d).

Under treatment of increasing DDP concentrations, A549 exo+si-NC significantly enhanced the viability of A549 or H1299 cells compared with PBS+si-NC, whereas A549 exo+si-PRPS2 dramatically suppressed the viability of these cells, and the concentration of 20 μg/ml presented a significant difference among different treatments (Figure 5e,f). Consistently, the enhanced colony numbers by A549 exo+si-NC treatment was significantly suppressed by A549 exo+si-PRPS2 treatment (Figure 5g). Under the exposure of 2 μg/ml DDP, A549 exo+si-NC treatment significantly inhibited cell apoptosis, detected by caspase-3 activity assay and nucleosome ELISA assay, whereas A549 exo+si-PRPS2 dramatically enhanced cell apoptosis of these cells (Figure 5h,i). These results demonstrated that exosomal PRPS2-mediated macrophage M2 polarization promoted DDP resistance of NSCLC cells.

**Discussion**

NSCLC is the major type of lung cancer, and one of the most common cancers in the world. Currently, various strategies are available for NSCLC treatment; however, the effect is limited and unsatisfactory due to metastasis and drug resistance of NSCLC. DDP treatment is the most commonly used chemotherapy for NSCLC in clinic and is effective for NSCLC at early stage. However, most NSCLC patients develop DDP resistance. TAMs with M2 phenotype in the TME is commonly believed to promote NSCLC progression. Exosomes from tumor cells are important for the communication between tumor cells and their TME. Moreover, accumulating evidence has demonstrated that PRPS2 functions as an oncogene in various cancers, but the effect of PRPS2 has not been studied in NSCLC. Therefore, we explored the role of PRPS2 in NSCLC and the potential mechanism. We found that enhanced PRPS2 was corrected with DDP resistance and poor prognosis of NSCLC patients. PRPS2 downregulation increased sensitivity of DDP-resistant NSCLC cells to DDP treatment. NSCLC cell-derived exosomes induced macrophage M2 polarization. PRPS2 was enriched in the exosomes of NSCLC cells, and the exosomal PRPS2 mediated macrophage M2 polarization to promote the DDP resistance of NSCLC cells. This research has linked PRPS2, TAM, exosomes and NSCLC, and provided evidence demonstrating that PRPS2 may serve as a potential target for DDP-resistant NSCLC treatment.

PRPS2 was reported to be an oncogene in many cancers. In prostate cancer, colorectal cancer and lymphoma, PRPS2 expression levels are significantly increased, and PRPS2 promotes cell migration and cell invasion of tumor cells by regulating matrix
metalloproteinase nine activities and E-cadherin expression (Cunningham et al. 2014). Moreover, PRPS2 controls the crosstalk of metabolism and protein synthesis to fuel tumor cell growth (Cunningham et al. 2014). Consistently, our study demonstrated that enhanced PRPS2 expression was correlated with DDP resistance and poor prognosis in NSCLC patients. PRPS2 was necessary for the DDP resistance of NSCLC. Mechanically, PRPS2 promoted DDP resistance of NSCLC through inducing macrophage M2 polarization.

In the TME, macrophage polarization is crucial for immune regulation and tumor progression. Based on the dichotomy, macrophages are proposed to be classical M1 and alternative M2 (Martinez and Gordon 2014). M1 macrophages promote inflammatory responses, while M2 macrophages suppress inflammatory responses and promote tumor growth (Martinez and Gordon 2014). TAMs with the M2 phenotype promote tumor progression, and are important for immune escape and tumor progression (Mantovani et al. 2002; Sica et al. 2006; Solinas et al. 2009). Therefore, strategies targeting TAMs are potential choices for anti-tumor treatment. Suppression of colony-stimulating factor 1 (CSF-1) receptor resulted in TAM death in vitro and significantly attenuated TAMs, promoted CD8+/CD4+ T cell ratio in animal models. CSF-1 receptor administration significantly suppressed TAMs in patients with diffuse-type giant cell tumors (Ries et al. 2014). TAM reduction in stromal tumor dramatically altered the TME and resulted in suppressed breast cancer progression (Luo et al. 2006). We found that co-culturing with DDP-resistant A549 or H1299 cells, or treatment using the exosomes from A549 or H1299 cells, induced M2 polarization of macrophages, with increased levels of IL-10, Arg-1, CD163 and CD206. Moreover, the M2 macrophages promoted DDP resistance of A549 or H1299 cells.

Exosomes are one of the extracellular vesicles secreted by various cells and can affect the function and behavior of distant cells that take up these exosomes (Kalluri and LeBleu 2020). We found that PRPS2 was enriched in the exosomes of NSCLC cells, and their exosomes promoted macrophage M2 polarization, and finally contributed to the DDP resistance of NSCLC cells.

THP-1-derived macrophages have been widely used as a human cell model in the immune microenvironment of multiple cancer types (Dong et al. 2019; Genin et al. 2015; Weng et al. 2019). The data with THP-1-derived macrophages are sufficient and consistent with discoveries in this study. Related research using RAW164.7 and human peripheral blood monocytes would be explored in the future study.

In summary, we explored the role of PRPS2 in NSCLC and the potential mechanism. High PRPS2 expression positively correlated with DDP resistance and poor prognosis of NSCLC patients. PRPS2 downregulation enhanced sensitivity of DDP-resistant NSCLC cells to DDP. NSCLC cell-derived exosomes induced M2 polarization of macrophages. The exosomal PRPS2-mediated M2 polarization promoted the DDP resistance of NSCLC cells.

**Conclusion**

PRPS2 potentiates DDP resistance in NSCLC via promoting exosome-mediated M2 polarization of macrophages. PRPS2 may serve as a potential target in NSCLC treatment.
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