Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.jzus.zju.edu.cn; www.springerlink.com
E-mail: jzus@zju.edu.cn

Kinesin KIF4A is associated with chemotherapeutic drug resistance by regulating intracellular trafficking of lung resistance-related protein*#

Li-na PAN[§1,2], Yuan ZHANG[§3], Chang-jun ZHU[1,2], Zhi-xiong DONG[†‡1,2]

([1]*Tianjin Key Laboratory of Animal and Plant Resistance, College of Life Sciences, Tianjin Normal University, Tianjin 300387, China*)

([2]*Key Laboratory of Molecular and Cellular Systems Biology, Tianjin Normal University, Tianjin 300387, China*)

([3]*Department of Cancer Research Institute, Cancer Affiliated Hospital of Xinjiang Medical University, Urumqi 830000, China*)

[†]E-mail: skydzx@tjnu.edu.cn

Received Mar. 10, 2017; Revision accepted May 30, 2017; Crosschecked Nov. 15, 2017

Abstract: Multidrug resistance (MDR) is the major impediment to cancer chemotherapy. The expression of lung resistance-related protein (LRP), a non-ATP-binding cassette (ABC) transporter, is high in tumor cells, resulting in their resistance to a variety of cytotoxic drugs. However, the function of LRP in tumor drug resistance is not yet explicit. Our previous studies had shown that Kinesin KIF4A was overexpressed in cisplatin (DDP)-resistant human lung adenocarcinoma cells (A549/DDP cells) compared with A549 cells. The expression of KIF4A in A549 or A549/DDP cells significantly affects cisplatin resistance but the detailed mechanisms remain unclear. Here, we performed co-immunoprecipitation experiments to show that the tail domain of KIF4A interacted with the N-terminal of LRP. Immunofluorescence images showed that both the ability of binding to LRP and the motility of KIF4A were essential for the dispersed cytoplasm distribution of LRP. Altogether, our results shed light on a potential mechanism in that motor protein KIF4A promotes drug resistance of lung adenocarcinoma cells through transporting LRP-based vaults along microtubules towards the cell membrane. Thus KIF4A might be a cisplatin resistance-associated protein and serves as a potential target for chemotherapeutic drug resistance in lung cancer.

Key words: KIF4A; Lung resistance-related protein (LRP); Drug resistance
http://dx.doi.org/10.1631/jzus.B1700129 **CLC number:** Q291

1 Introduction

Chemotherapy is one of the most effective strategies of cancer treatment. However, multidrug resistance (MDR) where cancer cells become resistant to different cytotoxic drugs is the major impediment to effective cancer chemotherapy (Kathawala *et al.*, 2015). There are many factors involved in this process, including overexpression of transport-associated proteins in tumor cells, such as P-glycoprotein (P-gp), multidrug resistance-associated protein 1 (MRP1), breast cancer resistance protein (BCRP), and lung resistance-related protein (LRP), all of which have been considered as major factors in

‡ Corresponding author
§ The two authors contributed equally to this work
* Project supported by the National Natural Science Foundation of China (Nos. 31271485 and 31301138), the Tianjin Research Program of Application Foundation and Advanced Technology (No. 12JC 2DJC21400), the Doctor Foundation of Tianjin Normal University (Nos. 52XB1104 and 52XB1005), the Joint Funds of the Xinjiang Uygur Autonomous Region Natural Science Foundation (No. 2016 D01C375), the Program for New Century Excellent Talents in University in China (No. NCET-11-1066), and the State Key Laboratory of Molecular Oncology (No. SKL-KF-2017-18), China
# Electronic supplementary materials: The online version of this article (http://dx.doi.org/10.1631/jzus.B1700129) contains supplementary materials, which are available to authorized users
ORCID: Zhi-xiong DONG, http://orcid.org/0000-0001-7281-671X; Li-na PAN, http://orcid.org/0000-0002-5332-2682

cancer chemotherapeutic resistance (Scheper *et al.*, 1993; Germann, 1996; Gottesman *et al.*, 2002).

LRP, also known as the major vault protein (MVP), is the main structural component of vault complexes which are the largest intracellular ribonucleoprotein particles (Scheffer *et al.*, 1995). LRP was found to be overexpressed in doxorubicin resistant non-small cell lung cancer cells which had no P-gp expression (Scheper *et al.*, 1993). High expression of LRP was observed in many tumor cells resistant to various cytotoxic drugs including mitoxantrone, doxorubicin, methotrexate, vincristine, etoposide, cisplatin, and cytarabine (Mossink *et al.*, 2003), suggesting that LRP played an important role in drug resistance. However, the detailed molecular mechanisms whereby LRP participates in drug resistance are still unclear. It was reported that LRP-based vaults were involved in nucleus/cytoplasm transport, and that this may decrease the nucleus/cytoplasm distribution ratio of drugs (Scheffer *et al.*, 1995). However, the majority of LRP-based vaults are localized in cytoplasm and no more than 5% of LRP-based vaults were associated with the nucleus (Chung *et al.*, 2005). In addition, some LRP-based vaults were observed moving rapidly along microtubules in cytoplasm (Slesina *et al.*, 2006; van Zon *et al.*, 2006; Yang *et al.*, 2010). These observations suggest that LRP-based vaults might be involved in intracellular transport in addition to nucleus/cytoplasm transport and function as a drug delivery vehicle which moves cytotoxic drugs away from their subcellular targets out of cells so as to contribute to the drug-resistance process in tumor cells. However, it is unknown if there are any motor proteins transporting LRP-based vaults to move along microtubules towards cell membrane inside of cells.

Motor protein KIF4A, a member of the Kinesin-4 family, is a microtubule-based plus-end directed motor protein. KIF4A has been reported to participate in a variety of cell processes, including intracellular trafficking of HIV gag and integrin-β_1 (Martinez *et al.*, 2008; Willemsen *et al*., 2014), mitotic progression (Zhu and Jiang, 2005; Zhu *et al.*, 2005; Wandke *et al.*, 2012), DNA damage response (Wu *et al.*, 2008) and brain neuron development (Midorikawa *et al.*, 2006). It has been also reported that KIF4A was upregulated in lung cancer and could serve as a prognostic biomarker and therapeutic target for lung cancer (Taniwaki *et al.*, 2007; Zhu *et al.*, 2015). Our previous studies showed that KIF4A is also involved in cisplatin resistance in lung cancer cells (Xiao *et al.*, 2016). However, the detailed mechanisms are still not clear.

In this study, to investigate whether the LRP and KIF4A are involved in the multidrug resistance of lung cancer cells, the expression of LRP and KIF4A was examined in cisplatin-resistant A549/DDP cells and A549 cells. The underlying mechanism was then investigated by examining how KIF4A regulated the intracellular distribution of LRP. These findings reveal the potential role of Kinesin KIF4A in regulating drug resistance in lung cancer cells by transporting LRP-based vaults-drug delivery vehicles.

2 Materials and methods

2.1 Cell culture and transfection

Human lung adenocarcinoma A549 cells and cisplatin (DDP)-resistant A549/DDP cells were cultured in RPMI 1640 medium containing 10% (0.1 mg/ml) fetal calf serum (Invitrogen, Carlsbad, CA, USA) at 37 °C and 5% CO_2. DDP (2 μg/ml; Sigma-Aldrich, St. Louis, USA) was added to the culture medium of A549/DDP cells. The cells were transfected with 50 nmol/L small interfering RNAs (siRNAs) and/or 0.2–1.0 μg of plasmids using the TurboFect transfection reagent (Thermo Fisher Scientific, Waltham, MA, USA). Cells were harvested or fixed for immunoprecipitation, immunoblotting, or immunofluorescence analysis 36–48 h after transfection.

2.2 Plasmids, siRNA, and antibodies

A full-length human LRP complementary DNA (cDNA; GenBank: X79882.2) was generated by polymerase chain reaction (PCR) and subcloned into *Eco*R1/*Bam*HI sites of the mammalian expression vector pEGFP-C1 or pFlag-C1 using the primers 5'-CCG GAA TTC ATG GCA ACT GAA GAG TTC ATC-3' and 5'-CGC GGA TCC GCG CAG TAC AGG CAC CAC GT-3'. KIF4A expression plasmids were generated as described previously (Zhu and Jiang, 2005). Fragments of KIF4A and LRP were subcloned into vectors of pEGFP-C1 or pFlag-C1, respectively. All plasmids were confirmed by DNA sequencing (GeneWiz, Suzhou, China). siRNA specifically targeting 3'-UTR of KIF4A (5'-GGA

ATGAGGTTGTGATCTT-3') was synthesized by GenePharma (Shanghai, China). Polyclonal rabbit anti-KIF4A antibodies were generated as previously described (Zhu and Jiang, 2005). Rat anti-α-tubulin, rabbit anti-green fluorescent protein (GFP), and mouse anti-Flag antibodies were purchased from Sigma-Aldrich. Mouse anti-LRP was purchased from BD Biosciences (San Jose, CA, USA). All secondary antibodies were obtained from Life Technologies Inc. (Grand Island, NY, USA).

2.3 Immunoprecipitation and Western blot

A549 cells were cultured in 60-mm plates and transfected with 3 μg of plasmids. Two days after transfection, the cells were lysed by NP-40 buffer (50 mmol/L Tris-Cl, 150 mmol/L NaCl, 1% (0.01 g/ml) NP-40, 1 mmol/L phenylmethylsulfonyl fluoride (PMSF), 1 mmol/L dithiothreitol (DTT), 10 U/ml aprotinin, 20 μg/ml leupeptin, 50 μmol/L Na_3VO_4, 5 mmol/L β-glycerophosphate, 5 mmol/L NaF, 10% (v/v) glycerol). Lysates were mixed with anti-KIF4A antibodies at 4 °C overnight and then incubated either with protein A (CST, USA) beads at 4 °C for 2 h or with anti-Flag M2 magnetic beads (Sigma-Aldrich) at 4 °C for 3 h. Immunoprecipitates were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), transferred to a polyvinylidene fluoride (PVDF) membrane, and then immunoblotted with various primary antibodies and secondary antibodies.

2.4 Immunofluorescence analyses

A549 or A549/DDP cells (3×10^4) were grown on glass coverslips in a 24-well plate and cultured at 37 °C in a 5% CO_2 incubator overnight. Then cells were transfected with 50 nmol/L KIF4A siRNA and/or 1 μg of plasmids. Thirty-six hours after transfection, cells were fixed in phosphate-buffered saline (PBS) containing 3% (0.03 g/ml) formaldehyde and 2% (0.02 g/ml) sucrose for 15 min followed by incubation in PBS containing 0.1 mol/L glycine for 5 min. Cells are penetrated and blocked with PBS containing 10% (0.1 g/ml) goat serum and 0.4% (4 g/L) TritonX-100 for 30 min. After washing with PBS-TX (PBS containing 0.1% (v/v) TritonX-100) three times, cells were incubated with primary antibody in PBS-TX for 2–3 h at room temperature followed by secondary antibody in PBS-TX for 1–2 h. Finally, cells were stained with 1 μg/ml of 4'6-diamidino-2-phenylindole (DAPI) in PBS-TX for 3 min and the coverslips were mounted in 4 μl of FluroGuard Antifade solution (Bio-Rad Inc., San Diego, California, USA) and sealed with Cytoseal-60 (Fisher Inc., Rockford, IL, USA).

3 Results

3.1 Kinesin KIF4A interacted with LRP on the microtubules in the cytoplasm

It was reported that LRP was involved in MDR by mediating drug transport processes inside cells (Steiner *et al.*, 2006). However, the regulation of intracellular distribution of LRP has remained unclear. Given the fact that LRP-based vaults can move along microtubules (van Zon *et al.*, 2006), we explored the potential binding partners of LRP among microtubule-based motor proteins, the Kinesin superfamily (Kifs), in A549/DDP cells. Previously, we found that Kinesin KIF4A was highly expressed in lung cisplatin-resistant A549/DDP cells and KIF4A participated in regulating cisplatin resistance of A549/DDP cells (Xiao *et al.*, 2016). We then investigated the relationship between LRP and KIF4A.

First, we examined the expression of LRP and KIF4A in A549 and A549/DDP cells. Consistent with previous reports, LRP and KIF4A were highly expressed in A549/DDP cells and much more than in A549 cells in both mRNA and protein levels (Trussardi *et al.*, 1998; Xiao *et al.*, 2016; Zhang *et al.*, 2016), as shown in Fig. S1. Then we ectopically expressed Flag-tagged LRP in A549/DDP cells and performed co-immunoprecipitation assay using anti-Flag antibodies followed by immunoblotting with anti-KIF4A antibody. Western blot results showed that KIF4A could co-immunoprecipitate with Flag-LRP (Fig. 1a). To confirm the interaction of KIF4A and LRP, we performed immunoprecipitation experiments using A549/DDP cell lysates with anti-LRP antibodies, which were then immunoblotted with anti-KIF4A antibodies. As shown in Fig. 1b, KIF4A could be immunoprecipitated with LRP by anti-LRP antibodies. These results indicated an interaction between KIF4A and LRP in A549/DDP cells.

It is well-known that KIF4A is a microtubule-associated motor protein. We used immunofluorescence

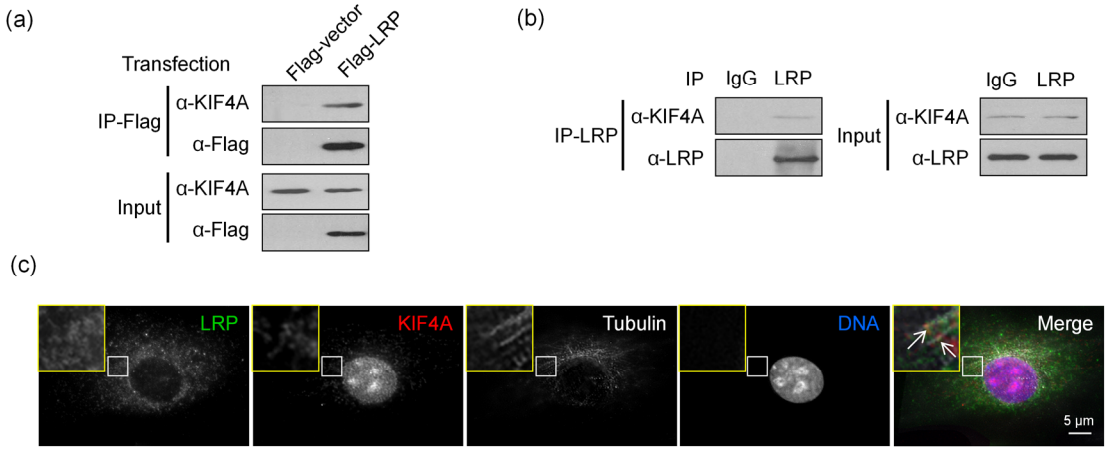

Fig. 1 LRP co-localized with KIF4A on microtubules

(a) A549 cells were transfected with pFlag-LRP plasmids or vector plasmids. Forty-eight hours post transfection, cells were lysed and immunoprecipitated by anti-Flag M2 magnetic beads followed by Western blot analysis using anti-Flag antibodies or anti-KIF4A antibodies as indicated. (b) A549/DDP cells were lysed and immunoprecipitated by anti-LRP or IgG antibodies. Western blot was performed with anti-LRP antibodies or anti-KIF4A antibodies as indicated. (c) A549/DDP cells were fixed and stained with mouse anti-LRP (green), rabbit anti-KIF4A (red), rat anti-α-tubulin (gray) antibodies, and DAPI (blue). KIF4A was partially co-localized with LRP on microtubules in the cytoplasm (arrows). IP-: immunoprecipitated; α-: anti- (Note: for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article)

analyses to examine the intracellular localization of LRP and KIF4A. As shown in Fig. 1c, KIF4A was partially co-localized with LRP on microtubules in the cytoplasm. Taken together, these results indicated that LRP interacted with motor protein KIF4A and was co-localized on microtubules with KIF4A in the cytoplasm.

3.2 Tail domain of KIF4A interacted with N-terminal of LRP

To further investigate which domain of KIF4A was necessary for its interaction with LRP, we constructed several plasmids expressing GFP-tagged truncated KIF4A. As shown in Fig. 2a, Kinesin KIF4A harbors multiple well-characterized functional domains. We respectively co-expressed Flag-LRP and GFP-KIF4A FL (full-length) or truncated version N351 (motor domain), N1018 (motor and coiled-coil/Stalk domain), C896 (coiled-coil/Stalk and tail domain) or C230 (tail domain) in A549 cells. Co-immunoprecipitation experiments showed that both KIF4A-C230 and KIF4A-C896 bound LRP similarly to KIF4A-FL. In contrast, the N-terminal of KIF4A (N351 and N1018) cannot bind LRP (Fig. 2b).

Previous studies have shown that LRP contains EF-hands (aa 131–143) interacting with calcium and a coiled-coil domain (aa 648–800) for intermolecular interaction (van Zon *et al*., 2002). We next constructed plasmids expressing Flag-tagged LRP-WT (full-length), N447 (N-terminal, aa 1–447) or C446 (C-terminal, aa 448–893) (Fig. 2c). Then we co-transfected these plasmids with pEGFP-KIF4A for immunoprecipitation assays. As shown in Fig. 2d, GFP-KIF4A was immunoprecipitated by LRP-WT and LRP-N447, while the LRP-C446 failed to bind GFP-KIF4A. These results suggested that the N-terminal of LRP was necessary for its interaction with KIF4A. Altogether, the results indicated that the tail domain of KIF4A could interact with the N-terminal of LRP.

3.3 KIF4A controlled intracellular trafficking of LRP

The N-terminal of LRP interacted with the motor domain of Kinesin KIF4A. Our previous studies had shown that Kinesin KIF4A played a part in cisplatin resistance of lung cancer cells (Xiao *et al*., 2016). We therefore proposed that motor protein KIF4A might be involved in regulation of the cytoplasm trafficking of LRP. To examine whether cytoplasmic distribution of LRP could be regulated by Kinesin KIF4A, siRNAs were used to knock down the endogenous KIF4A in A549/DDP cells. As shown in Fig. 3a, KIF4A was depleted by around 90% by transfection of siRNA specific targeted 3'-UTR of

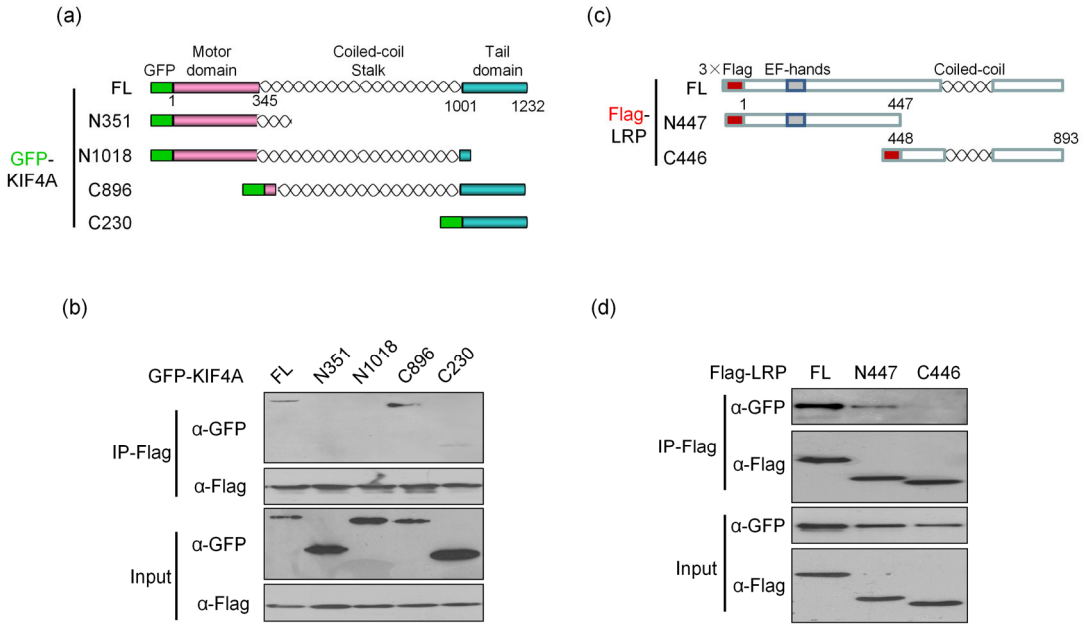

Fig. 2 N-terminal of LRP interacted with tail domain of KIF4A

(a) Schematic diagrams of GFP-KIF4A constructs. (b) A549 cells were co-transfected with plasmids of pFlag-LRP and pEGFP-KIF4A-truncations. Forty-eight hours post transfection, cells were lysed and immunoprecipitated by anti-Flag M2 magnetic beads. Anti-Flag antibodies and anti-GFP antibodies were used for Western blot as indicated. (c) Schematic diagrams of Flag-LRP constructs. (d) A549 cells were co-transfected with plasmids of pEGFP-KIF4A and pFlag-LRP-truncations. Forty-eight hours post transfection, cells were lysed and immunoprecipitated by anti-Flag M2 magnetic beads. Anti-Flag antibodies and anti-GFP antibodies were used for Western blot as indicated. IP-: immunoprecipitated; α-: anti-; FL: full-length

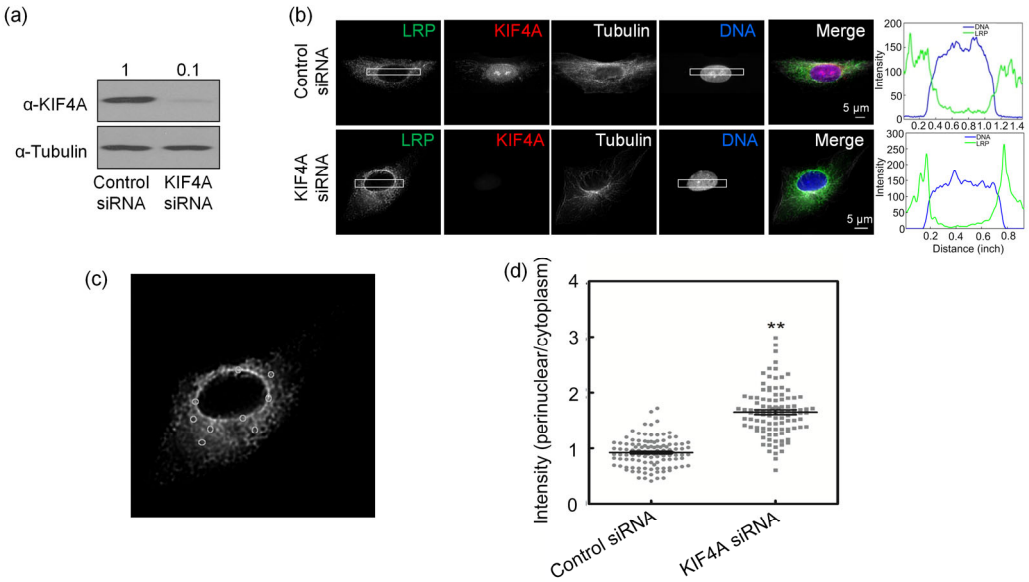

Fig. 3 Cytoplasmic distribution of LRP regulated by KIF4A

(a, b) A549/DDP cells were transfected with KIF4A or control siRNAs. Thirty-six hours post transfection, cells were either lysed for Western blot (a) or fixed and stained with mouse anti-LRP antibodies (green), rabbit anti-KIF4A antibodies (red), and rat anti-α-tubulin antibodies (gray); DNA is shown as blue (b). The box represents the region used to generate the line scans that indicate the localization of LRP (green) relative to DNA (blue). (c, d) Cytoplasmic distribution of LRP in KIF4A or control siRNAs treated A549/DDP cells was measured and quantified. Random 4–5 regions of LRP at cytoplasm or perinuclear were analyzed (c) and the ratio of LRP localization at perinuclear versus cytoplasm was illustrated as dots (d). Bar graphs represent the mean±standard deviation (SD) from at least three independent experiments with more than 20 cells. $^{**}P<0.01$, vs. control. α-: anti- (Note: for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article)

KIF4A in A549/DDP cells. Immunostainning experiments showed that LRP was dispersed throughout the cytoplasm in control siRNA transfected cells, while a great deal of LRP clustered in the perinuclear region in KIF4A-depleted cells (Fig. 3b). To display the intracellular distribution of LRP more clearly, we quantified 4–5 areas at random at the perinuclear region or the cytoplasm for optical intensity analysis in every cell (Fig. 3c) and calculated the intensity ratio of LRP localization at the perinuclear versus cytoplasm. As shown in Fig. 3d, more LRP clustered in the perinuclear region in KIF4A-depleted cells. These results suggest that dispersed cytoplasmic distribution of LRP depended on the motor protein KIF4A.

To examine the requirement of KIF4A in dispersed cytoplasmic distribution of LRP in A549/DDP cells, we performed RNA interference (RNAi) rescue experiments to transfect siRNA specifically targeting 3'-UTR of KIF4A and simultaneously expressing different KIF4A truncated mutants in A549/DDP cells. As shown in Fig. 4a, endogenous KIF4A was depleted efficiently while exogenous proteins of GFP-KIF4A-FL, GFP-KIF4A-N351, GFP-KIF4A-N1018, GFP-KIF4A-C896, or GFP-KIF4A-C230 were ectopically expressed in A549/DDP cells. We performed immunostaining experiments to investigate the cytoplasmic distribution of LRP in detail in these A549/DDP cells. Cells expressing GFP-KIFA-FL but lacking endogenous KIF4A displayed a normal cytoplasmic distribution of LRP. In contrast, LRP clustered in the perinuclear region in cells with depleted KIF4A but expressing truncated KIF4A mutants N351, N1018, C896, or C230 (Figs. 4b and 4c).

Taken together, all the results indicated that Kinesin KIF4A was involved in the cytoplasmic trafficking of LRP. As cargo, LRP interacted with the tail domain of KIF4A and then was transported along the microtubules towards cell membrane.

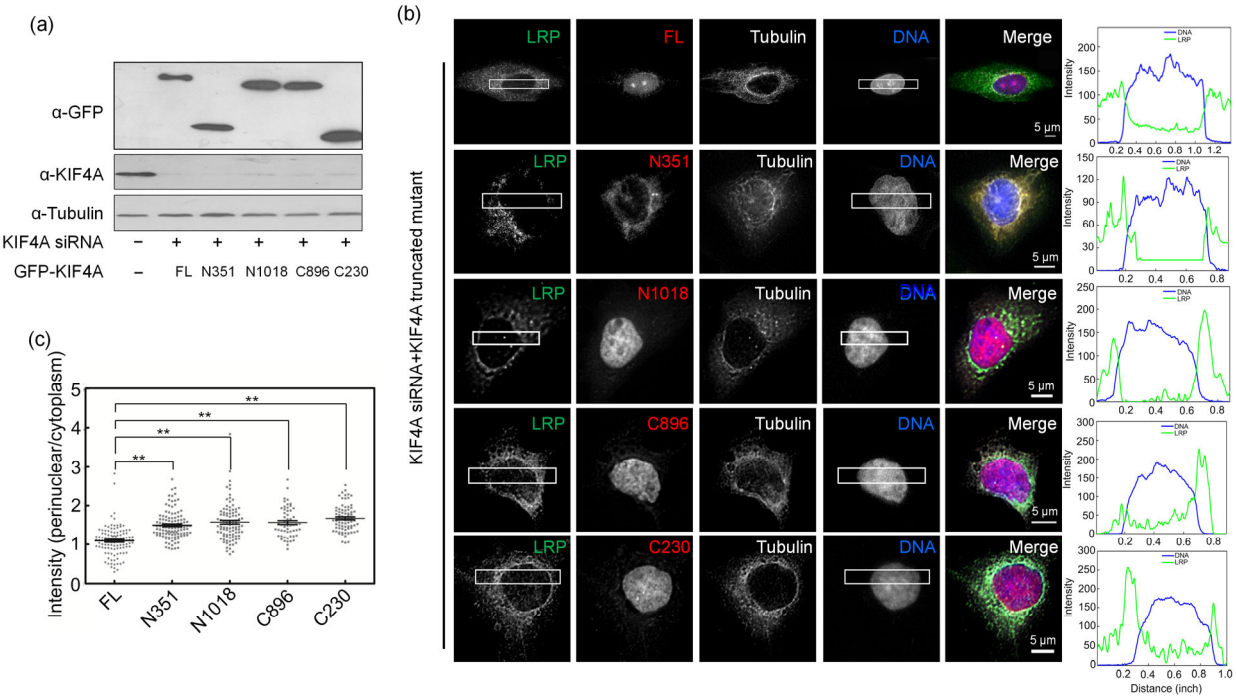

Fig. 4 Both motor and tail domains of KIF4A required for cytoplasmic distribution of LRP

(a, b) A549/DDP cells were transfected with KIF4A siRNA and plasmids of pEGFP-KIF4A-FL, pEGFP-KIF4A-N351, pEGFP-KIF4A-N1018, pEGFP-KIF4A-C896, and pEGFP-KIF4A-C230. Thirty-six hours post transfection, cells were either lysed for Western blot (a) or fixed and stained with mouse anti-LRP antibodies (green), rat anti-α-tubulin antibodies (gray), and GFP-fusion proteins (red); DNA is shown as blue (b). The box represents the region used to generate the line scans that indicate the localization of LRP (green) relative to DNA (blue). (c) Quantification of the ratio of LRP localization at perinuclear versus cytoplasm. Bar graphs represent the mean±SD from at least three independent experiments with more than 20 cells. $^{**}P<0.01$. α-: anti- (Note: for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article)

4 Discussion

KIF4A, a plus-ends directed microtubule-based motor protein, is essential for chromosome condensation, congression, and spindle microtubule dynamics during mitosis. Here, we report that Kinesin KIF4A promoted a drug-resistance process in lung cancer by binding and transporting LRP throughout the cytoplasm. We also determined that the tail domain (cargo-binding domain) of KIF4A interacted with the N-terminal of LRP (Fig. 2). Cytoplasmic distribution of LRP required full-length of KIF4A, suggesting that LRP-based vaults might be one of cytoplasmic cargos of motor protein KIF4A.

LRP has been identified as one of the MDR proteins given the fact that there is a significant association between LRP expression in tumor tissues and the clinical effect of chemotherapy in the survival rates of many types of cancer patients (Scheffer *et al.*, 2000). However there are still debates on whether LRP is involved in drug resistance directly. Mossink *et al.* (2002) reported that knockdown of LRP did not induce hypersensitivity to cytotoxic agents. However, other studies suggested that LRP is involved in drug resistance in human breast cancer MCF-7/ADR cells by directly transporting doxorubicin from the action target (nucleus) to cytoplasm (Han *et al.*, 2012). At the same time, as the MVP, LRP is the main structural component of vault complex. The other two vault proteins are vault poly (ADP-ribose) polymerase (VPARP) and telomerase-associated protein 1 (TEP1) (Mossink *et al.*, 2003). It was reported that LRP self-associated by its coiled-coil domain (CC domain, aa 648–800), and interacted with VPARP by its N-terminal domain (van Zon *et al.*, 2002). Since the ratio of VPARP to MVP molecules is 8/96 in the vaults, VPARP will not occupy all the N-terminal of LRP. Lots of EF-hand domains (aa 131–143) at the N-terminal may be exposed for interacting with other proteins (Yu *et al.*, 2002). The results in Fig. 2 indicated that the truncated mutant without the N-terminal of LRP (LRP-C446) cannot bind KIF4A, suggesting that the N-terminal of LRP was essential for interacting with KIF4A. Further studies are needed to determine if the EF-hand motif and the Ca^{2+} binding are essential for LRP-binding KIF4A.

Most vaults appeared very mobile throughout the cytoplasm as assessed by timelapse imaging with photoactivatable PA-GFP labeled LRP (Yang *et al.*, 2010). Though there are studies supporting the idea that LRP functioned on nucleocytoplasmic trafficking and transport, no more than 5% of vault particles were associated with the nucleus (Kickhoefer *et al.*, 2002; Chung *et al.*, 2005), suggesting that LRP might be involved in intracellular transport. Our results indicated that many LRP clustered in the perinuclear region after depletion of KIF4A (Fig. 3). This phenotype could be recovered by the expression of the full-length KIF4A, but not the motor domain or tail domain truncated mutants of KIF4A (Fig. 4). It is well-known that Kinesin motors are involved in the major microtubule-dependent intracellular transport pathways. Both the motor domain and the tail domain are necessary for this process. The Kinesin motor domain could utilize ATP to fuel their movement along microtubules and the tail domain is essential for cargo binding. In KIF4A-depleted cells expressing KIF4A-N351 or KIF4A-N1018, LRP cannot interact with KIF4A-N351 and KIF4A-N1018 so that LRP could not be transported by these KIF4A mutants. This results in perinuclear localization of LRP in these cells. However, in KIF4A-depleted cells expressing KIF4A-C896 or KIF4A-C230, LRP also clustered in the perinuclear region since these mutants cannot move along microtubules without the motor domain. These results suggest that the tail domain of KIF4A is needed for LRP binding, and the motor domain of KIF4A is essential for LRP intracellular trafficking.

Taken together, our results indicate that motor protein KIF4A can bind and transport LRP, the major component of vault complex, in cisplatin-resistant lung cancer cells. Given the fact that LRP-based vaults play an essential role in driving cytotoxic drugs away from their subcellular targets, our studies reveal a potential mechanism by which Kinesin KIF4A contributes to the drug-resistance process in lung cancer cells by transporting LRP-based vaults. In addition, Kinesin KIF4A might also serve as a novel therapeutic target in drug-resistant lung cancer.

Contributors

Li-na PAN and Zhi-xiong DONG designed research; Li-na PAN and Yuan ZHANG performed research; Li-na PAN, Chang-jun ZHU, and Zhi-xiong DONG analyzed data; Li-na PAN and Zhi-xiong DONG wrote the paper.

Acknowledgements

We would like to express our sincere thanks to Prof. Wei JIANG (State Key Laboratory of Molecular Oncology, Cancer Institute and Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China) for experimental technical guidance in this study.

Compliance with ethics guidelines

Li-na PAN, Yuan ZHANG, Chang-jun ZHU, and Zhi-xiong DONG declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Chung, J.H., Ginn-Pease, M.E., Eng, C., 2005. Phosphatase and tensin homologue deleted on chromosome 10 (PTEN) has nuclear localization signal-like sequences for nuclear import mediated by major vault protein. *Cancer Res.*, **65**(10):4108-4116. http://dx.doi.org/10.1158/0008-5472.CAN-05-0124

Germann, U.A., 1996. P-glycoprotein—a mediator of multidrug resistance in tumour cells. *Eur. J. Cancer*, **32**(6):927-944. http://dx.doi.org/10.1016/0959-8049(96)00057-3

Gottesman, M.M., Fojo, T., Bates, S.E., 2002. Multidrug resistance in cancer: role of ATP-dependent transporters. *Nat. Rev. Cancer*, **2**(1):48-58. http://dx.doi.org/10.1038/nrc706

Han, M., Lv, Q., Tang, X.J., *et al.*, 2012. Overcoming drug resistance of MCF-7/ADR cells by altering intracellular distribution of doxorubicin via MVP knockdown with a novel siRNA polyamidoamine-hyaluronic acid complex. *J. Control. Release*, **163**(2):136-144. http://dx.doi.org/10.1016/j.jconrel.2012.08.020

Kathawala, R.J., Gupta, P., Ashby, C.R., *et al.*, 2015. The modulation of ABC transporter-mediated multidrug resistance in cancer: a review of the past decade. *Drug Resist. Updat.*, **18**:1-17. http://dx.doi.org/10.1016/j.drup.2014.11.002

Kickhoefer, V.A., Poderycki, M.J., Chan, E.K., *et al.*, 2002. The La RNA-binding protein interacts with the vault RNA and is a vault-associated protein. *J. Biol. Chem.*, **277**(43):41282-41286. http://dx.doi.org/10.1074/jbc.M206980200

Martinez, N.W., Xue, X., Berro, R.G., *et al.*, 2008. Kinesin KIF4 regulates intracellular trafficking and stability of the human immunodeficiency virus type 1 Gag polyprotein. *J. Virol.*, **82**(20):9937-9950. http://dx.doi.org/10.1128/JVI.00819-08

Midorikawa, R., Takei, Y., Hirokawa, N., 2006. KIF4 motor regulates activity-dependent neuronal survival by suppressing PARP-1 enzymatic activity. *Cell*, **125**(2):371-383. http://dx.doi.org/10.1016/j.cell.2006.02.039

Mossink, M.H., van Zon, A., Franzel-Luiten, E., *et al.*, 2002. Disruption of the murine major vault protein (MVP/LRP) gene does not induce hypersensitivity to cytostatics. *Cancer Res.*, **62**(24):7298-7304.

Mossink, M.H., van Zon, A., Scheper, R.J., *et al.*, 2003. Vaults: a ribonucleoprotein particle involved in drug resistance? *Oncogene*, **22**(47):7458-7467. http://dx.doi.org/10.1038/sj.onc.1206947

Scheffer, G.L., Wijngaard, P.L., Flens, M.J., *et al.*, 1995. The drug resistance-related protein LRP is the human major vault protein. *Nat. Med.*, **1**(6):578-582. http://dx.doi.org/10.1038/nm0695-578

Scheffer, G.L., Schroeijers, A.B., Izquierdo, M.A., *et al.*, 2000. Lung resistance-related protein/major vault protein and vaults in multidrug-resistant cancer. *Curr. Opin. Oncol.*, **12**(6):550-556. http://dx.doi.org/10.1097/00001622-200011000-00007

Scheper, R.J., Broxterman, H.J., Scheffer, G.L., *et al.*, 1993. Overexpression of a M(r) 110000 vesicular protein in non-P-glycoprotein-mediated multidrug resistance. *Cancer Res.*, **53**(7):1475-1479.

Slesina, M., Inman, E.M., Moore, A.E., *et al.*, 2006. Movement of vault particles visualized by GFP-tagged major vault protein. *Cell Tissue Res.*, **324**(3):403-410. http://dx.doi.org/10.1007/s00441-006-0158-8

Steiner, E., Holzmann, K., Elbling, L., *et al.*, 2006. Cellular functions of vaults and their involvement in multidrug resistance. *Curr. Drug Targets*, **7**(8):923-934. http://dx.doi.org/10.2174/138945006778019345

Taniwaki, M., Takano, A., Ishikawa, N., *et al.*, 2007. Activation of KIF4A as a prognostic biomarker and therapeutic target for lung cancer. *Clin. Cancer Res.*, **13**(22):6624-6631. http://dx.doi.org/10.1158/1078-0432.CCR-07-1328

Trussardi, A., Poitevin, G., Gorisse, M.C., *et al.*, 1998. Sequential overexpression of LRP and MRP but not P-gp 170 in VP16-selected A549 adenocarcinoma cells. *Int. J. Oncol.*, **13**(3):543-548. http://dx.doi.org/10.3892/ijo.13.3.543

van Zon, A., Mossink, M.H., Schoester, M., *et al.*, 2002. Structural domains of vault proteins: a role for the coiled coil domain in vault assembly. *Biochem. Biophys. Res. Commun.*, **291**(3):535-541. http://dx.doi.org/10.1006/bbrc.2002.6472

van Zon, A., Mossink, M.H., Houtsmuller, A.B., *et al.*, 2006. Vault mobility depends in part on microtubules and vaults can be recruited to the nuclear envelope. *Exp. Cell Res.*, **312**(3):245-255. http://dx.doi.org/10.1016/j.yexcr.2005.10.016

Wandke, C., Barisic, M., Sigl, R., *et al.*, 2012. Human chromokinesins promote chromosome congression and spindle microtubule dynamics during mitosis. *J. Cell Biol.*, **198**(5):847-863. http://dx.doi.org/10.1083/jcb.201110060

Willemsen, M.H., Ba, W., Wissink-Lindhout, W.M., *et al.*, 2014. Involvement of the kinesin family members *KIF4A* and *KIF5C* in intellectual disability and synaptic function. *J. Med. Genet.*, **51**(7):487-494.

http://dx.doi.org/10.1136/jmedgenet-2013-102182

Wu, G., Zhou, L., Khidr, L., *et al.*, 2008. A novel role of the chromokinesin Kif4A in DNA damage response. *Cell Cycle*, **7**(13):2013-2020. http://dx.doi.org/10.4161/cc.7.13.6130

Xiao, N., Fu, C.H., Lin, Y.N., *et al.*, 2016. Overexpression of chromosome kinesin protein KIF4A enhance cisplatin resistance in A549/DDP cells. *Int. J. Clin. Exp. Pathol.*, **9**(3):3331-3339.

Yang, J., Kickhoefer, V.A., Ng, B.C., *et al.*, 2010. Vaults are dynamically unconstrained cytoplasmic nanoparticles capable of half vault exchange. *ACS Nano*, **4**(12): 7229-7240. http://dx.doi.org/10.1021/nn102051r

Yu, Z., Fotouhi-Ardakani, N., Wu, L., *et al.*, 2002. PTEN associates with the vault particles in HeLa cells. *J. Biol. Chem.*, **277**(43):40247-40252. http://dx.doi.org/10.1074/jbc.M207608200

Zhang, W., Zhou, H., Yu, Y., *et al.*, 2016. Combination of gambogic acid with cisplatin enhances the antitumor effects on cisplatin-resistant lung cancer cells by down-regulating MRP2 and LRP expression. *OncoTargets Ther.*, **9**:3359-3368. http://dx.doi.org/10.2147/OTT.S100936

Zhu, C., Jiang, W., 2005. Cell cycle-dependent translocation of PRC1 on the spindle by Kif4 is essential for midzone formation and cytokinesis. *Proc. Natl. Acad. Sci. USA*, **102**(2):343-348. http://dx.doi.org/10.1073/pnas.0408438102

Zhu, C., Zhao, J., Bibikova, M., *et al.*, 2005. Functional analysis of human microtubule-based motor proteins, the kinesins and dyneins, in mitosis/cytokinesis using RNA interference. *Mol. Biol. Cell*, **16**(7):3187-3199. http://dx.doi.org/10.1091/mbc.E05-02-0167

Zhu, C.L., Cheng, D.Z., Liu, F., *et al.*, 2015. Hepatitis B virus upregulates the expression of kinesin family member 4A. *Mol. Med. Rep.*, **12**(3):3503-3507. http://dx.doi.org/10.3892/mmr.2015.3792

List of electronic supplementary materials

Fig. S1 mRNA and protein expression of LRP and KIF4A in A549 and A549/DDP cells

中文概要

题　目：驱动蛋白 KIF4A 通过调节肺耐药相关蛋白 LRP 的胞内运输参与肿瘤耐药

目　的：探讨驱动蛋白 KIF4A 调节肺耐药相关蛋白 LRP 在细胞内分布的作用机制，及其在肿瘤耐药过程中的作用。

创新点：首次发现驱动蛋白 KIF4A 的 C 端区与肺耐药相关蛋白 LRP 的 N 端区结合，且 KIF4A 调节 LRP 在胞内分布依赖 KIF4A 的 N 端马达结构域。

方　法：应用免疫共沉淀和免疫荧光技术检测 KIF4A 与 LRP 的结合。根据 KIF4A 及 LRP 蛋白结构，构建绿色荧光蛋白（GFP）融合 KIF4A 截短突变质粒及 Flag 融合 LRP 截短突变质粒，免疫沉淀分析 KIF4A 与 LRP 相互作用区域。通过 RNA 干涉（RNAi）内源 KIF4A，外源转入 KIF4A 截短突变体质粒，检测 LRP 在胞内分布。

结　论：本实验中免疫沉淀及免疫荧光结果显示，KIF4A 与 LRP 结合，且在微管上有共定位（图 1）。截短突变体免疫沉淀实验结果表明，KIF4A 的 C 端尾部结构域与 LRP 的 N 端区结合（图 2），RNAi 敲降内源 KIF4A 表达导致 LRP 聚集在细胞核周围（图 3），外源表达全长 KIF4A 可恢复 LRP 在胞内弥散状定位，但外源表达 KIF4A 的 C 端或 N 端截短突变无法恢复 LRP 的胞内定位，仍聚集在核周区域（图 4）。综上所述，驱动蛋白 KIF4A 可与 LRP 结合，并调节 LRP 在胞内定位，KIF4A 的 C 端尾部结构域与 LRP 结合，N 端马达结构域促进 LRP 在胞内运输，二者缺一不可。

关键词：驱动蛋白（KIF4A）；肺耐药相关蛋白（LRP）；耐药