

Expression of CD147 in advanced non-small cell lung cancer correlated with cisplatin-based chemotherapy resistance

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CD147, a widely expressed cell surface glycoprotein in cancer, is associated with tumor invasiveness and chemotherapy resistance. Recently, CD147 is also regarded as a potential therapeutic target for cancer therapy. The aim of the study was to investigate CD147 expression in non-small cell lung cancer (NSCLC), and evaluate its correlation with cisplatin-based chemotherapy resistance. In this study, we examined immunohistochemically the expression of CD147 in 118 advanced NSCLC cases treated with cisplatin-based chemotherapy, and then the association of CD147 expression with clinicopathological characteristics was analyzed. Furthermore, RNA interference approach was used to silence CD147 expression in a cisplatin-resistant human lung cancer cell line A549/DDP, and the inhibition effect of cisplatin on tumor cells was assayed by MTT. In the overall series, positive CD147 expression was observed in 101/118 (85.6%) cases. A membranous CD147 pattern was identified in 76/101 (75.2%) of CD147 positive tumors. CD147 membranous expression, but not the overall CD147 expression, was associated with poor response to cisplatin-based chemotherapies and a poor prognosis in advanced NSCLC patients. In vitro results showed that silencing CD147 increased the proliferation inhibitory effect of cisplatin to A549/DDP cells. In conclusion, our study indicated that membranous CD147 expression is a predictive factor of the response to cisplatin-based chemotherapies, and the use of CD147-targeted therapeutic adjuvants might be considered in the treatment of advanced NSCLC patients.

Key words: CD147; non-small cell lung cancer; resistance

In China, more than half of the patients with non-small-cell lung cancer (NSCLC) were in their advanced stages at diagnosis [1]. Cisplatin-based regimens are most frequently used in the systemic chemotherapy of NSCLC, and have been shown to improve the survival. However, the response rate of cisplatin-based chemotherapies in advanced NSCLC patients is less than 30%, a major proportion of the patients exhibit a poor response [2]. Therefore, the treatment of these patients represents a major clinical problem in advanced NSCLC patients.

Intrinsic or acquired drug resistance mediated by cancer cells accounts for the lack of response to cisplatin-based chemotherapies [3, 4]. With the increasing number of novel targeted chemotherapeutic regimens available, the utility of these agents in NSCLC patients resistant to cisplatin-based chemotherapies deserves further investigation.

CD147 (basigin, EMMPRIN) is a multifunctional transmembrane glycoprotein, which was supposed to be connected in cancer invasion, metastasis, and drug resistance [5-7]. Recently,

several studies have identified CD147 as a novel potential therapeutic target for cancer therapy [8, 9]. Especially, a phase I/II clinical trial on a novel 131I-labeled CD147-specific monoclonal antibody Fab'2 fragment (Licartin) in hepatocellular carcinoma has been conducted [10, 11]. These data suggest that CD147 might represent a novel targeted treatment option in NSCLC.

In the present study, we examined the expression of CD147 in advanced NSCLC samples, and further assayed the association between silencing CD147 with cisplatin resistance in a cisplatin-resistant lung cell line A549/DDP. The aim of the study was to evaluate whether CD147 expression correlates cisplatin-based chemotherapies resistance in advanced NSCLC and which subgroup might benefit from CD147-targeted therapies.

Materials and methods

Patients and chemotherapy. One hundred and eighteen advanced (III/IV stage) NSCLC patients undergoing resection

or only biopsy in Shandong University Medical College and Tongji Hospital (Shanghai, China) between February 2000 and July 2007 were included in this study with informed consent. The study approved by the local ethics committee. All tumor specimens analyzed were collected before chemotherapy. The detailed clinicopathological characteristics of all patients are listed in Table 1. Only squamous cell carcinoma and adenocarcinoma histological subtypes were selected in our study.

All patients received cisplatin-based third-generation chemotherapy doublets. 39 cases received NP regimens (NVB 25 mg/m², days 1 and 8; DDP 20 mg/m², day 1–5), 44 had TP regimens (Taxol 135 mg/m², day 1; DDP 20 mg/m², day 1–5), and 35 were given GP regimens (Gemzar 1000 mg/m², days 1 and 8; DDP 20 mg/m², day 1–5). All chemotherapeutic drugs were administered intravenously and repeated every 3–4 weeks. Response to treatment was determined after 2–3 cycles by WHO criteria, which classified the responses into complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD).

Immunohistochemistry. Immunohistochemical staining was carried out on 4 µm of formalin-fixed, paraffin-embedded sections of NSCLC tumor tissues. Briefly, antigen retrieval was performed in 0.01 M citrate buffer (pH 6.0); endogenous peroxidase activity was quenched with hydrogen peroxide for 5 min at room temperature. After blocking, sections were incubated with a goat polyclonal antibody against human CD147 (Santa Cruz Biotech, 1:100 dilution) at 4°C overnight. Visualization was performed using DAKO EnVision system. Cytoblock paraffin sections of breast carcinoma cell line MCF-7 served as the positive control for immunocytochemistry, and nonspecific IgG was used as the negative control.

Immunostaining intensity was classified into no staining, weak, moderate, and strong. The number of tumor cells with different staining intensities was counted. The definition of overall CD147 staining was based on the following criteria: cases were defined as positive if the percentage of the moderately or strongly stained tumor cells was more than 25%; the rest was identified as negative. The cellular pattern of positive CD147 expression was further categorized into membranous and cytoplasmic expression pattern.

Cell culture and RNA interference. Human lung adenocarcinoma cell line A549 and its cisplatin-resistant subline A549/DDP (obtained from XiangYa Cell center, Changsha, China) were maintained in RPMI-1640 medium supplemented with 10% bovine calf serum, for A549/DDP cells, 2 µg/ml cisplatin was added. To silence CD147 expression, 1×10⁶ A549/DDP cells were transfected with RNAi control and CD147 specific siRNA Transfection Reagent Complex (Santa Cruz Biotech) according to the manufacturer's instruction as described previously [12]. Cells were double-transfected at 72h intervals and then were harvested for further analysis. Specific silencing of the targeted gene was validated by Western blot.

Western blotting. 30 µg extracted protein samples were separated by 12% SDS-polyacrylamide gel electrophoresis, and then nitrocellulose membrane transferring was conducted. The

membrane was blocked with 5% defatted milk and incubated with the same antibody against CD147 used in immunohistochemistry and β-actin (Santa Cruz Biotech) at 1:500 dilutions overnight at 4°C. Detection was performed using an ECL chemiluminescence reagent (Amersham).

Cisplatin sensitivity assay. A549/DDP, A549/DDP (control RNAi) and A549/DDP (CD147 RNAi) cells (1×10⁴) were plated in 96-well plates and were treated with different concentrations of cisplatin (0.2, 2, 20, 200 mg/ml). After 72h incubation, MTT assay were performed, and the inhibition ratio was calculated as described previously [13].

Statistical analysis. The correlation between CD147 expression and the clinicopathological characteristics of NSCLC were analyzed using Fisher's exact test. Continuous variables were analyzed by ANOVA test. The survival curves were estimated by the Kaplan-Meier method, and differences were compared by the log-rank test.

Results

Immunohistochemistry of CD147 in advanced NSCLC tumor. Positive CD147 staining was defined in 101/118 (85.6%) cases. All the positive tumors showed a moderate or strong staining in cytoplasmic or membranous compartments of the cells, but not in the surrounding stroma. In positive cases, membranous pattern was identified in 76/101 (75.2%) cases, and the rest of the CD147 positive tumors demonstrate cytoplasmic staining (Fig. 1).

Association of CD147 with clinicopathological characteristics. No association was observed between the overall CD147 expression and all the clinicopathological parameters examined (Table 1). Positive membranous CD147 expression was associated with a poor response to chemotherapy. Among NSCLC cases with chemotherapy resistance, 75.0% demonstrated positive membranous CD147 expression, higher than that in the chemotherapy-sensitive subgroup (56.1%, $P=0.016$) (Table 1). As seen in Figure 2, no difference was observed in the clinical outcome according to the overall CD147 expression status whereas positive membranous CD147 expression was strongly associated with poor survival for NSCLC patients. The median overall survival was 16 months for patients with membranous CD147 expression, and 21 months for the non-membranous expression subgroup (log rank test, $P=0.003$). No significant difference in overall survival was observed for the different chemotherapeutic regimens ($P=0.896$). Furthermore, Membranous CD147 expression was associated with poor survival for patients with adenocarcinoma subtype ($P=0.002$), whereas the staining pattern had no influence on outcome for patients with squamous cell carcinoma ($P=0.603$) Fig. 2.

Silencing of CD147 inhibits the proliferation of cisplatin-resistant A549/DDP cells. As shown in Fig. 3, A549/DDP cells expressed a higher level of CD147 expression than A549 cells in Western-blot analysis. Down-regulation of CD147 by RNA interference could increase the inhibitory effect of

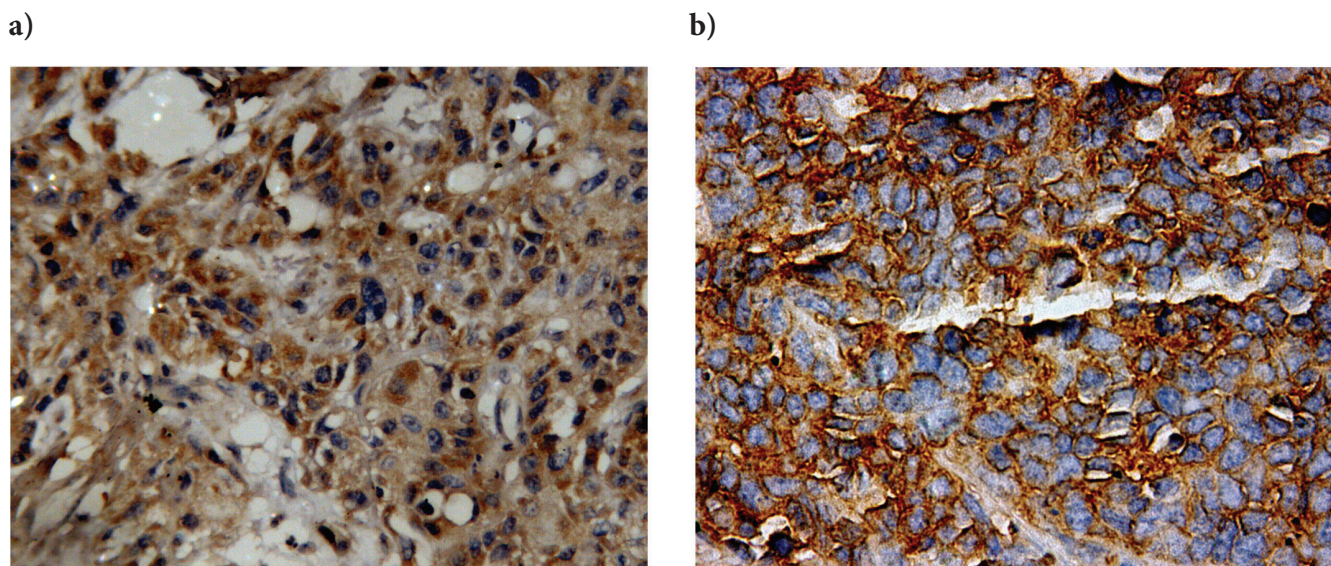


Figure 1. Immunostaining of CD147 expression in primary advanced NSCLC cancer tissues. (a) positive cases with cytoplasmic staining; (b) positive cases with membranous staining (x200 magnification).

Table 1. Correlation of CD147 expression with clinicopathological characteristics in 118 advanced NSCLC patients.

Characteristics	Overall CD147 expression		P value	Membranous CD147 expression		P value
	Negative (n=17)	Positive (n=101)		Negative (n=42)	Positive (n=76)	
Age (year)						
<60	60	9	0.852	25	35	0.161
≥60	58	8		17	41	
Gender						
Male	102	14	0.595	36	66	0.864
Female	16	3		6	10	
Stage						
IIIA	21	3	0.993	9	12	0.225
IIIB	54	8		22	32	
IV	43	6		11	32	
Histology type						
Squamous cell carcinoma	66	8	0.426	26	40	0.331
Adenocarcinoma	52	9		16	36	
Chemotherapy regimens						
NVB+DDP	39	6	0.751	12	27	0.737
Taxol+DDP	44	5		17	27	
Gemzar+DDP	35	6		13	22	
Response to cisplatin-based Chemotherapy						
CR, PR	66	11	0.431	29	37	0.016
SD, PD	52	6		13	39	

cisplatin in A549/DDP cells in a dose-dependant manner. As shown in Fig. 4 MTT assay results showed that CD147 RNAi A549/DDP cells had a higher inhibition rate than the negative RNAi and untransfected cells after treatment with increasing concentrations of cisplatin for 72h (ANOVA, P<0.05).

Discussion

CD147, also named extracellular matrix metalloprotease inducer (EMMPRIN) is a member of the immunoglobulin family and a tumor surface glycoprotein that is overexpressed

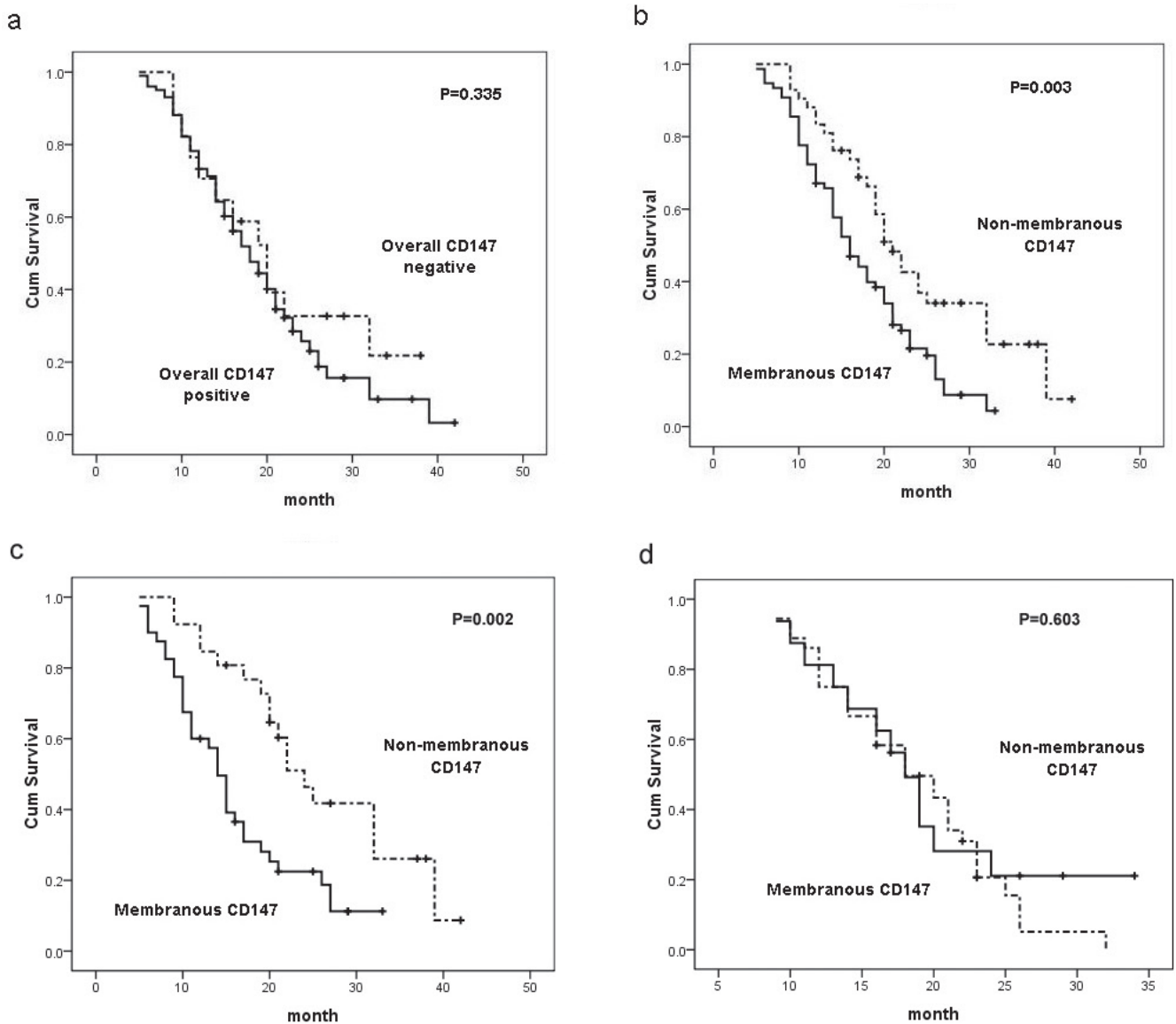


Figure 2. Kaplan-Meier survival curves for overall (a) and membranous (b) CD147 expression in advanced NSCLC patients. Survival according to membranous CD147 expression in patients with adenocarcinoma (c) or squamous cell carcinoma (d).

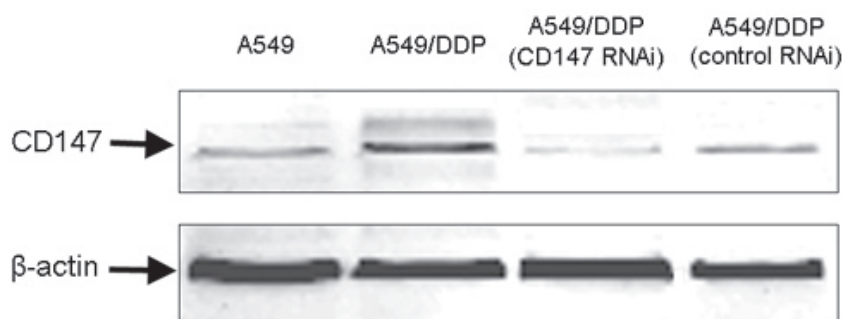


Figure 3. Western-blot results showed that cisplatin-resistant A549/DDP cells had a higher CD147 expression than A549 cells; CD147 expression was down-regulated after RNA interference compared with control.

in multiple cancer tissues, such as head and neck cancer, NSCLC, cervical cancer, and hepatocellular carcinoma [14]. Recently, CD147 has been implicated as a novel regulator of the canonical Wnt/beta-catenin signaling pathway in lung cancer [15]. Kong et al. [16] identified that transcription factor Sp1 is essential for regulating the CD147 gene expression in lung cancer. Siene et al. [17] found that CD147 was overexpressed in NSCLC and membranous location of CD147 correlated with a shorter survival in patients with adenocarcinoma. In this study, we also found that positive membranous CD147 were seen in more than half (64.4%) of the advanced NSCLC tumors and correlated with a worse prognosis. We also found that membranous CD147 expression was associated with shortened survival in patients with adenocarcinoma, but not in squamous cell carcinoma, and these results are consistent with Siene's study. Furthermore, we identified that membranous CD147 expression was more frequently observed in NSCLC patients with resistance to cisplatin-based chemotherapies.

Our in vitro results also demonstrated cisplatin-resistant lung cancer cells A549/DDP showed a higher level of CD147 expression than cisplatin-sensitive A549 cells. Moreover, down-regulation of CD147 by RNAi could sensitize cisplatin-resistant A549/DDP. Several other in vitro studies also indicate that silencing CD147 could increase the sensitivity of cancer cells to a variety of chemotherapeutic agents including cisplatin [18-19]. Therefore, our in vivo and in vitro findings indicated an association between membranous CD147 expression and cisplatin-based chemoresistance in advanced NSCLC.

Yang et al. [20] discovered that the CD147 and CD98hc complexes could inhibit intracellular cisplatin accumulation. Toole et al [7, 21] proposed that CD147 might participate in drug resistance by stimulating the production of hyaluronan on the cytoplasmic membrane, which has been reported to be associated with various drug resistance mechanisms in cancer cells. These findings indicate a mechanism that may link CD147 and cisplatin resistance. In particular, several recent in vitro studies found that monocarboxylate transporters (MCT) could regulate maturation and trafficking of CD147 to the plasma membrane in cancer cells [22] and membranous CD147 could also contribute to the expression of MCT and the regulation of MCT localization in the plasma membrane of cancer cells [23]. The co-expression of CD147 and some MCT subtypes at membrane has been implicated in the substrate availability, the metabolic pathway of lactate and pH balance within the tumor, which contribute to drug resistance in tumor cells [24]. Therefore, these findings suggest that membranous CD147 may be functionally linked to cisplatin resistance as a chaperone to MCTs.

In summary, our study indicated that advanced NSCLC with cisplatin resistance had a high frequency of membranous CD147 expression; secondly, our findings also suggest that down-regulated or blocking CD147 function might sensitize

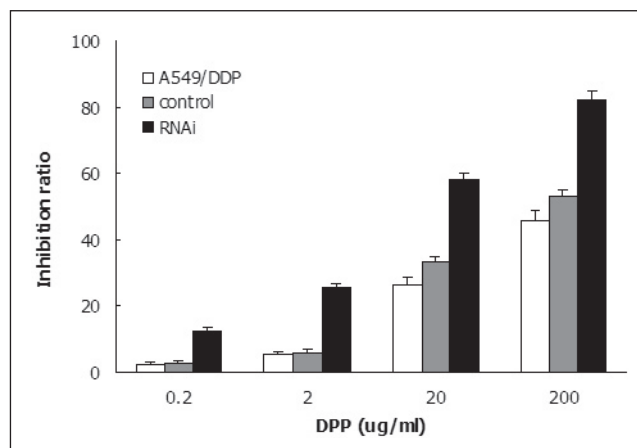


Figure 4. MTT assay results showed that CD147 RNAi A549/DDP cells had a higher inhibition rate than the negative RNAi and untransfected cells after treatment with increasing concentrations of cisplatin (ANOVA, $P < 0.05$).

response to cisplatin in resistant lung cancer cells. Therefore, we propose that chemotherapy resistant advanced NSCLC might benefit from the emergent CD147-targeted therapy, and the effect of CD147-targeted therapy on NSCLC patients deserves further investigation.

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