Differences in Fatty Acid Oxidation between Nab-Paclitaxel- and Solvent-Based Paclitaxel-Treated A549 Cells Based on Metabolomics

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ABSTRACT: The pharmacokinetics, safety, and anticancer efficacy profiles of nanoparticle albumin-bound (nab)-paclitaxel formulations are superior to those of solvent-based paclitaxel formulations. The aims of the present study were to study the effects of nab-paclitaxel and solvent-based paclitaxel formulations on the metabolic profiles of the model cell line (A549) and attempt to elucidate the associated metabolic pathways. A mass spectrometry-based cell metabolomics approach and viability evaluation were used to explore the potential difference. Western blotting was utilized to measure the levels of relevant proteins, and carnitine palmitoyltransferase 1 (CPT1) activities were quantified. Fold changes normalized to controls in levels of carnitine and several acylcarnitines were significantly different ($p < 0.05$) between A549 cells treated with nab-paclitaxel and those treated with solvent-based paclitaxel. Relative to the controls, there were also significant fold change differences in palmitic and linoleic acid levels in the cell lysates, mitochondrial CPT1 activities, and mitochondrial medium-chain acyl-CoA dehydrogenase (MCAD) protein levels in the A549 cells subjected to the nab-paclitaxel and solvent-based paclitaxel formulations. Results suggested that the two formulations differentially modulated fatty acid oxidation in the A549 cells. While cell viability results did not reveal significant differences, the findings implied that a mass spectrometry-based cell metabolomics approach could be a sensitive tool to explore the differences caused by formulation changes without using animals. Since uncertainties of products containing nanomaterials warrant holistic screening to address safety concerns, the aforementioned approach may be of regulatory importance and is worth further investigation.

INTRODUCTION

Much attention has been directed toward the application of nanotechnology and/or nanomaterials in the development of novel therapeutic agents. Some of these products have been approved by stringent regulatory authorities, such as the European Medicines Agency (EMA) and the United States Food and Drug Administration (USFDA). While nanomaterials are highly versatile, it is challenging to evaluate their safety and efficacy as each formulation has unique properties. Hence, more efficient platforms need to be developed that can effectively assess products applying nanotechnology and/or nanomaterials for quality, safety, efficacy, and compliance with regulatory bodies.

The formulation containing nanoparticle albumin-bound paclitaxel (nab-paclitaxel) (Abraxane) was originally developed to eliminate the use of the toxic solvent, polyoxyl 35 castor oil, in the solvent-based paclitaxel formulation. Abraxane administration obviates the requirement for antihistamine and steroid premedication used to prevent immunoreactions to polyoxyl 35 castor oil, shortens infusion time, and improves pharmacokinetics and anticancer efficacy profiles. The improvement was attributed to enhanced endothelial cell binding, transcytosis, and intratumor delivery, which may be related to the binding of albumin to the gp60 receptor on the endothelial cells and the secreted protein, acidic and rich in cysteine (SPARC). A quantitative proteomics study demonstrated that A549 cells treated with nab-paclitaxel and paclitaxel significantly differed in terms of their glucosamine 6-phosphate N-acetyltransferase 1 (GNPNAT1/GNA1) expression. However, the mechanisms underlying the clinically observed difference between nab-paclitaxel and solvent-based paclitaxel formulations remain to be clarified. More studies are required to explain the distinct behavior of nab-paclitaxel and assess the impact of nanomaterials.

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Metabolomics is an “omics” approach designed to improve the holistic understanding of biological systems. It involves the study of metabolites that are intermediate- and end products of cellular metabolic processes. Cell metabolomics is the metabolic analysis of cell cultures. It supports studies on specific cell types under controlled conditions, mitigates variability among treatments, and is more ethical and cost-effective than animal experimentation and human clinical trials. It also supports a data-driven, preconception-free approach that facilitates the exploration of unknown mechanisms and the formulation and testing of hypotheses.

Both the nab-paclitaxel and solvent-based paclitaxel formulations are indicated for the treatment of non-small cell lung cancer (NSCLC). Hence, we used A549 cells as a model system. The aims of the present study were to use cell metabolomics to study the effects of nab-paclitaxel and solvent-based paclitaxel formulations on the metabolic profiles of A549 cells and attempt to elucidate the associated metabolic pathways. Considering the high sensitivity and the versatility of mass spectrometry, the mass spectrometry-based metabolomics approach was chosen. To the best of our knowledge, this study is the first to clarify the relative differences in the impact on A549 cells between the nab-paclitaxel and solvent-based paclitaxel formulations via a mass spectrometry-based cell metabolomics approach. The present study identified the distinct effects and the potential metabolic pathways associated with them. The methodology implemented herein could also be used in future research regarding nanoformulations.

**RESULTS**

Effects of Paclitaxel, the Solvent-Based Formulation Containing Paclitaxel, and Nab-Paclitaxel on Cell Viability. The cell viability revealed no significant differences among A549 cells treated with paclitaxel, the solvent-based formulation containing paclitaxel, or nab-paclitaxel after 24 h (Figure 1). The treatments with concentrations equivalent to 100 nM paclitaxel resulted in around 50% of cell growth in comparison with the control groups. In view of the cell viability assay results and the range of serum paclitaxel concentrations in clinical use, the concentration 100 nM was selected for use in the subsequent experiments.

Metabolic Profiles Demonstrating Altered Carnitine and Certain Acylcarnitine Levels. We evaluated the effects of the various formulations at the metabolic level using a mass spectrometry-based cell metabolomics approach. We obtained metabolic profiles for A549 cells treated with 100 nM paclitaxel in dimethyl sulfoxide (DMSO), the solvent-based formulation containing paclitaxel, and nab-paclitaxel for 24 h and compared them against that for the control. We quenched and extracted the cell samples, applied the MIID method to normalize the concentration, and conducted the metabolic profiling by UHPLC-QTOF-MS. We identified 36 metabolites with reproducible signals but the treatments differed mainly in terms of their carnitine levels and acylcarnitine types and levels. Fold changes normalized to the control group for the levels of carnitine and acylcarnitines including acetylcarnitine, propionylcarnitine, butyrylcarnitine, valerylcarnitine, hexadecanoylcarnitine, and oleoylcarnitine were significantly different between treatment groups (Table 1). A significant difference for the aforementioned metabolites was also observed between cells treated with the solvent-based formulation containing paclitaxel and those treated with nab-paclitaxel assessed by Tukey’s honestly significant difference (HSD) post hoc test comparisons.

UHPLC-QqQ-MS was applied to assess the fold changes in the levels of carnitine and acylcarnitines relative to the control (Figure 2). Carnitine and acetylcarnitine were quantitated via calibration curves of the standards and internal standards of these metabolites. Among the carnitine and acylcarnitines evaluated in the present study, the levels of carnitine and short-chain acylcarnitines (the number of carbon atoms for the acyl group: 2−5) were lower in the treatment groups in comparison with those of the control group, while those of long-chain acylcarnitines (the number of carbon atoms for the acyl group: 14−18) were higher. Significant differences of the fold changes normalized to the control group between A549 cells treated with the solvent-based formulation containing paclitaxel and those with nab-paclitaxel were observed for carnitine and most acylcarnitines including acetylcarnitine, propionylcarnitine, butyrylcarnitine, valerylcarnitine, hexanoylcarnitine, octanoylcarnitine, decanoylcarnitine, tetradecenoylcarnitine, hexadecanoylcarnitine, hexadecenoicarnitine, stearoylcarnitine, and oleoylcarnitine. The fold changes for carnitine and the aforementioned acylcarnitines were larger for A549 cells treated with nab-paclitaxel except for octanoylcarnitine. The carnitine system plays a major role in cancer metabolic plasticity, enabling tumor cells to withstand harsh environments and to meet their high energy demand. Results demonstrated that the carnitine system of A549 cells responded differently to each paclitaxel formulation.

Altered Long-Chain Fatty Acid Levels. Carnitine transfers long-chain fatty acids to the mitochondria for β-oxidation and acylcarnitine biosynthesis. As the carnitine and acylcarnitine levels varied among treatments, we used UHPLC-QTOF-MS to measure the levels of long-chain fatty acids in A549 cells treated with 100 nM paclitaxel in DMSO, the solvent-based formulation containing paclitaxel, or nab-paclitaxel for 24 h. The levels of the long-chain fatty acids with reproducible signals, namely, palmitic acid, linoleic acid, oleic acid, and stearic acid, are displayed in Figure 3 (see the Mass Spectrometry Analyses of Long-Chain Fatty Acids section). The levels of these long-chain fatty acids were higher in the treatment groups in comparison with those in the control group. Relative to the control, there were significant fold change differences in the palmitic and linoleic acid levels.
Table 1. Metabolic Profiles of A549 Cells Treated with 100 nM Paclitaxel in Dimethyl Sulfoxide, the Solvent-Based Formulation Containing Paclitaxel, and Nab-Paclitaxel\(^a\)

| Retention time (min) | Metabolite          | PTX/CTL (A)          | s-paclitaxel/CTL (B) | nab-paclitaxel/CTL (C) | Fold change \(\pm\) standard deviation | \(p\) value \(d\) |
|----------------------|---------------------|----------------------|----------------------|------------------------|----------------------------------------|------------------|
| 0.83                 | Carnitine           | 0.66 ± 0.05          | 0.56 ± 0.03          | 0.80 ± 0.04            | 0.0015**                                | 0.0684          |
| 1.36                 | Acetylcarnitine     | 0.41 ± 0.07          | 0.38 ± 0.04          | 0.51 ± 0.01            | 0.0390*                                | 0.7483          |
| 2.67                 | Propionylcarnitine  | 0.43 ± 0.07          | 0.33 ± 0.03          | 0.54 ± 0.03            | 0.0052**                                | 0.0899          |
| 4.37                 | Butyrylcarnitine    | 0.34 ± 0.06          | 0.28 ± 0.05          | 0.44 ± 0.05            | 0.0336*                                | 0.4374          |
| 5.34                 | Valerylcarnitine    | 0.44 ± 0.05          | 0.28 ± 0.04          | 0.51 ± 0.11            | 0.0228**                                | 0.0901          |
| 12.26                | Hexadecanoylcarnitine | 4.92 ± 0.93         | 3.20 ± 0.80          | 5.50 ± 0.44            | 0.0229*                                | 0.0702          |
| 12.45                | Oleoylcarnitine     | 5.23 ± 0.47          | 2.88 ± 0.33          | 4.81 ± 0.66            | 0.0027**                                | 0.0030**        |

\(^a\)CTL, control; nab-paclitaxel, nanoparticle albumin-bound (nab)-paclitaxel formulation; s-paclitaxel, the solvent-based formulation containing paclitaxel; PTX, paclitaxel; RT, retention time; \(*p < 0.05\) and \(**p < 0.01\). \(\text{Isomers could not be separated by the present analytical method. Only the levels of the metabolites in the treatments presenting with significant fold change differences relative to the control are shown. Three independent experiments were conducted. For each experiment, three independent samples were prepared per condition. Each sample was analyzed twice using ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry. Data are expressed as the fold change relative to the controls and are presented as means ± standard deviations (\(n = 3\)). \(Fold change differences were analyzed by one-way analysis of variance (ANOVA), followed by all pairs, Tukey’s honestly significant difference (HSD) post hoc test comparisons.}\)

Figure 2. Levels of carnitine and acylcarnitines of A549 cells treated with 100 nM paclitaxel in dimethyl sulfoxide, the solvent-based formulation containing paclitaxel (s-paclitaxel), or nab-paclitaxel for 24 h. Three independent experiments were conducted. For each experiment, three independent samples were prepared per condition. Each sample was analyzed twice using UHPLC-QqQ mass spectrometry. Carnitine and acetylcarnitine were quantitated via calibration curves of the standards and internal standards of these metabolites. Data are expressed as fold changes relative to the controls and are presented as means ± standard deviations (\(n = 3\)). Fold change differences were analyzed by one-way analysis of variance (ANOVA), followed by all pairs, Tukey’s honestly significant difference (HSD) post hoc test comparisons. \(*p < 0.05\) and \(**p < 0.01\).

in A549 cells treated with the solvent-based formulation containing paclitaxel and those treated with nab-paclitaxel.

Protein Levels in Cell Lysate Mixtures. We compared the CPT1A and CPT1C protein levels among the controls and A549 cells treated with paclitaxel in DMSO, the solvent-based formulation containing paclitaxel, and nab-paclitaxel for 24 h. However, no significant differences in the protein levels were detected among the treatments (Figure 4A,B). Therefore, the CPT1A and CPT1C expression levels were not significantly affected by paclitaxel regardless of formulations.

The MCAD protein level was lower in A549 cells treated with the solvent-based formulation containing paclitaxel than it was in those subjected to the other treatments (Figure 4C). The fold change in the MCAD protein level in A549 cells treated with the solvent-based formulation containing paclitaxel was significantly lower than that for the A549 cells treated with paclitaxel in DMSO.

Protein Levels in Mitochondrial Fractions. Under the experimental conditions of the present study, cytochrome \(c\) was localized mainly to the mitochondria (Figure 5A). There were no significant fold change differences among treatments in terms of their mitochondrial cytochrome \(c\) levels. However, the cytochrome \(c\) levels of all treatments were higher than that of the control (Figure 5A). Similar to the results for the cell lysate mixtures (Figure 4A), there were no significant fold change differences in the mitochondrial CPT1A levels among treatments compared with the controls (Figure 5B). However, the mitochondrial MCAD protein level\(^{11}\) was lower in A549 cells treated with the solvent-based formulation containing paclitaxel than in the A549 cells subjected to the other
treatments (Figure 5C). The fold change in the MCAD protein level in A549 cells treated with the solvent-based formulation containing paclitaxel was significantly less than that in the A549 cells treated with nab-paclitaxel (Figure 5C).

Altered Carnitine Palmitoyltransferase 1 (CPT1) Activities. The fold changes in the CPT1 protein level did not significantly differ among treatments. Nevertheless, the fold change in mitochondrial CPT1 activity was significantly lower in A549 cells treated with the solvent-based formulation containing paclitaxel than in the A549 cells subjected to the other treatments (Figure 6).

**DISCUSSION**

The elucidation of the impact of nanomaterials on safety and efficacy has been a long-sought goal complicated by the versatility of nanomaterials and the unpredictability of the associated effects. The commercially available product nab-paclitaxel containing albumin-bound nanoparticles with a mean particle size of approximately 130 nm is one of the successful examples demonstrating improved safety and efficacy profiles. Ilinskaya et al. demonstrated that paclitaxel formulated with the traditional vehicle and the solvent used in the traditional formulation, polyoxyl 35 castor oil, but not nab-paclitaxel, induced the production of interleukin-8. In a large-scale randomized phase III trial on patients with advanced NSCLC, nab-paclitaxel plus carboplatin was associated with a significantly greater objective response rate than solvent-based paclitaxel plus carboplatin. Median overall survival was significantly longer for the former in patients aged equal to or above 70 years. To explore the metabolic pathways that might contribute to the observed differences, similar to previous metabolomics studies devoted to the

Figure 4. Representative western blot images and fold changes in carnitine palmitoyltransferase 1A (CPT1A) (A), CPT1C (B), and medium-chain acyl-CoA dehydrogenase (MCAD) (C) expression relative to the control in A549 cells treated with 100 nM paclitaxel in dimethyl sulfoxide, the solvent-based formulation containing paclitaxel (s-paclitaxel), and nab-paclitaxel for 24 h. For (A) and (B), three independent samples were prepared per condition. For (C), three independent experiments were conducted. For each experiment, three independent samples were prepared per condition. All data are expressed as fold changes relative to the controls and are presented as means ± standard deviations (n = 3). Fold change differences were analyzed by one-way analysis of variance (ANOVA), followed by all pairs, Tukey’s honestly significant difference (HSD) post hoc test comparisons. *p < 0.05.

Figure 5. Representative western blot images and fold changes relative to the control for the expression levels of cytochrome c (A), carnitine palmitoyltransferase 1A (CPT1A) (B), and medium-chain acyl-CoA dehydrogenase (MCAD) (C) proteins in the mitochondria of A549 cells treated with 100 nM paclitaxel in dimethyl sulfoxide, the solvent-based formulation containing paclitaxel (s-paclitaxel), or nab-paclitaxel for 24 h. Three independent samples were prepared per condition. Data are expressed as fold changes relative to the controls and are presented as means ± standard deviations (n = 3). Fold change differences were analyzed by one-way analysis of variance (ANOVA), followed by all pairs, Tukey’s honestly significant difference (HSD) post hoc test comparisons. *p < 0.05.
Fatty acid oxidation is vital to cancer cell survival.\textsuperscript{17} CPT1 is a key rate-limiting enzyme in the fatty acid oxidation pathway.\textsuperscript{17} CPT1A is the primary isoform in the lung.\textsuperscript{18} CPT1C downregulation in senescent human pancreas epithelial carcinoma PANC-1 cells may be associated with decreases in the levels of carnitine and short-chain acylcarnitines and suppression of tumorigenesis in these cells presenting with mitochondrial dysfunction.\textsuperscript{19} CPT1C was upregulated in human non-small cell lung carcinoma.\textsuperscript{20} CPT1C-depleted A549 cells were relatively more sensitive to metabolic stress.\textsuperscript{21} However, the levels of CPT1A and CPT1C protein in the cell extracts and the CPT1A levels in the mitochondria did not significantly differ among paclitaxel treatments (Figure 4). Liepinsh et al.\textsuperscript{21} investigated ischemia/reperfusion-induced damage in heart mitochondria. They established that acylcarnitines with 12–18 carbon atoms in their acyl groups accumulated to toxic concentrations in the mitochondrial intermembrane space. This effect was attributed to increased CPT1 and decreased CPT2 activity in ischemic myocardium.\textsuperscript{21}

Here, we detected greater long-chain acylcarnitine accumulation (Figure 2) in the cells exposed to nab-paclitaxel than in those subjected to the solvent-based formulation containing paclitaxel. Meanwhile, the mitochondrial CPT1 activity of A549 cells treated with nab-paclitaxel was significantly higher than that in the A549 cells exposed to the solvent-based formulation containing paclitaxel. These findings suggested that a reduction in mitochondrial CPT1 activity might account for the relatively lower long-chain acylcarnitine levels (Figure 2) in the A549 cells treated with the solvent-based formulation containing paclitaxel. The mitochondrial CPT1 activity in the A549 cells treated with the solvent-based formulation containing paclitaxel was lower than that of the control. In contrast, we observed higher levels of long-chain acylcarnitines in A549 cells treated with the solvent-based formulation containing paclitaxel than those treated with nab-paclitaxel. The solvent-based formulation containing paclitaxel may be more aggressive than the solvent-based formulation containing nab-paclitaxel, which may account for the differences in the long-chain acylcarnitine levels (Figure 2).

While long-chain fatty acid oxidation depends upon the carnitine shuttle, short-chain- and medium-chain fatty acid oxidations do not.\textsuperscript{10} Fold changes in the levels of all short-chain- and medium-chain acylcarnitines observed herein, except tiglylcarnitine, significantly differed between A549 cells treated with the solvent-based formulation containing paclitaxel and those with nab-paclitaxel (Figure 2). An increase in octanoylcarnitine may be attributed to a deficiency of medium-chain acyl-CoA dehydrogenase (MCAD).\textsuperscript{22} The lower MCAD protein levels in cell lysate mixtures or mitochondrial fractions (Figures 4C and 5C) may account for the higher octanoylcarnitine levels in A549 cells treated with the solvent-based formulation containing paclitaxel (Figure 2).

Cytochrome c is a mitochondrial respiratory chain protein.\textsuperscript{23} Certain pro-apoptotic stimuli induce the release of cytochrome c.\textsuperscript{24} Herein, the mitochondrial cytochrome c levels of all treatments were higher than those of the controls (Figure 5A). This finding corroborates previously reported results.\textsuperscript{23} The observed increases in mitochondrial cytochrome c might indicate early cellular defense against anticancer drugs.

Nonetheless, the present study has limitations. First, we explored the impact of different formulations by exposing A549 cells to a single representative concentration of paclitaxel, solvent-based paclitaxel, or nab-paclitaxel so as to focus on the potential mechanisms involved in a more simplified setting. However, multiple-dose animal studies would be essential in the future since they could more closely mimic the conditions in clinical practice. Furthermore, we studied the formulation only in its entirety in the present study. While results implied...
that the differential impact exerted by different formulations was related to fatty acid oxidation, the detailed mechanisms and the effects of each excipient remain to be unraveled. For example, as mentioned earlier, the solvent, polyoxyl 35 castor oil, in the solvent-based paclitaxel formulation was previously demonstrated to induce interleukin-8 in human peripheral blood mononuclear cells and whole blood through a mechanism bypassing gene expression. Whether the difference in fatty acid metabolism was affected by the specific paclitaxel delivery vehicle or by a single excipient is worth further elucidation. Therefore, investigation on the mechanisms of the effects of each excipient, such as polyoxyl 35 castor oil and albumin, as well as their impacts on the cellular uptake profiles of various formulations in the future is essential and worthy so as to have a more detailed and comprehensive understanding of the contribution of each excipient and the mechanisms behind them.

## CONCLUSIONS

Several of the challenges associated with developing injectable hydrophobic paclitaxel formulations have been overcome. The present study disclosed relative differences between A549 cells exposed to nab-paclitaxel and solvent-based paclitaxel in terms of fatty acid oxidation. This discovery may elucidate the mechanisms underlying the clinically observed differences in efficacy between the solvent-based paclitaxel and nab-paclitaxel formulations. This study also confirmed the feasibility of a cell metabolomics approach to investigate and compare the impacts of nanoformulations. The potential of its application in the holistic profiling of nanomaterials for regulatory purposes is worth further investigation.

## EXPERIMENTAL SECTION

### Chemicals. The nab-paclitaxel formulation was purchased from Celgene Corporation (Summit, NJ). The solvent-based formulation containing paclitaxel (Phyxol) was a generic version of Taxol (paclitaxel) Injection obtained from Sinphar Pharmaceutical Co., Ltd. (I-Lan, Taiwan). Paclitaxel, sodium bicarbonate, 5,5′-dithio-bis-(2-nitrobenzoic) acid (DTNB), and palmitoyl-coenzyme A (CoA) were purchased from Sigma-Aldrich (St. Louis, MO). The 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) was purchased from J.T.Baker (Phillipsburg, NJ). Penicillin G sodium salt/streptomyacin sulfate/amphotericin B cocktail was obtained from Biological Industries (Cromwell, CT). Please refer to the Supporting Information for further details.

### Cell Line and Culture Conditions. The human non-small cell lung cancer (NSCLC) cell line (A549) was obtained from the American Type Culture Collection (ATCC; Manassas, VA) and maintained in the Roswell Park Memorial Institute (RPMI)-1640 medium (Gibco, Waltham, MA) supplemented with 10% (v/v) fetal bovine serum (FBS; Gibco, Waltham, MA), 100 units/mL penicillin G sodium salt, 100 µg/mL streptomycin sulfate, 250 ng/mL amphotericin B, 2.0 g/L sodium bicarbonate, and 10 mM HEPES. The A549 cells were incubated at 37 °C in a humidified incubator with 5% CO2 in air.

### Cell Viability Assay. A sulforhodamine B (SRB) assay was used to measure the viability of A549 cells treated with 1, 3, 10, 30, or 100 nM paclitaxel in dimethyl sulfoxide (DMSO), the solvent-based formulation containing paclitaxel, or nab-paclitaxel for 24 h. The controls (culture media containing 0.1% (v/v) DMSO for the paclitaxel group; culture media alone for all others) were processed in the same manner. Please refer to the Supporting Information for further details.

### Mass Spectrometry (MS) Analyses of Metabolic Profiles. A549 cells were treated with 100 nM paclitaxel in DMSO, the solvent-based formulation containing paclitaxel, or nab-paclitaxel for 24 h before being washed with double-distilled water (37 °C), followed by cold (−20 °C) mass-grade methanol. The A549 cells were then transferred with cell scrapers to Eppendorf tubes (Eppendorf, Hamburg, Germany), and the solvent was removed by drying under a nitrogen stream. The extraction procedure for analysis was similar to that previously described by Chen et al. Briefly, an Agilent 1290 ultra-high performance liquid chromatography (UHPLC) system (Agilent Technologies, Santa Clara, CA) coupled to an Agilent 6460 QqQ (triple quadrupole) mass spectrometer (Agilent Technologies, Santa Clara, CA) was used, and hexakis(2,3,3,3-tetrafluoropropoxy)phosphazene was the ion suppression indicator (ISI).

The procedure used to analyze the metabolite profiles was previously described, with certain modifications. In brief, an Agilent 1290 UHPLC system coupled to an Agilent 6540 QTOF (quadrupole time-of-flight) mass spectrometer (Agilent Technologies, Santa Clara, CA) was used. An Agilent 1290 UHPLC system coupled to an Agilent 6460 QqQ mass spectrometer was used to verify the carnitine and acylcarnitine signals. Calibration curves were established using various known concentrations of l-carnitine inner salt and acetyl-d3-carnitine hydrochloride standard solutions. Stable-isotope-labeled (SIL) analogs, l-carnitine-methyl-d3, hydrochloride, and acetyl-d3-l-carnitine hydrochloride served as internal standards.

### MassHunter Workstation Software Profiler version B.06.00 (Agilent Technologies, Santa Clara, CA) was used to analyze the metabolite profiles via UHPLC-QTOF-MS. Metabolites were identified using an in-house library with accurate masses and retention times. Data were converted to comma-separated values (.csv) format and processed in Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA). To improve signal reliability and reproducibility, the relative standard deviations for the peak heights of the extracted signals were set to <25%. The signal values must be greater than that of the blank (solvent; 50% (v/v) methanol) by a factor of ≥2000. Similar methods were implemented to analyze carnitine and acylcarnitines via UHPLC-QqQ-MS. However, MassHunter Workstation Software Quantitative Analysis version B.07.00 (Agilent Technologies, Santa Clara, CA) was used, and peak areas were analyzed instead of peak heights. The relative standard deviations for the peak areas of the extracted signals were <25%. The corresponding metabolic pathways were analyzed with the Human Metabolome Database.

Please refer to the Supporting Information for further details.

### Mass Spectrometry Analyses of Long-Chain Fatty Acids. Sample preparation was similar to that used for the MS analyses of the metabolic profiles. The extraction method resembled a previously described procedure, with certain modifications. A precursor ion scan of 184 method similar to a precursor ion scan of 184 method was implemented to analyze the fatty acylcarnitine signals. Calibration curves were established using various known concentrations of acylcarnitines via UHPLC-QqQ-MS. However, MassHunter Workstation Software Quantitative Analysis version B.07.00 was used, and peak areas were analyzed instead of peak heights. The relative standard deviations for the peak areas of the extracted signals were <25%. The corresponding metabolic pathways were analyzed with the Human Metabolome Database. Please refer to the Supporting Information for further details.
1290 UHPLC system coupled to a Bruker maXis QTOF-MS (Bruker Daltonics, Bremen, Germany) and Bruker Data Analysis software version 4.1 (Bruker Daltonics, Bremen, Germany) were used to analyze the long-chain fatty acid profiles. Metabolites were identified using an in-house library with accurate masses and retention times. Data were exported to .XY files and processed in Microsoft Excel 2016 (Microsoft Corporation, Redmond, WA). To enhance signal reliability and reproducibility, the relative standard deviations of the peak areas of the extracted signals were <25%. Please refer to the Supporting Information for further details.

Western Blotting. A549 cells were treated with 100 nM paclitaxel in DMSO, the solvent-based formulation containing paclitaxel, or nab-paclitaxel for 24 h; harvested; and analyzed by western blotting. A549 cells treated with the culture medium served as the control. The protein content of the mitochondrial fraction was also evaluated. The mitochondria were collected with a mitochondria isolation kit for cultured cells (Thermo Scientific, Waltham, MA). Please refer to the Supporting Information for further details.

Carnitine Palmitoyltransferase 1 (CPT1) Activity Measurement. CPT1 activity was measured as previously described.19,20 Briefly, the mitochondria were collected with a mitochondria isolation kit for cultured cells (Thermo Scientific, Waltham, MA). CPT1 activity was measured using a spectrophotometer (SpectraMax Paradigm, Molecular Devices, San Jose, CA) at 412 nm. The reaction comprised the release of CoA-SH from palmitoyl-CoA in the presence of DTNB. CPT1 activity was quantified in nmol CoA-SH released/min/mg protein. Total protein concentrations were determined with a Bio-Rad protein assay kit (Bio-Rad, Hercules, CA) based on the Bradford protein assay.21 Please refer to the Supporting Information for further details.

Statistical Analysis. Treatments were compared by one-way analysis of variance (one-way ANOVA), followed by Tuckey’s honestly significant difference (HSD) post hoc test comparisons. Statistical analyses were performed in JMP version 14.0.0 software (SAS Institute Inc., Cary, NC). Data are presented as means ± standard deviations. p < 0.05 was considered statistically significant.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c04385.

Detailed experimental procedures: chemicals; cell viability assay; mass spectrometry (MS) analyses of metabolic profiles; mass spectrometry analyses of long-chain fatty acids; mitochondria isolation and sample preparation; western blotting; and carnitine palmitoyltransferase 1 (CPT1) activity measurement (PDF)

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J.-W.H.: formal analysis; investigation; methodology; and writing, review and editing. C.-H.K.: methodology; resources; and writing, review and editing. H.-C.K.: methodology and writing, review and editing. J.-Y.S.: resources and writing, review and editing. T.-W.T.: formal analysis and writing, review and editing. L.-C.C.: conceptualization; formal analysis; funding acquisition; methodology; resources; supervision; writing, original draft; and writing, review and editing.

Notes

The authors declare no competing financial interest.

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