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Graphene and graphene oxide as a solid matrix for extraction of membrane and membrane-associated proteins

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Abstract
The extraction of membrane proteins remain a challenge due to innate hydrophobicity, dynamic discrepancy, and restrain effect of membrane lipids. Nanomaterials with high surface area have competency of hydrophobic-hydrophobic lipid interactions. It is shown here that both graphene and graphene oxide dissolved in solubilization buffer are viable sorbents for efficient extraction of membrane proteins. LC-MS/MS analysis further revealed that graphene (50–200 nm) and graphene oxide (50–200 nm) can enrich more kinds of membrane proteins than a commercially available kit. Graphene was further applied to the enrichment of membrane proteins of normal cells as well as cancer cells, and 1079 and 872 proteins were identified, respectively, among which 56.5% and 60.5% were membrane proteins. In particular, 241 proteins were significantly regulated in cancer cells. Gene expression of 15 proteins was verified by qRT-PCR, and 4 of them were further quantified by immunoassay. These data collectively demonstrate that graphene has great potential to improve membrane protein extractions and thus can serve downstream cancer proteomics.

Keywords Carbon nanomaterials · Cellular membrane protein · Proteomics · Preconcentration · Microextraction · Mass spectrometry · Hydrophobic-hydrophobic interaction · Cancer · Immunoassay

Introduction

Graphene is a two dimensional carbon material that has unique physiochemical properties such as extraordinary electrical and optical properties and high specific surface area. Graphene and its derivatives can be used as potential biomedical device [1, 2] as well as biosensor [3, 4], enzyme immobilizer [5, 6], preconcentrator [7, 8] and for targeted drug delivery [9, 10]. They can also be covalently grafted on targeted biomolecules for affinity enrichment of targeted proteins

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[11–14]. Graphene has been used as an efficient sorbent for the enrichment of low abundance peptides or proteins [12, 13, 15]. For example, graphene and graphene oxide can extract cytochrome C with high efficiency [16, 17]. Moreover, graphene oxide can serve as a nano-carrier for delivery of water insoluble aromatic anticancer drugs into cells [18]. Graphene and graphene oxide have been explored as a sorbent for membrane lipid extraction and cytotoxicity analysis. In particular, graphene and graphene oxide sheet can penetrate into *E. coli* cell membrane via hydrophobic-hydrophobic interaction and vigorously extract phospholipids on nanosheets, while cell membranes lose integrity [19].

Membrane proteins (MPs) are crucial mediators of matter and information between cells and intracellular compartment as well as organ systems [20, 21]. Despite the tremendous progress in analysis of soluble proteins, membrane proteins have lagged behind and under-represented in most datasets due to their hydrophobic nature and inherent low abundance [22]. In human proteome, MPs have an active role in oncogenic processes and they are targets of almost 70% of cancer therapies in use or under study. Specifically, G-protein-coupled receptors (GPCRs) are the prime and most diverse group of membrane receptors found in eukaryotes, which

have been explored as model for novel drug screening [23]. Considering the importance of MPs in diagnosis and treatment, an efficient solubilization, lower lipid interference, better extraction and enrichment procedure for membrane proteins is therefore urgently demanded.

To address these issues, a number of strategies have been developed for the isolation and enrichment of MPs, which include: centrifugation (differential centrifugation, sucrose gradient centrifugation [24] and aqueous polymer two phase systems [25]), targeted enrichment (biotin directed affinity [26], cell surface capture [27]), and alternative approaches (ionic liquids [28], detergent free copolymer [29] and integrated systems [30]). Centrifugation methods are widely used for membrane protein extraction because these methods are easily adjustable to the requirements of downstream analytical technique. Besides, other targeted enrichment methods required additional pretreatments and special precaution to improve extraction and identification of bona fide membrane proteins.

Since nanomaterials graphene and graphene oxide can efficiently extract lipids, we hypothesized that nanomaterials can serve as solid extraction matrix for membrane proteins (MPs) by reducing lipid intrusion effect on membrane protein solubilization and extraction. Graphene and graphene oxide were mixed with solubilization buffer to reduce lipid interference for membrane proteome profiling. LC-MS/MS based quantification was utilized for protein identification and relative quantification from cultured cells (MRC-5, BEAS-2B, and 95-D). Through this strategy, the cellular membrane proteome was efficiently extracted and comprehensively analyzed. Differentially expressed proteins with crucial cellular functions between cancer and control were discovered and further validated by immunoassay. The utility of the method in membrane proteome analysis is also discussed.

Materials and methods

Cell culture

Human lung fibroblast MRC-5, normal BEAS-2B, and metastatic 95-D cells were obtained from American Type Culture Collection (Manassas, VA, USA) and cultured in RPMI modified medium (GE Healthcare, Life Science, Lagun-utah, Germany, http://www.gelifesciences.com.cn) supplemented with 5% fetal bovine serum (Atlanta Biologicals, Lawrenceville, Germany, https://www.atlanta-biologicals.com/) and antibiotics at 37 °C. The cells were maintained in 5% CO_2 and 95% air. The cells having 95% confluence were harvested.

Extraction of membrane proteins (MPs) using a commercial kit

The extractions were performed according to the extraction protocol of Mem-PER™ plus Protein Extraction Kit (Thermo scientific, USA, http://www.thermofisher.com).

Extraction of membrane proteins (MPs) using graphene and graphene oxide as a sorbent

MPs were extracted with graphene and graphene oxide (from Xianfeng Nano Inc., Nanjing, China, http://en.xfnano.com/). Briefly, cells were washed with cell wash buffer (three times) at 300 g for 5 min and the supernatant was removed. The washed cells were then incubated with ice-cold cell permeabilization buffer at 4 °C for 10 min with constant mixing to proper penetrate the cell membrane. After permeabilization, cell suspensions were centrifuged at 16000 g for 15 min at 4 °C and the supernatant containing cytosolic proteins was removed carefully. The remaining cell membrane pellets were solubilized with solubilization buffer containing graphene or graphene oxide. This mixture was incubated at 4 °C for 30 min with constant mixing to the solubilized cell membrane. The cell membrane solubilized suspension were centrifuged at 16000 g for 15 min at 4 °C and the supernatant containing membrane and membrane associated proteins was transferred to new collection tubes. The graphene or graphene oxide pellets were further resuspended with 250 mM SDS and sonicated for 2 min with 2 s interval. The SDS resolubilized membrane suspensions were further centrifuged at 16000 g for 15 min and the supernatant was collected as membrane proteins bounded on nanomaterials.

SDS-PAGE analysis of MPs

For SDS-PAGE, 2 μg of membrane proteins were loaded to a 4–12% NuPAGE Bis-Tris gel (Invitrogen, Carlsbad, CA, USA, http://www.thermofisher.com). The silver staining of the gel was carried out by following the kit (Beyotime, Shanghai, China, http://www.beyotime.com) manual instructions, to visualize and track the protein migration.

Insolution digestion for LC-MS/MS

The FASP digestion was performed as previously described [31]. Briefly, 20 μg MPs were precipitated; the precipitate MPs were resuspended in a reducing solution (50 mM NH_4HCO_3 + 6 M $NH_5N_3.HCl$ + 100 mM DTT) and incubated at 60 °C for 60 min. After reduction, MPs were alkylated with 100 mM iodoacetamide for 40 min in dark. The protein samples were transferred to 10 kDa ultrafilter. 1 μg trypsin (Promega, Madison, WA, USA, https://www.promega.com.cn/) was added to digest protein and digested peptide was

collected. 1 μL formic acid (1%) was added to stop reaction. Desalting of the peptide was carried out by using Zip Tip C18 column and samples were further eluted in 40%ACN/0.1%FA solution.

Nano LC-MS/MS analysis and data acquisition

Thermo Scientific EASY-nLC 1000 Nano-flow UHPLC interfaced to Thermo Scientific Q Exactive hybrid quardrapole-Orbitrap mass spectrometer (Thermo Fisher, San Jose, CA) were used for protein analysis. The sample peptides were loaded on trapping column and eluted onto a 75 μm analytical column with a flow rate of 300 nL/min. The trapping and analytical column were packed with Jupiter Proteo resin (3 μm, C18, 300 Å, Phenomenex, Torrance, CA, http://www.phenomenex.com/). The mass spectrometer was operated in data independent mode and analyzed data were acquired by collision induced dissociation (CID) mode. Orbitrap analyzer used for acquisition of MS spectra in the m/z range of 300 and 1800 at 60,000 resolutions.

Protein identification and quantification

Mass spectrometry raw files were searched with Proteome Discoverer version 1.3 (Thermo Fisher) against Human SwissProt protein database (V3.82, 184,208 entries), and the result of intensity, based on intensity–based absolute quantification method (iBAQ) was obtained by searching with MaxQuant (version 1.5.3.30) against the UniProtKB human database. For both of the two search engines, the parameters were set as follows. The maximum of missed cleavages was set as two and trypsin was selected for protein digestion. Cysteine carbamidomethylation was set as static modification, whereas methionine oxidation and protein N-terminal acetylation were set as variable modifications. For protein identification, fragment tolerance was set at 0.50 Da with parent tolerance up to 10 ppm, whereas for quantification, a mass tolerance of 20 ppm for fragment ions was set. The maximum false discovery rate (FDR) for peptide and proteins were limited to 1%. Protein expression from each type of cell lines and extraction methods were compared by using label-free iBAQ value, based on at least two unique peptides. The BEAS-2B sample was set as the reference, and data processing was aligned. Statistically significant proteins were detected by the two-tailed unpaired Student's t-test ($p < 0.05$) and protein abundances that with less than 2-fold (95-D/BEAS-2B) were discarded.

Bioinformatics analysis

The assignment of protein cellular localization and function was performed based on Human Membrane Protein Analysis System (HMPAS) and Gene Ontology Consortium Database.

STRING protein-protein interaction analysis was performed by using online available tools (http://string-db.org/). Gene ontology assessment was performed by using DAVID 6.7 version (https://david-d.ncifcrf.gov/tools.jsp) and Panther tools (http://www.pantherdb.org/).

Immunoblotting

MPs were extracted from cells by kit method or graphene method, as described in the method section. Total cell lysates were prepared by using RIPA cell lysis buffer. Equal amount of proteins were loaded and resolved via SDS-PAGE and immunoblotting were performed by following the instructions of antibodies manufacturer (LAMP-1, CAV-1, and LYAR, Immunoway Biotechnology, USA, http://www.immunoway.com/) and NDUFB10 (Abcam, USA, http://www.abcam.com).

qRT-PCR

Cellular RNA was extracted from normal and cancer cells by using Trizol (Life Technologies, Carlsbad, CA, https://www.thermofisher.com/). After RNA extraction, genomic DNA was removed by using RNase free Recombinant DNase I. The total RNA concentration was measured by NanoDrop spectrophotometer. First stand cDNA were prepared from purified RNA with RevertAid First Strand cDNA synthesis kit protocol. Following first stand cDNA synthesis, Hieff™ qPCR SYBR$^{(R)}$ Green Master Mix were used to qRT-PCR sample preparation.

Results and discussion

Method development

Biologically important membrane proteins are embedded in lipid bilayers and there are two key rate-limiting steps involve in membrane protein extraction, namely suppression effect of soluble cytosolic proteins and membrane lipid interference. Therefore, cytosolic proteins were removed by permeabilization buffer, nanomaterials (Graphene and Graphene oxide) were mixed with membrane solubilization buffer and used to minimize the interference of membrane lipids and enhance membrane protein extraction. Graphene with cell membrane lipid complex formation that were characterized by AFM, and this is shown in the Electronic Supplementary Material (Fig. S1).

Commercial kit based extraction of membrane and associated proteins

A simplified overview on MP extraction by using the commercially available Mem-PER™ kit is shown in Fig. 1a. The
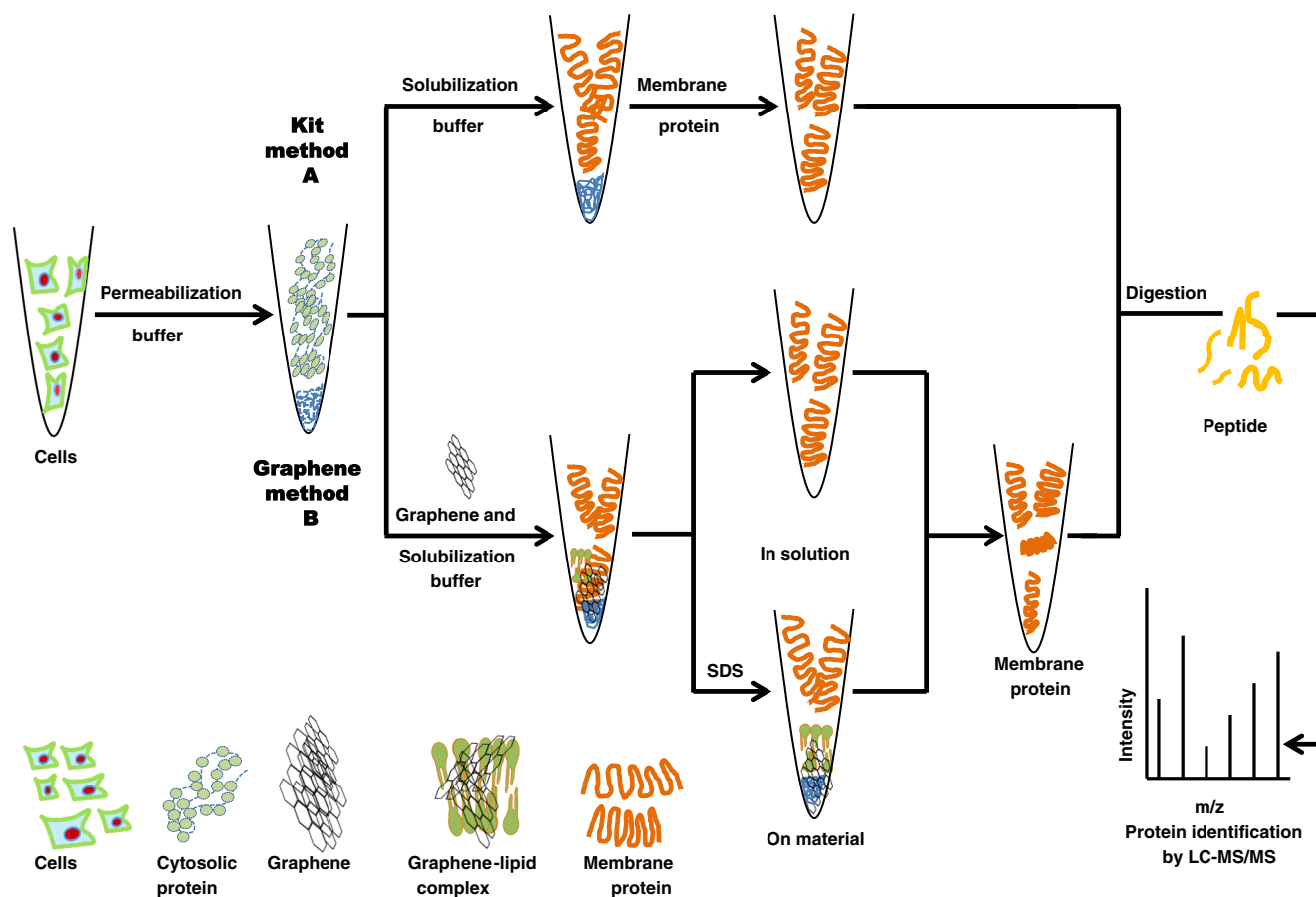
Fig. 1 Schematic diagram for membrane protein extraction. **a** Membrane protein extraction by commercial kit. **b** Nanomaterials modified extraction of membrane proteins

MP experiments were carried out in triplicate. The results shows that the kit can extract an average of 233 ± 15.5 μg of proteins from about 5×10^6 MRC-5 cells (Table 1).

Extraction of modified membranes and associated proteins

Overviews of nanomaterial assisted extraction method is shown in Fig. 1b. Nanomaterial assisted membrane protein extraction from MRC-5 cells was executed with graphenes and graphene oxides having the size of 50–200 nm and 50–500 nm, respectively. The results are summarized in Table 1. About 5×10^6 MRC-5 cells were used for total membrane protein (MPs and MAPs) extraction. When compared with kit extraction method, the results reveal that larger size graphene and graphene oxide (with size of 50–500 nm) can extract 81% and 70% more MPs. In contrast, graphene and graphene oxide with size of 50–200 nm can extract 100% and 81% more MPs, respectively. More specifically, MPs extraction by using graphene and sraphene oxide both in solution and on materials were evaluated. For the solution part, the

Table 1 Membrane protein extraction with nanomaterials (G and GO) and commercial kit from normal lung cells (MRC-5)

Methods	Average MPs in solution (μg)	Average MPs on material (μg)	Average total MPs with RSD (μg)	Extraction (%)	P-value
Kit extraction	233	0	233 ± 15.5	100	0.000
Graphene (50–200) nm	232	236	468 ± 66.0	200	0.0013
Graphene (50–500)nm	110	312	422 ± 75.3	181	0.0017
Graphene oxide (50–200) nm	106	318	424 ± 23.6	181	0.0002
Graphene oxide (50–500)nm	105	292	397 ± 32.5	170	0.0014

About 5×10^6 cells were used for each extraction. The comparison between groups was carried out by using the one way ANOVA test

results demonstrate that the highest average MP extraction was 232 μg from graphene (50–200 nm). For the "on materials" part, the highest average MP extraction was 318 μg from graphene oxide (50–200 nm) (Table 1).

It is also found that graphene and graphene oxide increase total membrane protein extraction when compared with commercial kit method. Moreover, the method exhibits enhanced protein extraction efficiency as compared to other previously known methods [32]. For example, Bünger et al. extracted MPs from colorectal carcinoma cells (SW620) (8–10 X 10^7 cells) by using five different kit methods, but they only acquired 0.02 to 4.8 mg of MPs, respectively.

Based on the extraction yield (in solution and on material), we selected graphene and graphene oxide of smaller size (50–200 nm) for further experiments.

## Membrane protein identification using graphene and graphene oxide

MPs extracted by both commercial kits and by graphene and graphene oxide were analyzed by SDS-PAGE (Fig. 2a). The results of protein extraction (both in solution and on materials) were also compared to those obtained with a commercial kit. SDS PAGE analysis shows that in solution protein extraction, some protein bands were with lower intensity as compared to the on material protein extraction (higher intensity bands). SDS-PAGE results indicate that our method can extract and release more MPs and MAPs, which generate higher yields of total MPs.

LC-MS/MS based shotgun proteomics was carried out to analyze the MPs and MAPs in MRC-5 cells. For the kit method, 1602 proteins were identified, among which 920 are MPs. The results reveal that 1646 and 1435 proteins can be identified from graphene (50–200 nm) in solution and on materials, respectively, with 860 shared proteins. The MPs and MAPs extracted by graphene oxide (50–200 nm) in solution and on material were identified as 1335 and 1763 proteins, respectively, with 710 shared proteins. In total, 2221 proteins were from graphene method and 2388 were extracted from graphene oxide method, respectively (Fig. 3a). Overall, 1105 proteins were shared by all three-extraction methods. Additionally, graphene and graphene oxide method shared 1672 proteins (Fig. 3b). The number of total identified membrane proteins was raised up to 238 (25%) for graphene and 304 (32%) for graphene oxide (Fig. 3c). The improved extraction and number of identified membrane proteins indicate the superiority of the method. Considering the extraction yields as well as MPs percent, graphene (50–200 nm) was selected for following experiments.

Comparison of MP extraction yield between normal lung cells and lung cancer cells

The MP extraction from BEAS-2B and 95-D was carried out by commercial kit as well as graphene method (in solution and on materials). The extraction experiments were carried out in triplicates and the results of these two methods were compared. The average extraction of commercial kit method was 75 ± 10.56 μg (BEAS-2B) and 98 ± 22.57 μg (95-D) of proteins from about 1.2×10^6 cells, respectively. However, graphene method extracted 164 ± 9.98 μg (BEAS-2B) and 170 ± 19.63 μg (95-D) membrane proteins from the same number of cells (Fig. 4a). The results indicated that the yield was elevated by 152% and 76% in BEAS-2B and 95-D, respectively, when compared with the commercial kit method. The SDS–PAGE of membrane proteins extracted by graphene
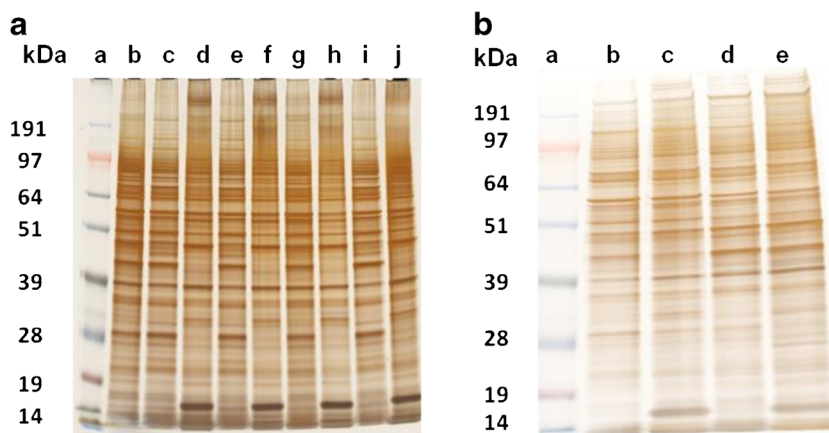
Fig. 2 Protein separation by SDS-PAGE and proteomic analysis of normal and lung cancer cell (MRC-5, BEAS-2B, and 95D). **a** SDS-PAGE of membrane proteins from MRC-5 cells. (a) protein ladder, (b) Kit extracted membrane proteins, (c) GO (50–200) nm in solution extraction, (d) GO (50–200) nm on materials extraction, (e) GO (50–500) nm in solution extraction, (f) GO (50–500) nm on materials extraction, (g) G(50–200) nm in solution extraction, (h) G (50–200) nm on materials extraction, (i) G (50–500) nm in solution extraction, (j) G (50–500) nm on materials extraction. **b** SDS-PAGE of membrane proteins from normal (BEAS-2B) and Lung cancer (95-D) cells. (a) Protein ladder, (b) kit normal membrane protein, (c) G normal membrane protein, (d) kit cancer membrane protein, (e) G cancer membrane proteins. K and G set for kit and G method, respectively
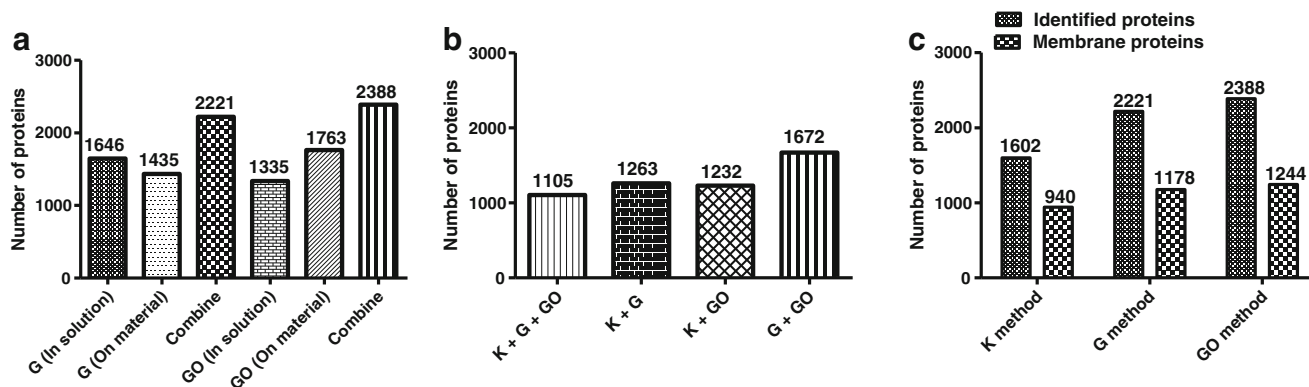
Fig. 3 Proteomic analysis of normal lung cells (MRC-5). **a** In solution and on materials identified proteins by G and GO (50–200) nm. **b** Common identified proteins by Kit, G and GO methods **(C)** Total identified proteins and their relative membrane proteins identified by G, GO and kit method. K, G, and GO represent for kit, G and GO methods, respectively

method and commercial kit from both BEAS-2B and 95-D are shown in Fig. 2b. The electrophoretic analyses of extracted proteins exhibit some visible differences in protein profiling (Fig. 2b). Furthermore, the above finding concluded that graphene can significantly improve the MPs and MAPs extraction efficiency. However, extraction efficiency may vary between different cell types.

Membrane and membrane associated proteome analysis of normal lung cells and lung cancer cells

The MPs and MAPs isolated from both BEAS-2B and 95-D was further analyzed through using label free quantification approach. According to triplicate experiments, graphene method revealed that 1079 proteins (BEAS-2B) and 872 proteins (95-D) were present in all replicates, whereas the commercial kit method extracted 1019 (BEAS-2B) and 829 (95-D) proteins, respectively. We observed that graphene method shared 78.5% and 80% of total extracted proteins with commercial kit method (Fig. 4b).These results indicate graphene method generated 20% variation of total identified proteins in normal and cancer cells.

Proteins identified by graphene and kit method were selected for following downstream analysis. The details of the identified MPs and MAPs from BEAS-2B and 95-D are shown in Fig. 4c and d. The graphene method extracted 610 MPs from

Fig. 4 Systemic comparison of yields and identified proteins in BEAS-2B and 95-D cells by G and kit method. **a** Histogram of mean membrane protein extraction. **b** Comparison of consistently identified proteins. **c** Comparison of identified membrane proteins. **d** Comparison of differentially expressed proteins and their relative membrane proteins. K and G represent for kit and G method, respectively
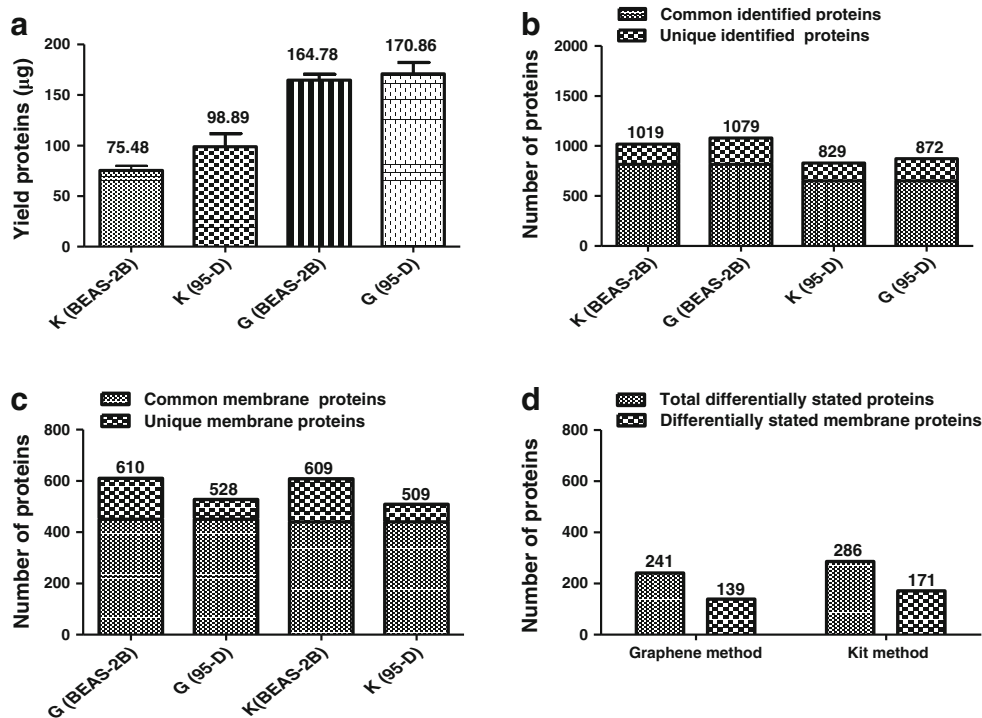
BEAS-2B and 528 from 95-D cells. Among these MPs, 451 proteins were found common in both BEAS-2B and 95-D cells. Contrariwise, kit method was extracted 609 MPs from BEAS-2B and 509 from 95-D cells. Among these MPs, 440 proteins were found common in both BEAS-2B and 95-D cells (Fig. 4c). Furthermore, total identified MPs by both graphene method and commercial kit method from BEAS-2B and 95-D cells were 687 and 678, respectively. When compared with kit method, graphene method (BEAS-2B + 95-D) extracted 125 unique MPs, respectively. Among them, 50 proteins were shared by both normal and cancer cells, 47 were unique to normal cells and 28 were unique to cancer cells. STRING protein-protein interaction analysis of these 125 unique proteins showed that 88% of them were systemically interacted and these results demonstrated their extraction occurred in a systemic approach (Fig. S2). We further analyzed the functional annotation of these uniquely identified proteins by DAVID. The results revealed that 74.4% of these were phosphoproteins (Data not shown). In addition, we analyzed these 28 proteins that graphene method uniquely identified for cancer cells (95D). The results revealed that some of them are lung cancer prognosis biomarker (e.g Caveolin-1 [33]), tumor growth and metastasis marker (e.g Integrin linked protein kinase [34]), respectively.

We anticipated isolating additional variety of MPs or MAPs through the nanomaterial method. However, proteomics analysis did not show increase in total number of identified MPs or MAPs, partially due to the graphene method is capable to extract similar MPs and MAPs as kit method.

Downstream analysis of differential expressed proteins in normal lung cells and lung cancer cells

To identify differentially expressed protein profiles between BEAS-2B and 95-D cells, the consistently identified proteins by both methods were compared. The comparison results revealed that graphene method and kit method extracted 241 and 286 differentially expressed proteins in BEAS-2B and 95-D cells, respectively. Out of these differentially expressed proteins 139 and 171 were MPs whereas other differentially expressed proteins may be belongs to MAPs class (Fig. 4d).

Gene ontology analysis of differentially expressed MPs and MAPs identified by both methods is provided in supplementary material (Fig. S3). The cellular component analysis in both methods revealed that 96% of genes identified are from cell part, macromolecular complex, organelle membrane and membranes. Biological process and pathway analysis was performed for graphene method and results showed that 29% and 12% of gene were involved in cellular process and integrin signaling pathways, respectively. The gene ontology analysis of graphene

extracted identified MPs and MAPs leads to the conclusion that these proteins are involved in various biological processes.

In-depth comparative analysis of identified proteins from graphene method, we revealed some differentially expressed candidates (e.g CD proteins, Annexin proteins, Transgelin and Catenin proteins), which can be potential biomarkers for lung cancer. The details of differentially expressed MPs and MAPs are provided in Table S1 and Table S2 includes the differentially expressed proteins obtained by commercial kit method. In concise, 43% of total differential expressed proteins upregulate in graphene method and only 26% are in kit method. These differential expressed proteins belong to MPs and MAPs.

Validation of differentially expressed MPs and MAPs

To confirm the differentially expressed MPs discovered only by graphene method and validate the utility of the method, qRT-PCR and immunoblotting assays were carried out. qRT-PCR was performed for 50 proteins gene expression, which were selected based on quantification data as well as literature survey. Fifteen proteins were successfully verified, which were consistent with our LC-MS/MS data (Table 2). Among these 15 verified candidates, four biologically important MPs and MAPs i.e. (Caveolin-1, LAMP-1, LYAR and NDUFB10) were chosen for immunoblotting assessment, as shown in Fig. 5. In graphene method, we found significant higher expression of LAMP-1, CAV-1, LYAR and NDUFB10 in 95D cells. For kit extraction method, we found significant higher expression of CAV-1 and LYAR in 95D cells. The results also revealed that LAMP-1 and NDUFB10 are deficient in the kit extraction method (Fig. 5). The densitometric analysis of differential expressed membrane and membrane associated proteins (CAV-1, LYAR, NDUFB10 and LAMP-1) from BEAS-2B and 95-D cells is provided in supplementary material (Fig. S4).

When compared with kit method, we revealed that 4 targeted proteins expression in graphene method were consistent with LC-MS/MS results as well as and qPCR results. However, 2 down regulated proteins (LYAR and NDUFB10) were inconsistent with western blot results. In addition, we revealed that targeted proteins and their gene expression were more variable in kit method (Fig. 5, Table 2, and Table S1 and S2). Therefore, graphene method is competence with existing kit method for membrane proteome profiling.

The nanomaterial method improved the total membrane protein extraction yield from limit number of cells, which is valuable for different types of analysis (e.g. identification, quantification, and detection). When compared with kit method, graphene method can extract important MPs that may not be isolated by kit method, as evidenced by western blot analysis. The nanomaterial graphene method for membrane proteins profiling devoted to the following virtues: (I) reduced

Table 2 List of q-RTPCR verified differentially expressed proteins from LC-MS/MS

Acc. no.	Protein name	Gene	U.pep (BEAS-2B)	S. cov (BEAS-2B)	U.pep (95D)	S.cov (95D)	P-value	LC-MS/MS (95D/ BEAS-2B)	q-RTPCR (95D/ BEAS-2B)
Q03135	Caveolin-1	CAV1	2	17.4	4	36	0.015	9.31	1.248
P11279	Lysosome-associated membrane glycoprotein 1	LAMP1	3	8.6	3	8.6	0.007	3.31	2.950
O94776	Metastasis-associated protein MTA2	MTA2	3	6.1	7	14.4	0.008	3.65	5.328
P08574	Cytochrome c1, heme protein, mitochondrial	CYC1	7	35.4	5	24	0.007	0.38	0.140
P10515	Dihydrolipoyllysine-residue acetyltransferase component of pyruvate dehydrogenase complex, mitochondrial	DLAT	10	27.5	8	21.8	0.019	0.44	0.691
C9JAB2	Serine/arginine-rich splicing factor 7	SRSF7	6	29.4	3	17.9	0.046	0.43	0.346
P00505	Aspartate aminotransferase, mitochondrial	GOT2	16	51.4	12	44.7	0.003	0.45	0.546
P23368	NAD-dependent malic enzyme, mitochondrial	ME2	14	41.3	6	17.1	0.03	0.32	0.694
Q00059	Transcription factor A, mitochondrial	TFAM	6	25.2	2	15.4	0.006	0.41	0.391
O43837	Isocitrate dehydrogenase [NAD] subunit beta, mitochondrial	IDH3B	10	25.5	6	39.7	0.014	0.42	0.129
P38646	Stress-70 protein, mitochondrial	HSPA9	33	54.4	31	54.8	0.002	0.49	0.882
Q96DI7	U5 small nuclear ribonucleoprotein 40 kDa protein	SNRNP40	10	45.1	7	32.2	0.008	0.39	0.453
O96000	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 10	NDUFB10	6	44.8	3	20.9	0.013	0.22	0.727
Q9NX58	Cell growth-regulating nucleolar protein	LYAR	10	32.7	6	22.7	0.001	0.15	0.479
Q15006	ER membrane protein complex subunit 2	EMC2	7	35.4	4	18.2	0.013	0.4	0.706
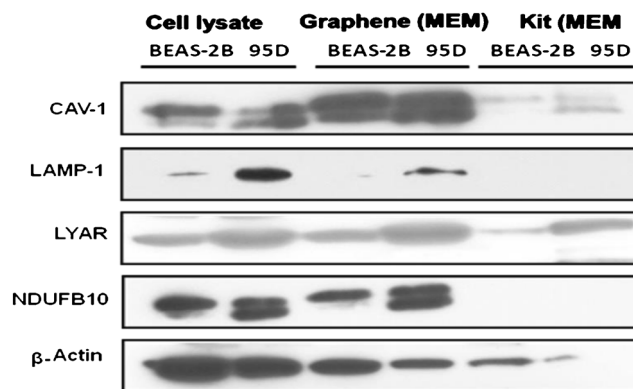
Fig. 5 Immunoblotting validation of differential expressed proteins on lung cancer (95-D) and normal (BEAS-2B) cells identified by LC-MS/MS. β-actin was used as a loading control. Data is normalized by BEAS-2B cells

restrain effect of high abundance proteins and membrane lipids; (II) nanomaterials can serve as an extraction matrix; (III) convenient and higher membrane protein extraction capacity; and (IV) enrich and identify unique membrane proteins that related to diseases and drug targets.

Conclusions

In conclusion, nanomaterials are powerful agents to reduce lipid-protein interface of biological membranes and allows more solubilization and separation of membrane proteins from small number of cells. Graphene and graphene oxide were applied to extract membrane proteins from different cell lines. Through quantitative proteomics analysis, the graphene based extraction method is capable to extract membrane protein from different cell lines and elevated the extraction respectively. Furthermore, differentially expressed proteins were successfully validated. One potential obstacle is that extracted membrane proteins on materials should be detached by strong detergent, which should be removed prior to mass spectrometry analysis. Finally, the nanomaterials method will not only benefit for low abundant protein extraction but also for the understanding of mechanism related to disease progression and therapies.

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