Detection of tyrosine-phosphorylated proteins in hepatocellular carcinoma tissues using a combination of GST-Nck1-SH2 pull-down and two-dimensional electrophoresis

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Abstract. Tyrosine-phosphorylated proteins govern a host of cell functions, such as growth, division, adhesion and motility. We previously identified a group of Nck Src homology 2 (SH2) domain-binding proteins by combining the GST-Nck1-SH2 pull-down method with two-dimensional electrophoresis (2-DE) in hepatocellular carcinoma (HCC) tissues. In the present study, different methods and conditions for key procedures of GST-Nck1-SH2 pull-down and 2-DE were investigated and optimized. High-resolution results were obtained using the following conditions: a total amount of 100 μl GST-Nck1-SH2 fusion proteins/10 mg liver proteins to execute the pull-down procedure; 7 M urea and 2 M thiourea as lysis buffer; ultrafiltration depletion of interferential materials. Moreover, we performed a negative control experiment using GST-4T3 during the pull-down procedure, and further demonstrated that the proteins obtained using the aforementioned method interacted with Nck in a tyrosine phosphorylation-dependent manner. The optimized method offers a rapid, efficient alternative for the high-quantity screening of tyrosine-phosphorylated protein expression and solubility, which in turn facilitates future studies on tyrosine-phosphorylated proteins.

Introduction

Tyrosine-phosphorylated proteins govern a host of cell functions, such as growth, division, adhesion and motility (1). These proteins are critical regulators of signaling in the majority of eukaryotic cellular pathways, and deregulated phosphorylation is involved in an array of diseases (2), including cancer (3).

Given their importance, a number of techniques have been used to study tyrosine-phosphorylated proteins, such as MS-based phosphoproteomic methods (4), redox-based probes (5), an Src homology 2 (SH2) profiling method based on far-western blot analysis (6), and the use of Grb2-SH2 domain binding proteins with SILAC (7).

The Nck adaptor protein consists of three SH3 domains followed by a C-terminal SH2 domain, and is capable of binding to numerous receptor tyrosine kinases via its SH2 domain (8). Dierck *et al* have developed an alternative phosphoproteomic method (termed SH2 profiling) to explore phosphotyrosine signaling in cancer cells (9), and have demonstrated that it is an ideal method to detect phosphotyrosine, due to its being highly sensitive and throughput.

However, these aforementioned studies were performed in cell lines. Therefore, the state of tyrosine-phosphorylated proteins in tumor tissues remains unknown. Our previous study successfully combined the GST-Nck1-SH2 pull-down with two-dimensional electrophoresis (2-DE) to detect tyrosine-phosphorylated proteins in liver tissues (10).

Although the combination of GST-Nck1-SH2 pull-down and 2-DE was able to detect tyrosine-phosphorylated proteins, it continues to involve numerous challenges. The greatest of those challenges was how to harvest samples from GST-Nck1-SH2 pull-down that are also compatible with downstream 2-DE. The efficiency of the GST-Nck1-SH2 pull-down method requires improvement in order that it yields sufficient tyrosine-phosphorylated proteins for downstream 2-DE. In addition, the samples obtained though the GST-Nck1-SH2 pull-down method include different types of interferential material, such as fragments of GST beads and iron, which are likely to affect the success of isoelectric focusing (IEF). Therefore, there is a requirement to deplete the interferential materials, while retaining the activity of the tyrosine-phosphorylated proteins. At present, to the best of our knowledge, few effective and detailed methods have been devised to obtain tyrosine-phosphorylated proteins from

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tissues by the use of the GST-Nck1-SH2 pull-down method. The present study set out to explore the effective techniques of GST-Nck1-SH2 pull-down, and to search for detailed methods regarding sample preparation for downstream 2-DE.

Materials and methods

Hepatocellular carcinoma (HCC) patient samples. HCC tissues were collected from 21 HCC patients who underwent hepatectomy at the First Affiliated Hospital of Sun-Yat Sen University (Guangzhou, China). None of these patients had received preoperative chemotherapy or radiotherapy. Normal liver tissues were obtained from 8 patients diagnosed with liver hemangioma or cholelithiasis. Specimens were obtained with written informed consent from all patients. The study was conducted with prior approval from the Committees for Ethical Review of Research involving Human Subjects of the First Affiliated Hospital of Sun-Yat Sen University.

Plasmid constructs and transfection. The full-length dermcidin cDNA was amplified and cloned into the pReciever M06 expression vector (FulenGen Co., Ltd., Guangzhou, China). The GST-tagged SH2 domain of Nck was generated by PCR amplification of the human Nck template, and then ligated into the pGEX-4T-3 expression vector.

GST fusion protein purification. *Escherichia coli* (BL21) was transformed with pGEX-4T-3 or pGEXNck-SH2 incubated with 0.2 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG) for 4 h. The GST fusion proteins were purified from bacterial lysates with GSH-Sepharose 4B beads, according to the manufacturer's instructions (Amersham Biosciences Corp., Picataway, NJ, USA). Tissue/cell lysates were prepared and spun at 15,000 x g for 15 min.

GST pull-down. The liver proteins were incubated with GST-Nck1-SH2-conjugated sepharose beads for 2 h at 4˚C. Following incubation, the supernatant was removed and the beads were washed with a Tris-sucrose solution (10 mM Tris-HCl; 150 mM NaCl; 1% Triton X-100, pH 7.5) to remove any non-specific or non-covalently bound proteins. The fusion proteins were eluted with 2 ml 2-D lysis buffer (10 mM reduced glutathione in 50 mM Tris-HCl, pH 8.0) and desalted by ultrafiltration.

2-D clean-up. 2-D clean-up was performed according to the manufacturer' instructions (Amersham Biosciences Corp.).

Ultrafiltration. Samples were loaded into centrifuge tubes with a 10-kDa membrane, in order to concentrate the proteins and remove small interference molecules. Samples were centrifuged at 5,000 x g, at 4˚C for 1 h.

2-DE and image analysis. Protein samples (250 μg) were diluted to 450 μl with a rehydration solution (7 M urea, 4% CHAPS, 0.5% IPG ampholyte, 65 mM DTE, 2 M thiourea, and 0.0002% bromophenol blue), and then loaded onto IPG gel strips (pH 3.0-10.0 linear, 24 cm long; Amersham Biosciences Corp.). The first dimensional separation was performed using the IPGphor system (Amersham Biosciences Corp.) at 18˚C with 8,000 V, for a total of 90 k VHS. Following IEF, the IPG strips were subjected to reduction with 2% DTE in equilibration solution (50 mM Tris-HCl, pH 8.8; 6 M urea; 2% SDS; 30% glycerol), followed by alkylation with 2.5% iodoacetamide in the same buffer. The gels were stained with Silver Staining kit (Amersham Biosciences Corp.) according to the manufacturer's instructions. The developed gels were scanned as 2-DE images using an image scanner, and then analyzed using ImageMaster software (Amersham Biosciences Corp.).

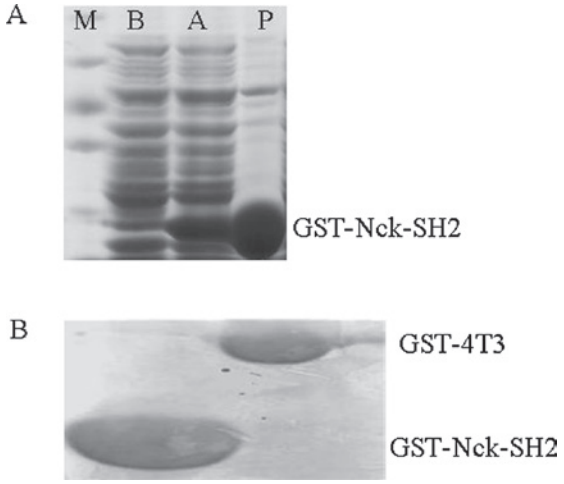

Figure 1. Induction and purification of GST-Nck1-Src homology 2 (SH2). *Escherichia coli* (BL21) was transformed with pGEX-4T-3 or pGEXNck-SH2, and incubated with 0.2 mM IPTG for 4 h. The GST fusion proteins were purified from bacterial lysates with GSH-Sepharose 4B beads, then 15 ml was loaded on each lane of the gel and stained with Coomassie blue. (A) Induction and purification of GST-Nck1-SH2. M, marker; B, before IPTG induction; A, after IPTG induciton; P, GST-Nck-SH2 fusion proteins. (B) Comparison of GST-Nck1-SH2 and GST-4T3.

In-gel digestion and protein identification. 2-DE gels of interest were washed in water/acetonitrile (ACN; 1:1) and then dehydrated in ACN. The gel pieces were air-dried and rehydrated in 20 μl of 10 mM DTT and 0.1 M NH_4HCO_3. Reduction of disulfide bonds was performed at 56˚C for 45 min. The supernatant was discarded and cysteine residues were modified to S-carboxyamidomethylcysteine in 55 mM iodoacetamide and 0.1 M NH_4HCO_3. After washing with 0.1 M NH_4HCO_3/ACN (1:1) for 15 min, followed by ACN, the gel pieces were air-dried, rehydrated in chilled 50 mM NH_4HCO_3 and 12.5 ng/μl trypsin, and then incubated at 37˚C overnight. The supernatant was collected and peptides were extracted twice from the gel with 50 mM NH_4HCO_3/ACN (1:1) followed by 5% formic acid/ACN (1:1). The combined extracts were evaporated to dryness in a vacuum centrifuge. Prior to mass spectrometric analysis, peptides were re-dissolved in 10 μl of 0.1% formic acid. Online peptide separation was performed after trapping each sample on a 180 μm x 20 mm Symmetry® C18 Nano Acquity™ UPLC™ column with 1% ACN and 0.1% formic acid at a 15 ml/min flow rate; following separation on a 75 μm x 250 mm BEH130 column (Nano Aquity™ UPLC™) with a 50-min gradient from 5 to 95% ACN and 0.1% formic acid, at a flow rate of 300 nl/min. A tapered fused silica was used as an emitter. Mass analyses were performed with a quadrupole time-of-flight mass spectrometer (QTOF, Waters Corp., Milford, MA, USA). The

Table I. Approximate yield using different methods.

Method	GST-Nck1-SH2 fusion protein (μl)	Liver protein (mg)	Yield (μg)	Protein spots
A	100	1	100±5	50±5
B	200	1	123±6	70±15
C	100	10	1202±28	200±38
D	100	20	1301+32	238±43

SH2, Src homology 2.

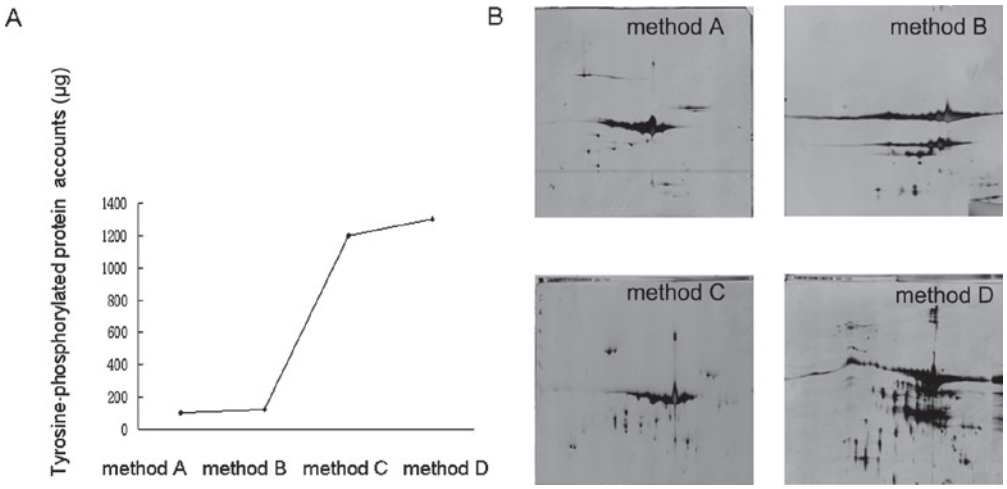

Figure 2. Different strategies employed to yield tyrosine-phosphorylated proteins. (A) Comparison of different strategies to yield tyrosine-phosphorylated proteins. Protein yields obtained by methods A, B, C and D were 100±5, 123±6, 1202±28 and 1301±32 μg, respectively. (B) Two-dimensional electrophoresis (2-DE) was used to confirm the effect of the above strategies. Protein spots (50±5, 70±15, 200±38 and 238±43) were detected on the 2-DE gels of methods A, B, C and D, respectively.

mass spectrometer was operated in a data-dependent mode to automatically switch between MS and MS/MS acquisition. Survey MS spectra (m/z 400-1800) were acquired in the QTOF, and the four most intense ions in each survey scan were fragmented and analyzed. Proteins were identified by automated database searching (Spectrum Mill; Agilent Technologies UK Ltd., Wokingham, UK) and MASCOT (Matrix Science, London, UK), of all MS and MS/MS spectra using the IPI Human, Swiss-Prot and NCBinr databases. Raw data files were converted to .pkl files by the Protein Lynx Global Server (PLGS; Waters Corp.). Search parameters were set as follows: MS accuracy, 0.15 Da; MS/MS accuracy, 0.15 Da; two missed cleavage allowed. Variable carbamidomethyl modification of cystine and variable oxidation of methionine, and all entries of the databases were searched.

Results

Induction and purification of GST-Nck1-SH2. The first key step of our study was to induce and purify the GST-Nck1-SH2 fusion proteins. As shown in Fig. 1A, following induction with isopropyl-β-D-1-thiogalactopyranoside (IPTG), the level of GST-Nck1-SH2 fusion proteins notably increased. To confirm the specificity of tyrosine-phosphorylated proteins which are the 'prey' proteins that are pulled down by by GST-Nck1-SH2, we performed negative control experiments with GST-4T3, under the same conditions. The comparison between GST-4T-3 and GST-Nck1-SH2 is shown in Fig. 1B. These results demonstrated that the induction and purification of GST-Nck1-SH2 fusion proteins were successful.

Comparison of different strategies for capturing tyrosine-phosphorylated proteins by the GST-Nck1-SH2 pull-down method. In order to yield sufficient tyrosine-phosphorylated proteins by GST-Nck1-SH2 pull-down, we explored different pull-down strategies to detect the 'prey' proteins in liver tissues (Table I). According to the general guidelines of GST pull-down in cells, we employed 100 μl GST-Nck1-SH2 fusion proteins to pull down the 'prey' tyrosine-phosphorylated proteins among 1 mg liver proteins in method A. However, in method B, we optimized the quantity of GST-Nck1-SH2 fusion protein to 200 μl; while in methods C and D, 10 and 20 mg of liver protein was added, respectively. Protein yields pulled down by methods A, B, C and D were 100±5, 123±6, 1202±28 and 1301±32 μg, respectively. As demonstrated in Fig. 2A, method C (100 μl fusion protein/10 mg liver protein) and method D (100 μl fusion protein/20 mg liver protein) were able to markedly increase the protein accounts, while methods A and B yielded insufficient quantities of protein for downstream tests.

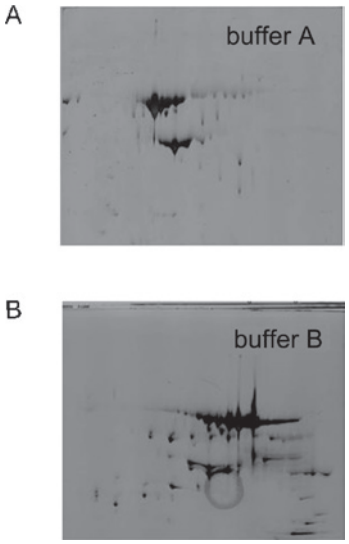

Figure 3. Comparison of different types of lysis buffer for dissolving proteins. The sample result from GST-Nck1-Src homology 2 (SH2) pull-down was dissolved by lysis buffer A (8 M urea, 4% CHAPS, 50 mM DTT) and B (7 M urea and 2 M thiourea, 4% CHAPS, 50 mM DTT). Subsequently, 100 μg protein was separated over a pH range of 3.0-10.0 (24-cm strips) and 12.5% SDS-polyacrylamide gel. The gel was visualized by silver staining. (A) Two-dimensional (2-D) gel result from lysis buffer A; 89±40 protein spots. (B) 2-D gel result from lysis buffer B; 200±38 protein spots.

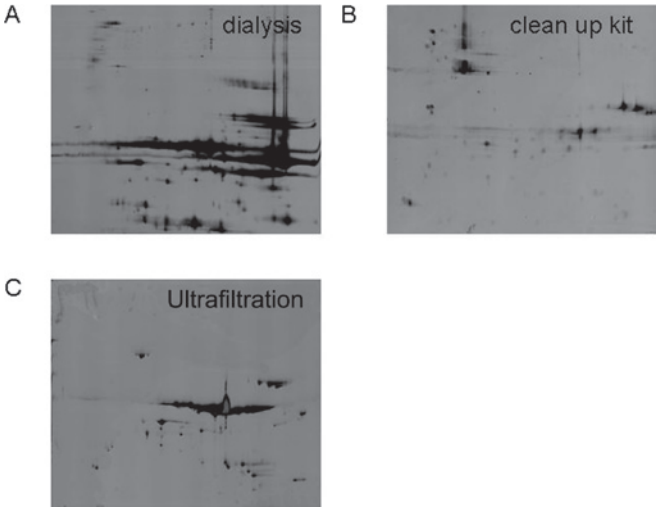

Figure 4. Comparison of depletion efficiency for interferential materials by two-dimensional electrophoresis (2-DE). A mini dialysis kit, a 2-D clean-up kit and ultrafiltration were employed to deplete the interferential materials. Subsequently, 100 μg protein, following deletion, were loaded onto 2-DE gels. For each gel, 100 μg protein was separated over a pH range of 3.0-10.0 (24-cm strips) and 12.5% SDS-polyacrylamide gel. The gel was stained with silver. (A) 2-D gel for the mini dialysis kit depletion shows horizontal streaks and blurry protein spots. (B) 2-D gel for the 2-D clean-up kit depletion has a limited number of remaining protein spots. (C) 2-D gel result from ultrafiltration depletion has 210±18 protein spots.

We set out to further investigate the effect of the previous strategies; samples pulled down by the aforementioned methods were loaded to 2-DE gels. As shown in Fig. 2B, protein spots of method A, B, C and D were 50±5, 70±15, 200±38 and 238±43, respectively. The 2-D gel results for methods C and D contained more protein spots than methods A and B; however, the 2-D gel for method D exhibited horizontal streaks and blurry protein spots. Additionally, the 2-D gel for method B exhibited only large GST beads. Overall, method C was selected for further study.

Comparison of different types of lysis buffer for dissolving proteins. Commonly, there are two types of lysis buffer for dissolving proteins. The standard cocktail contains 8 M urea (chaotropic), 4% CHAPS (detergent) and 50 mM DTT (lysis buffer A); while the other buffer contains 7 M urea and 2 M thiourea (lysis buffer B) in place of 8 M urea. The latter buffer type is capable of increasing the solubility of certain proteins and producing more spots, which is consistent with our results: Buffer A, 89±40 protein spots; buffer B, 200±38 protein spots (Fig. 3A and B). Overall, buffer B was used for further sample preparation.

Comparison of different methods for depleting the interferential materials. The resulting samples from GST-Nck1-SH2 pull-down include different types of material that affect the success of IEF. Therefore, we selected three types of typical methods for deleting the interferential materials to explore. These methods included a mini dialysis kit (8 kDa, Amersham Biosciences Corp.), a 2-D clean-up kit (Amersham Biosciences Corp.) and ultrafiltration (10 kDa, Millipore, Biosciences, NJ, USA). To delete the interferential materials, the samples must be loaded onto 2-DE gels. As shown in Fig. 4A, the 2-D gel for the mini dialysis kit was of poor quality, with horizontal streaks and blurry protein spots, suggesting that IEF had failed due to an incomplete removal of salts and GST fragments by the kit. However, the 2-D clean-up kit removed the fragments of the GST beads effectively, while also cleaning the majority of the tyrosine-phosphorylated proteins (Fig. 4B); only a few protein spots remained following depletion. As shown in Fig. 4C, 210±18 spots were detected after ultrafiltration depletion. Overall, ultrafiltration was chosen to be further tested.

Negative control of the GST-Nck1-SH2 pull-down procedure. An appropriate control experiment should be conducted during the GST-Nck1-SH2 pull-down procedure. In the present study, we performed a negative control experiment by the use of GST-4T3. The proteins pulled down by GST-Nck1-SH2 fusion or GST-4T3 underwent removal of the interferential materials by ultrafiltration, and were then loaded onto 2-DE gels under the same conditions. As shown in Fig. 5, the 2-D gel for GST-Nck1-SH2 separated 200±38 protein spots while that of GST-4T3 possessed GST fragments and few protein spots.

Proteins obtained using our method interacted with Nck in a tyrosine phosphorylation-dependent manner. To demonstrate whether the proteins that were pulled down by our method interacted with Nck in a tyrosine phosphorylation-dependent manner, we firstly identified two proteins by MALDI-TOF/TOF MS [Fig. 5B; lane 1, dermcidin (DCD); lane 2, engulfment and cell motility proteins (Elmo1)]. These results were published in our previous study (10).

Co-immunoprecipitation (co-IP) experiments were used to examine the correlation between endogenous Elmo1/DCD and Nck in SK-HEP-1 cells. As demonstrated in Fig. 6A, DCD was detected by anti-DCD antibody in the anti-Nck immunoprecipitate, but not in the precipitate obtained by IgG. In Fig. 6B,

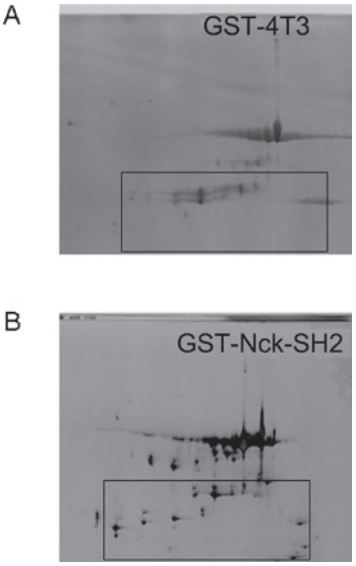

Figure 5. Two-dimensional (2-D) gel of GST-4T-3 and GST-NCK-Src homology 2 (SH2). For each gel, 100 μg protein were separated over a pH range of 3.0-10.0 (24-cm strips) and 12.5% SDS-polyacrylamide gel. The gel was visualized by silver staining. 2-D gel of (A) GST-4T3 control and (B) GST-Nck1-SH2. The rectangle emphasizes the different protein patterns in the two groups; the 2-D gel of GST-4T3 has numerous GST fragments and few protein spots while that of GST-Nck-SH2 has 200±38 protein spots.

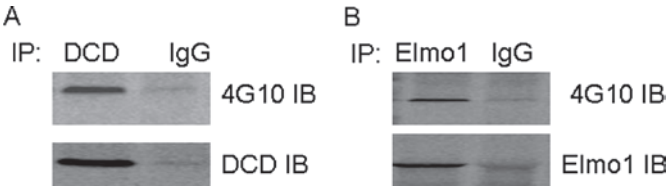

Figure 6. Coimmunoprecipitation was used to confirm the interaction of dermcidin (DCD) and Nck1, engulfment and cell motility proteins (Elmo1) and Nck1, in a tyrosine phosphorylation-dependent manner. SK-HEP-1 cell lysates were immunoprecipitated with an anti-Nck1 antibody or IgG as a control, followed by anti-DCD or anti-Nck immunoblot. (A) DCD was detected by anti-DCD antibody in the anti-Nck1 immunoprecipitate, but not in the precipitate obtained by IgG. (B) Elmo1 was detected by anti-Elmo1 antibody in the anti-Nck immunoprecipitate, but not in the precipitate obtained by IgG.

Elmo1 was detected by anti-Elmo1 antibody in the anti-Nck immunoprecipitate, but not in the precipitate obtained by IgG. Subsequent tests used to demonstrate that the proteins interact with Nck in a tyrosine phosphorylation-dependent manner have been published in our previous study (10).

Discussion

Given the importance of signaling mediated by tyrosine-phosphorylated proteins, there is significant interest in strategies to define or profile the global state of tyrosine phosphorylation in the cell (11). A limited number of studies have focused on tyrosine-phosphorylated proteins in tumor tissues thus far, although these regulate many important cancer-related activities, including cell proliferation, survival, invasion/metastasis and angiogenesis (12). Therefore, profiling the global state of tyrosine-phosphorylated proteins in a tumor is likely to provide a wealth of information that may be used to classify tumors for prognosis and prediction (13). To detect the state of tyrosine-phosphorylated proteins in tumor tissues, we selected liver tissue from HCC patients for further study. HCC is one of the most common and aggressive human malignancies (14), and is also regulated by tyrosine-phosphorylated proteins.

Machida *et al* demonstrated that SH2 binding methods may serve as a valuable complement in large-scale proteomic analyses (6). GST pull-down is an important tool for the validation of suspected protein-protein interactions, or for identifying novel protein interactions. 2-DE is one of the most commonly used methods in proteome analysis and classified tumors (15). Given this background, we employed GST-Nck1-SH2 pull-down to detect tyrosine-phosphorylated proteins in liver tissues, and then combined this with 2-DE to separate the proteins.

Although 2-DE is a powerful way to separate proteins for proteomics analysis, it presents a challenge for sample preparation (16). Based on the present study, the major barriers include the quantity of protein, depletion of interferential materials and certainty that the proteins obtained by GST-Nck1-SH2 pull-down are tyrosine-phosphorylated proteins.

Protein amounts. Yielding sufficient proteins is the initial step required for 2-DE, as the protein concentration of the loading sample for silver-stained 2-DE gels should not be <0.5 μg/μl (17). To capture sufficient tyrosine-phosphorylated proteins by GST-Nck1-SH2 pull-down, we explored four different methods. The first method involved following the guidelines for GST pull-down in cells. However, our results demonstrated that this method was not suitable for tissue samples as it only yielded a limited number of tyrosine-phosphorylated proteins. Subsequently, we optimized the quantity of GST-Nck1-SH2 particles or liver proteins in methods B, C and D, respectively. The quantity of tyrosine-phosphorylated protein in methods C and D markedly increased. We further confirmed the effect of the four different strategies by 2-DE. Overall, our results demonstrated that protein accounts obtained by GST pull-down were dependent on the ratio of GST-Nck1-SH2 fusion proteins to liver proteins. Only with an appropriate ratio (method C) are ideal accounts obtained, employing a greater quantity of liver proteins (method D) or fusion proteins (method B) did not lead to a greater amount of tyrosine-phosphorylated proteins being obtained. By contrast, using a large excess of particles or liver proteins would result in non-specific interactions between the proteins and particles.

Protein denaturization is another key step of 2-DE that is often achieved by the addition of chaotropic agents, such as urea and thiourea. Variations in the components and concentration of chaotropic agents markedly affect protein amounts and patterns. Thiourea is known to be able to increase the solubility of certain proteins and produce more protein spots, which is consistent with the present results.

Depletion efficiency and compatibility for downstream 2-DE. 2-DE is often limited by the presence of non-protein impurities in the samples. Excess salts originate from sample preparation and may render the solution too conductive for effective IEF. The samples resulting from GST-Nck1-SH2 pull-down included iron in the wash buffer and GST particle fragments, all of which negatively impact IEF. Therefore, depletion of such interferential material is an important step to insure successful IEF. To ensure

samples pulled down by GST-Nck1-SH2 may be used for downstream proteomics studies, we selected three common methods, acetone, a 2-D clean-up kit and ultrafiltration, to deplete the aforementioned materials, and then loaded the samples onto 2-DE gels.

To evaluate the depletion method, the depletion efficiency and the protein yield post-depletion were assessed. Dialysis is a simple and straightforward technique to de-salting with a dialysis membrane. The capped tube with the sample is inverted in a stirred beaker containing the solution against salts and molecules smaller than the molecular weight cut-off of the dialysis membrane exchange. However, our results demonstrated that dialysis could not be used for present study. A possible reason for this is that the samples resulting from GST-Nck1-SH2 pull-down included the majority of the GST bead fragments, which would block the dialysis membrane and result in failure of dialysis.

The 2-D clean-up kit is the classical depletion kit and it may be used to prepare proteins from sources that are diluted, and that contain high levels of salt and other interfering substances. However, our study demonstrated that this method was not suitable for tyrosine-phosphorylated proteins as a limited number of protein spots remained following depletion. We suggest that the reason for this finding is that the 2-D clean-up kit applies chemicals to the precipitant proteins, and the tyrosine-phosphorylated proteins require a tender method in order to retain their activity, thus any type of chemical modification would result in a loss of activity.

Ultrafiltration is a mild method for desalting and removal of materials of low molecular weight by centrifugalization that does not require a phase change. Thus, it is able to maintain the activity of tyrosine-phosphorylated proteins effectively. Overall, we suggest that ultrafiltration is a more appropriate method compared with the other methods.

Confirmation that the proteins obtained by GST-Nck1-SH2 pull-down are tyrosine-phosphorylated proteins. The disadvantage of GST-4T3 pull-down is the interferential interaction by any non-specific or non-covalently bound proteins. To eliminate false positives resulting from non-specific interactions, we performed a negative control experiment using GST-4T3 during the pull-down procedure, and subsequently loaded the samples pulled down by both GST-4T3 and GST-Nck1-SH2 onto 2-DE gels. Our results demonstrated that the 2-D gel for GST-Nck1-SH2 harvested more protein spots than that of GST-4T3.

We further identified DCD and Elmo1 by MALDI-TOF/TOF MS on the 2-D gel and demonstrated that the proteins pulled down by our method, which interacted with Nck in a tyrosine phosphorylation-dependent manner, were either DCD or Elmo1. As the SH2 domain is a small, modular protein domain that binds specifically to tyrosine-phosphorylated peptide ligands, we suggest that the present strategy is effective for identifying novel SH2 domains associated with phosphorylated proteins in tumor tissues.

In summary, we have optimized the GST-Nck1-SH2 pull-down procedure to obtain tyrosine-phosphorylated proteins in tumor tissues, and the sample preparation for downstream 2-DE. Moreover, the successful identification of protein spots by MALDI-TOF/TOF MS and the proteins pulled down by our method, which interacted with Nck in a tyrosine phosphorylation-dependent manner, demonstrated that GST-Nck1-SH2 pull-down combined with 2-DE is an effective molecular diagnostic approach to identifying novel SH2 domains associated with phosphorylated proteins in tumor tissue, thus facilitating future research.

Acknowledgements

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