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Development of Deuterated-leucine Labeling with Immunoprecipitation to Analyze Cellular Protein Complex

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Abstract

The deuterated-leucine (Leu-d_3) labeling is one kind of stable isotope labeling by amino acids in cell culture (SILAC), which has been widely used to compare and quantify protein relative expression. We expanded an integrated immunoprecipitation (IP) coupled with SILAC approach (SILAC-IP) to differentiate the specific binding partners associated with a bait protein in two populations of cells. By this SILAC-IP strategy, the identified specific-binding proteins were quantified by tracking pairs of Leu-d_3 labeled and unlabeled peptides from the mass spectra, which could differentiate specific-binding proteins from nonspecific partners in high confidence. We applied SILAC-IP method to differentiate specific-binding proteins associated with 14-3-3â in human hepatocellular carcinoma cell line QGY7703 between those in the liver cell line QSG7701. The proteins including HSP86, SKB1hs, GADPH and MEP50 were identified to associate with 14-3-3â in QGY7703 with high binding level than in QSG7701 cells.

Keywords: Deuterated-leucine (Leu-d_3) labeling; Stable isotope labeling by amino acids in cell culture (SILAC); Mass spectrometry; Immunoprecipitation; Protein complex

Introduction

The deuterated-leucine (Leu-d_3) labeling is one kind of stable isotope labeling by amino acids in cell culture (SILAC), which is a simple and accurate approach to compare and quantify protein relative expression based on mass spectrometry (MS) in cell system (Ong et al., 2002). In this method, one cell population is grown in medium containing normal amino acids, while another population is cultured in non-radioactive Leu-d_3 labeling medium lacking corresponding normal amino acid leucine (Leu-d_0). The Leu-d_3 labeling cells and the normal control cells are equally combined to extract proteins to digest and run MS analysis, then the pair of Leu-containing peaks appears with a mass split of 3n/z (n represented the number of Leu in the peptide, and z was the number with the charges of the peptide). One set of heavy isotope peaks is derived from Leu-d_3 labeling cells, while the other set of light isotope peaks comes from normal Leu-d_0-containing cells. And the peak intensity of heavy and light forms can reflect the different expression of proteins in the two groups of cells.

Now, SILAC strategies have been successfully applied in many fields of life sciences due to its advantages in quantitation, such as in identification of lipid raft proteins (Foster et al., 2003), signal-dependent proteins (Blagoev et al., 2003) and prostate cancer research (Everley et al., 2004) etc. The advantages of Leu-d_3 labeling compared with other stable isotope labeling were mainly included in the following (Mann, 2006). (1) Leu-d_3 labeling is one kind of metabolic labeling, with relative high-abundance of leucine in living cells. As we know, leucine is the most abundant amino acid to allow about 50% tryptic peptides containing Leu amino acid, which is more accurate and sensi-

tive for quantification. (2) The Leu-d_3 labeling can distinguish effectively between isoleucine and leucine, compared with other essential amino acid lebelling, such as lysine, methionine or tyrosine-labeling. (3) Compared with the chemical labeling, such as ICAT, SILAC method allows mixing of labelled with unlabelled cells, and therefore subsequent fractionation and purification steps will not introduce any errors in quantitation. (4) Mammalian cell lines are easily labelled by providing the SILAC amino acid with high labelling degree, and the quantitation is straightforward in the MS. Recently, SILAC method coupled with immunoprecipitation (IP) was used to identify proteins interacting in an attachment-dependent manner with focal adhesion proteins under adhesion and detachment conditions (de Hoog et al., 2004), which was applied in different conditions for one same cell line.

In this report, we expanded SILAC and IP method to differentiate specific-binding proteins associated with bait protein between two cell lines. To validate the methodology, we applied it to compare 14-3-3β complex in human hepatocellular carcinoma cell line QGY7703 between the liver cell line QSG7701 by this SILAC-IP strategy. 14-3-3β is a member of 14-3-3βprotein family in all eukaryotic cells which has seven isoforms named β,γ,ε,η,σ,θ and ζ (van Heusden, 2005). 14-3-3β was high expression in some tumor cell lines and human lung cancer tissues (Qi et al., 2005), and reduction of 14-3-3β could suppress tumor cell growth in vitro and in vivo (Sugiyama et al., 2003), which shows14-3-3β has oncogenic potential (Takihara et al., 2000). QGY7703 and QSG7701, which derived from primary liver cancerous and para-cancerous tissues respectively, have different biological characteristics (Wang, 1981; She et al., 1991; Zhu and Wang, 1979). QGY7703 is a liver cancer cell line, while QSG7701 is regarded as a normal liver cell line. In order to show the feasiblity of using SILAC method to identify the changes of protein complex that may correlate with liver carcinogenesis between two cell lines, we applied this technique to the analysis of specific members of protein complex associated with 14-3-3β in QGY7703 cells. The stable isotope Leu-$d_3$labeling was taken as internal markers for quantitative protein interactors because the ratio of the Leu-d_3 labeling isotopic peaks versus the normal Leu-$d_0$isotopic peaks derived from a same peptide was refelected the binding sepecificity status of partners associated with the bait. Theoretically, a heavy versus light isotope enrichment ratio significantly greater than 1 corresponds to an increased abundance of a particular protein around the bait, while the concentration of non-specifically distributed proteins surrounding the bait protein remains at similar level. This in-spectra quantitative markers of amino acid tags coupling with IP method can precisely identify those genuine interacting partners with minimum requirement of validation using other molecular/cellular approaches.

Materials and Methods

Cell culture and leu-d_3 labeling

Hepatocellular carcinoma cell line QGY7703 and liver cell line QSG7701 were obtained from the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences of China (Wang et al., 1981; Zhu and Wang, 1979). QSG7701 and QGY7703 cells were maintained with the media RPMI1640 containing 10% fetal bovine serum, and incubated at 37°C in a humidified atmosphere with 5% CO_2.

As for Leu-d_3 labeling for QGY7703, QGY7703 cells were cultured with Leu-d_3 labeled RPMI1640, in which only the Leu-d_3 amino acid (Cambridge Isotope Laboratories) replaced the normal Leu component, and 10% dialyzed fetal bovine serum was added. The labeling QGY7703 cells were used for SILAC-IP manipulation. Cells were harvested when they became 80%-100% confluence. The collected cell pellets were first washed with phosphate-buffered saline (PBS), then collected by centrifugation at 1200 rpm for 3 min at 4°C, and washed twice in PBS. After centrifugation, the supernatant was removed and the cell pellet was stored at -80°C until further use.

Protein extraction

10^6-10^7 harvested cells were mixed with 1 ml of lysis buffer (50 mM Tris-HCl, pH7.4, 150 mM NaCl, 1% NP-40) supplemented with 10μl protease inhibitor cocktail (Sigma #P-8340). The cells were sonicated on ice by 10 sec pulses of sonication for 3 min, then the cell lysate was centrifuged for 25 min at 12000×g , and the supernatant was collected for further use.

Immunoprecipitation

The 14-3-3β polyclonal antibody agarose suspension (Santa Cruz , sc-629) was washed with PBS by centrifuge for two times, then 6-8 ml protein extraction was added into 10 μl packed agarose (i.e., 20 μl original suspension) and incubated at 4°C overnight with mixing. The agarose pellet was collected by centrifugation at 2,500 rpm for 2 min at 4°C, and gently washed 2–3 times with 1.0 ml RIPA buffer

(10 mM Tris-Cl [pH 8.0], 1 mM EDTA, 0.5 mM EGTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS, 140mM NaCl), each time repeating centrifugation step above. After final wash, the pellet was resuspended in 30 µl of 2×electrophoresis sample buffer (sc-24945) to boil samples for 2–3 min, and the supernatant was subject to SDS-PAGE analysis.

For conventional IP, the protein mixture extracted from normal QSG7701, QGY7703 cells respectively was performed IP separately. Following 1-D SDS-PAGE separation of complex components, the bands differentially visualized by silver staining between QGY7703 and QSG7701 samples were determined as the 14-3-3β-specific interactors in QGY7703 cells to identify by MS. As for SILAC-IP experiments, same amount of proteins from normal QSG7701 cells and labeling QGY7703 cells were mixed to perform immiunoprecititation as the above described methods. The heavy versus light isotope enrichment ratio significantly greater than 1 was defined as specific binding proteins, while the ratio near or below 1 meaned that the protein is nonspecifically associated with the 14-3-3β bait protein.

Sample preparation for MS analysis

Proteins were separated by 12% SDS-PAGE and visualized by silver staining. Silver-stained bands were excised, and in-gel digestion was performed mainly as described by Shevchenko et al. (1996) (Shevchenko et al., 1996), with some modification. The gel slices were destained with the mixture of 15 mM $K_3Fe(CN)_6$ and 50 mM $Na_2S_2O_3$, and washed with deionized water for 3 times. The pieces were then dehydrated with acetonitrile (ACN) for 2-3 times. The dried slices were digested with 12.5 ng/µl of sequencing grade, modified trypsin in 20 mM ammonium bicarbonate overnight at 37° C . Following digestion, tryptic peptides were extracted twice with 50% ACN/ 0.1% trifluoroacetic acid (TFA) for 15 min each time with moderate sonication. The extracted solutions were pooled and evaporated to dryness under vacuum. The dry peptide samples were redissolved in 0.1% TFA.

Protein identification and quantification by MALDI-TOF/TOF-MS

The peptide samples were spotted onto the MALDI target plate mixing with a saturated matrix solution of α-cyano-4-hydroxycinnamic acid (CHCA). The 4700 Proteomic Analyzer TOF/TOF (Applied Biosystems, Framingham Boston, MA, USA) was used to identify the unknown proteins by peptide mass finger-printing (PMF) or MS/MS analysis. Mass spectra were acquired for the mass range of 700-3200 Da by the 4700 Explorer and searched by the GPS Explorer (containing the MASCOT as a search engine), PMF mass tolerance was set as 0.2 Da with mass filter of S/N20 and MS/MS tolerance was set as 0.3 Da with mass filter of S/N 10.

Identified proteins were quantified by tracking pairs of labeled and unlabeled peptides from the MS spectra, and it required at least a Leu-containing peptide to quantify. Protein abundance was calculated as ratios of the peak intensity of the fragment ions from the labeled *versus* the unlabeled peptides. Ratios were calculated from the average of all quantified peptides for a single protein.

Semiquantitative RT-PCR

To confirm the quantitative binding proteins, one higher binding protein GAPDH in QGY7703 was chosen to do semiquantitative RT-PCR in cells to validate its expression level. Total RNA was extracted from cell samples using Trizol regent (Invitrogen). 1 µg of total RNA was used as templates for RT-PCR assay with 10pmol of both forward and reverse primers. The forward primer was 5'-TCA TCT CTG CCC CCT CTG-3', and the reverse primer was 5'-CCT GCT TCA CCA CCT TCT TG-3'. The RT-PCR amplication for GAPDH was performed as follows: reverse transcription at 45 ºC for 45 min, initial PCR activation step at 95 ºC for 2 min, 28 cycles at 94 ºC for 30 s, 58 ºC for 45 s, 72 ºC for 30 s and a final extension cycle of 72 ºC for 5 min. 5µl of PCR products were resolved by electrophoresis in 1.2% agarose gels, stained with Goldview(Sigma), and visualized by UV fluorescence. The β-actin was performed parallelly and taken as the control.

Results

Leu-d_3 labeling detection

The ratio of Leu-d_3 labeling was gradually raised within the cell growth extension in Leu-d_3 labeled medium.The labeling ratio, also incorporation rate of Leu-d_3, was defined as the ratio of peak intensity of Leu-$d_3$labeling *versus* the sum of Leu-d_3- and Leu-d_0–labeling peak intensity from proteins. The housekeeping gene β -actin was always selected to monitor the labeling status. It was about 44% cells labeled after first passage based on the ratio of isotope peaks (m/z 1793, m/z 1790) from the peptide 'SYELPDGQVITIGNER' of actin (Figure 1A). The incorporation rate of Leu-d_3 was about 64% after three

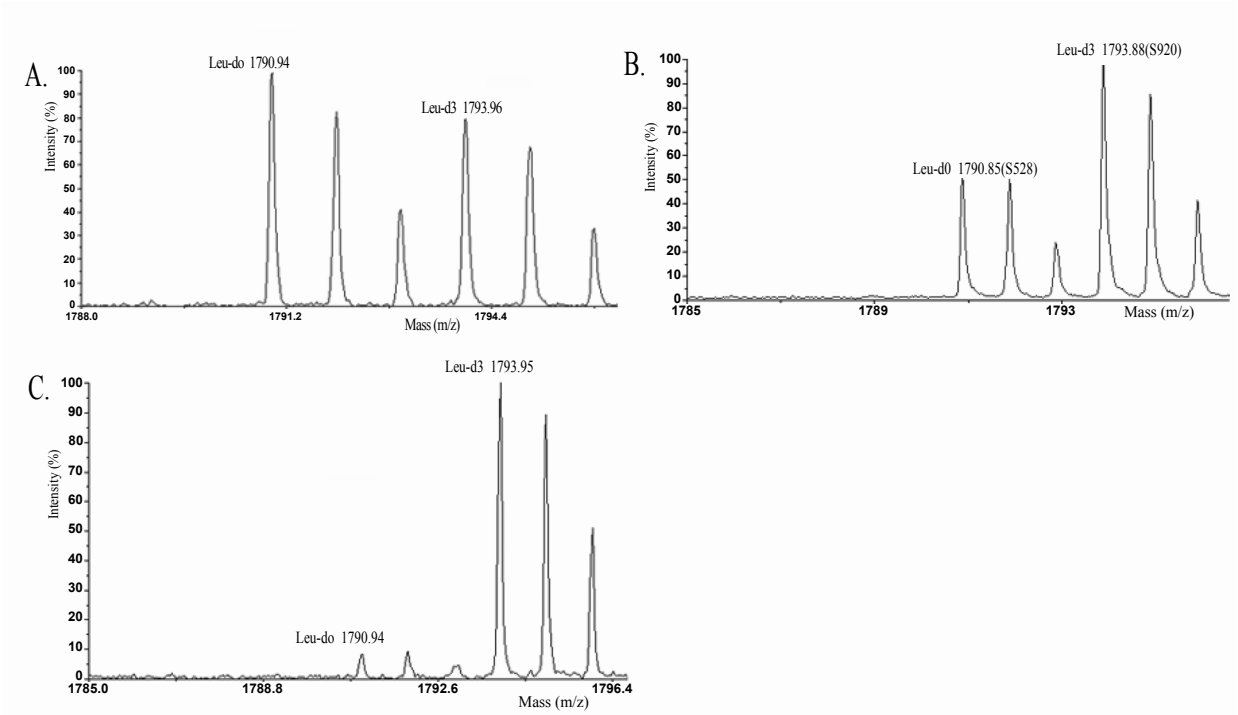

Figure 1: The incorporation rate of Leu-d_3 in actin at various cell passages. Almost complete incorporation of Leu-d_3 in actin after five growth passages (C). The m/z 1793 and 1790 represented respectively the Leu-d_3 labeling and Leu-d_0 non-labeling peptide in actin. A, B and C respectively represented Leu-d_3 labeling status after 1, 3 and 5 cell passages.

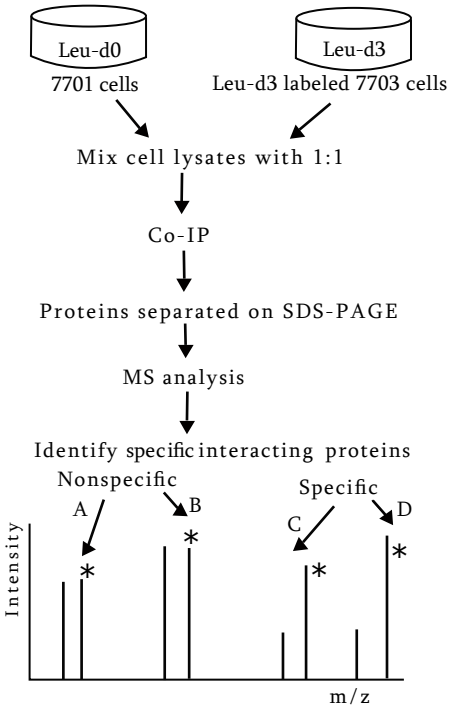

Figure 2: The strategy of immunoprecipitation in combination with SILAC method to analyze proteins specifically associated with 14-3-3β in QGY7703 cells. The same amount of proteins from Leu-d_3-labeling QGY7703 and normal QSG7701 cells were mixed to perform immunoprecipitation. And the protein complex was isolated on SDS-PAGE. The bands were cut to identify by MS. Proteins specifically bound with 14-3-3 βin QGY7703 cells were distinguished by the increased intensity of labeled mass peaks (C-D) from samples cultured in Leu-d_3-containing media. The " * " represented the Leu-d_3-labeling peaks from QGY7703 cells.

cell passages (Figure 1B). Leu-d_3 labeling was accessed to over 95% (Figure 1C), which indicated Leu-d_3 was almost completely incorporated into QGY7703 cells after five cell passages. This was one example to show Leu-d_3 labeling in actin. Generally, several different Leu-d_3-containing peptides were quantified in our experiments.

Immunoprecipitation and protein identification

The general strategy by SILAC-IP to identify proteins specifically associated with 14-3-3βin QGY7703 cells was illustrated in figure 2. Equal amount of cellular proteins extracted from QGY7703 cells with Leu-d_3 labeling and that from normally cultured QSG7701 cells was mixed uniformly. Then the protein mixture was incubated with the beads of anti-14-3-3β antibody, and the protein complex associated with 14-3-3β was eluted by boiling from the beads. The visible bands were cut and identified by MALDI-TOF/TOF MS analysis. In the MS spectrum, the intensity ratio of heavy isotope peaks *versus* its counterpart of light isotope peaks (SILAC ratio) can reflect the binding profile of the identified protein to the bait 14-3-3β. Theoretically, if the ratio is significantly larger than 1, it means the protein binds specifically with the bait in QGY7703 cells. Otherwise, the ratio with 1 means that the protein is nonspecifically associated with the beads coupled with 14-3-3β antibody.

Based on this criteria, the proteins of heat shock protein 86 (HSP86), SKB1hs, MEP50 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were specifically associated with 14-3-3β in QGY7703 due to its SILAC ratio significantly higher than 1. The SILAC ratio was respectively 5.7, 1.9, 1.75, 3.7 for HSP86, SKB1hs, MEP50 and GAPDH. While the β-actin, with isotope labeling intensity ratio of 0.9, was identified as a nonspecific binding protein with 14-3-3β in QGY7703, and the band of the IgG of 14-3-3β antibody with the isotope labeling intensity ratio was zero, which indicated that it was exogenous from the degradation of agarose-coupled 14-3-3β antibody. The representative quantification peptides were shown in figure 3. In addition, the expression level of 14-3-3β bait protein was similar between QSG7701 and QYG7703 cells based on the SILAC ratio of 14-3-3β in the protein complex, which was consistent with that 14-3-3β mRNA expression was near to same levels in these two cell lines by RT-PCR analysis (data not shown).

As for the separate conventional IP manipulation, proteins extracted from QGY7703 and QSG7701 cells were performed parallelly to do IP respectively. Then same amount of protein complex purified from QGY7703 and QSG7701 cells were parallelly separated on SDS-PAGE, and 5 different bands from QGY7703 cells, which were stronger than those from QSG77017701 cells, were visible through silver-staining (Figure 4). The strong bands from QGY7703 cells were cut and digested for MS identification. Similarly, the band 1 to band 5 in figure 4 was respectively identified as HSP86, SKB1hs, â-actin , MEP50 and GAPDH, and the band a was identified as the IgG of antibody (data not shown).

Validation for GADPH expression by semiquantitative RT-PCR

The semiquantitative RT-PCR analysis for GADPH expression was shown that its RNA expression level in QGY7703 cells was higher than that of QSG7701 (data not shown), which was consistent with its MS identification for protein binding profiles by our SILAC-IP approach. While β-actin was no change in RNA level in QGY7703 and QSG7701 cells.

Comparison of detection accuracy between IP and quantitative SILAC-IP approach

The direct comparison of the sensitivity and accuracy for detecting the components was made for the complexes isolated through IP and SILAC-IP strategy respectively. In the conventional IP design, following a 1-D SDS-PAGE separation of complex components, the bands visualized more strongly by silver staining were determined as the 14-3-3β-specific interactors in QGY7703 cells(Figure 4). In addition to identification of one strong nonspecific IgG band from the anti-14-3-3β antibody beads(Figure 4, band a), here the identified proteins including HSP86, SKB1hs, β -actin, MEP50 and GAPDH were regarded as specific associating ones with 14-3-3β in QGY7703 according to the band strength different from that in QSG7701. But as presented above, in comparison, assisted by heavy amino acid tags (Leu-d_3) as the in-spectra quantitative marker, β-actin was identified as a nonspecific binding protein in QGY7703, while the other 4 proteins were also successfully identified to bind with 14-3-3β in high intensity ratio of heavy versus light isotope peaks. Therefore, the bait-specific interacting proteins can be unambiguously identified by our SILAC-IP method.

Discussion

The multi-protein complexes are emerging as important entities of biological activity inside cells that exert functional diversity by contextual combination of gene products, and

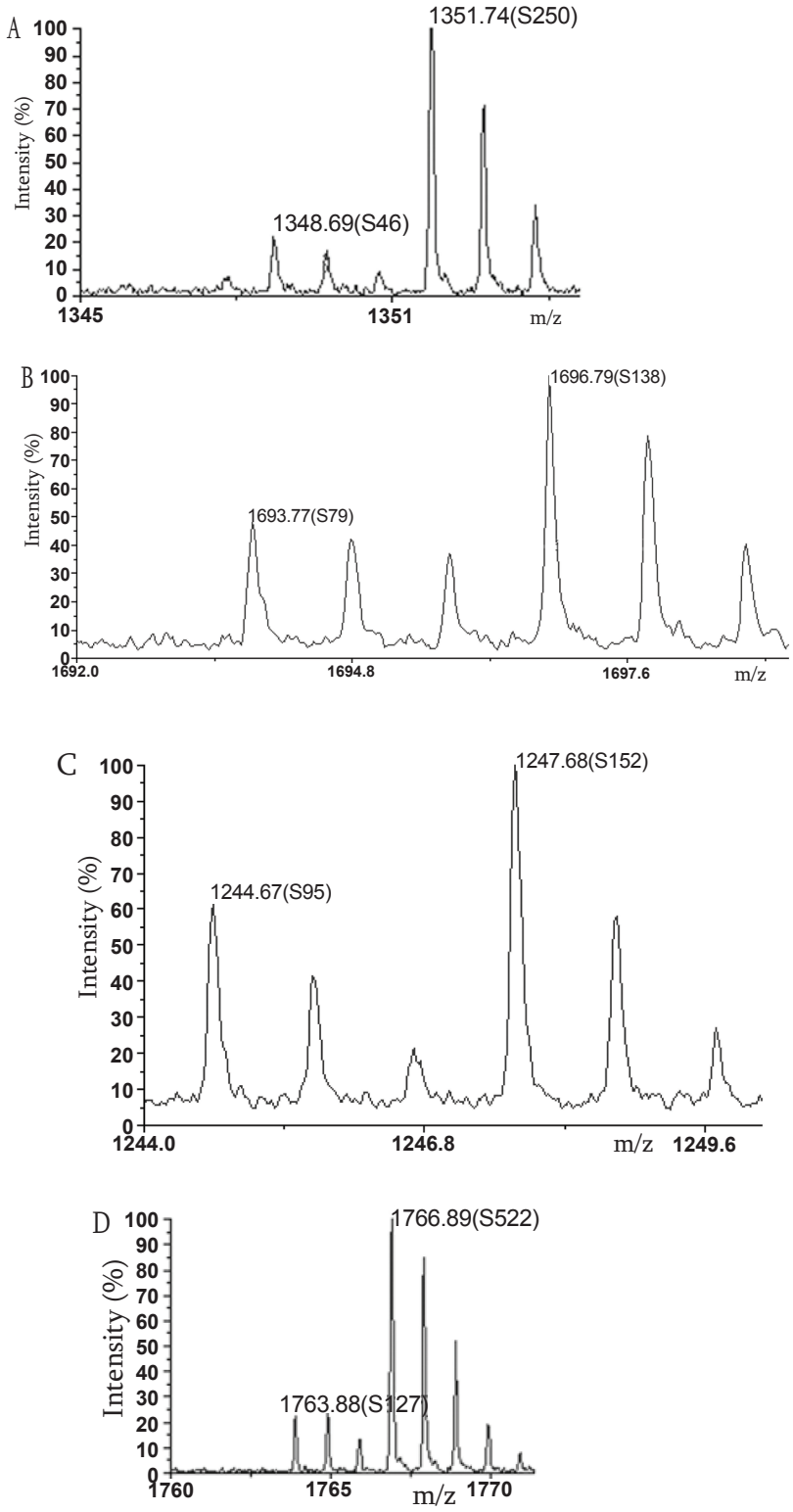

Figure 3: Mass spectra of several representative peptides from identified proteins of HSP86 (A), SKB1Hs(B), MEP50(C) and GAPDH(D) were used to quantify specifically binding proteins with 14-3-3β in QGY7703 cells. The pair of heavy and light isotope labeling peptides respectively came from QSG7701 and QGY7703 cells.

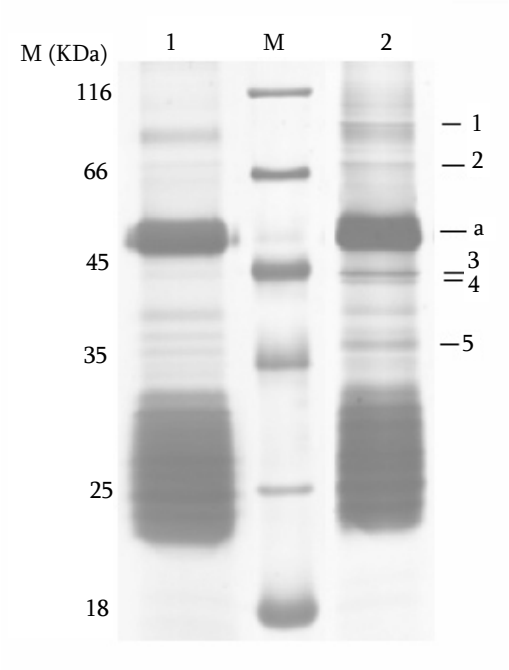

Figure 4: The protein complex associated with 14-3-3β by conventional immunoprecipitation was seprated on SDS-PAGE. Lane1, 2 was the sample respectively from QSG7701 and QGY7703 cells. M was the protein marker. The different band 1-5 was respectively identified as HSP86, SKB1hs, β-actin , MEP50 and GAPDH, and the band a was identified as the IgG of antibody.

organize several different proteins into functional units (Bauer and Kuster, 2003). Studying such complexes allows to place proteins into a functional context that is provided by their associated partners, which can help us understand protein functions more clearly. IP is a commonly used method to purify protein complexes, however, the main difficulty in IP was how to determine the nonspecific partners associated with antibody in large scale complex analysis. To solve this problem, we modified IP with the novel quantitative Leu-d_3 labeling to distinguish nonspecific binding proteins. The identified proteins specifically associated with the bait protein were quantified by tracking pairs of labeled and unlabeled peptides from the MS spectra, which can differentiate specific from nonspecific-binding proteins in high confidence. Our SILAC-IP method was used in two similar populations of cells, which expanded the application of SILAC-IP.

The SILAC-IP method was effectively integrated on the immunoprecipitation (IP) and SILAC, therefore any factors affecting IP or SILAC manipulation will influence the results in SILAC-IP. As for IP, the steric interference at the ligand binding site(s) due to antibody occupancy, and the washing procedures as well as elution steps were all probably result in capturing different specific-binding proteins with the bait. The antibody with good specificity and binding ability should be chosen for IP. In addition, the washing and elution steps will be optimized to allow the weak, transient and low-abundant binding partners to remain in the protein complex. In our experiments, due to the monoclonal antibody of 14-3-3β coupled with the agarose was not available in our IP experiment, therefore the commerical polyclonal 14-3-3β antibodies coupled with the agarose (Santa Cruz , sc-629) was chosen to perform IP, which showed several backgrounds in the conventional IP in the Figure 4. In addition, Because the protein complex combined with14-3-3β was eluted from the agarose by boiling, the IgG heavy and light chain of 14-3-3β antibody were also denatured and eluted from agarose. Therefore the strong IgG heavy chain appeared on SDS-PAGE and the denatured light chain was smeared with other protein bands. Future studies will also be needed to solve this issue by

using more mild elution methods such as acid elution with glycine.

Furthermore, the accuracy of the SILAC-based quantitative approach not only depends on the integrity of labeled cells, equal mixture of two group of proteins, but also the abundance and signal-to-noise ratio of the peptide pair (Ong and Mann, 2005; Mann, 2006). To maximize the number of proteins identified and quantified, MS sensitivity is also crucial, except for the quality of proteins, the use of materials, operation and so on. Therefore, we should adopt MALDI-TOF/TOF MS or ESI-LC/MS-MS based on the sample complexity to enhance the accuracy for protein identification and quantification.

Note that our aim was to expand SILAC technique into conventional IP to effectively distinguish nonspecific backgrounds from the really interacting partners among the protein complex. By means of the major advantage of in-spectra quantitative characterization through SILAC-MS, we can precisely identify those genuine interacting partners with minimum requirement of validation using other molecular/cellular approaches. Therefore, the SILAC-IP strategy can be widely applied in studying signaling pathways and molecular action mechanism of drugs, etc. Due to its capability to quantify both the proteome and its modifications in response to stimuli and perturbations, SILAC in combination with other biochemical method, such as IP, will become an important and powerful tools for discovering protein-protein interactions involving various functional pathways, action mechanisms of molecule (drug) intervention in system biology (Mann, 2006). This will provide a new insight for looking for novel targets for diagnosis and therapeutic intervention.

In this report, the proteins including HSP86, Skb1Hs, MEP50 and GAPDH were identified to bind with 14-3-3β in QGY7703 cells with high binding level than in QSG7701. The identification of specific protein-protein interactions will serve as a starting point for further understanding of the molecular mechanisms on liver carcinogensis. The HSP86 is one member of HSP90 family, and varys in response to signals for growth and development (Dale et al., 1997), therefore it is essential for cell viability under normal growth conditions. Skb1Hs, human Skb1 homolog, has been shown to associate with several different proteins in mammalian cells, including the tyrosine kinase JAK2, pICln etc (Krapivinsky et al., 1998). In addition, Skb1Hs and MEP50 are the components of the methylosome. MEP50 may function to mediate the interaction of multiple substrates with the methylosome, which plays important roles in the assembly of small nuclear ribonucleoprotein core particles and pre-mRNA splicing (Friesen et al., 2002). As for GAPDH, one key kinase in glycolysis, is reported to increased express in lung cancer (Tokunaga et al., 1987), prostate cancer (Epner and Coffey, 1996) and other cancer cell lines of human hepatocarcinoma and kidney carcinoma (Arcari et al., 1984) etc. It was found to bind with 14-3-3β in QGY7703 cells and its RNA expression level was also increased than that in QSG7701 cells, which indicates14-3-3β can involve in the increased glycolysis for cell growth through interaction with GAPDH in cancer cells. From these functional linkage among the identified interacting proteins in the proteomic datasheet, these proteins are potentially regulated by direct or indirect interactions with 14-3-3β and play important roles in cell growth and function in QGY7703 cells, which represents a new strategy to discover 14-3-3β functions by identifying biologically relevant protein complexes with 14-3-3β.

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References

1. Arcari P, Martinelli R, Salvatore F (1984) The complete sequence of a full length cDNA for human liver glyceraldehyde-3-phosphate dehydrogenase: evidence for multiple mRNA species. Nucl Acids Res 12: 9179-9189. » CrossRef » Pubmed » Google Scholar

2. Bauer A, Kuster B (2003) Affinity purification-mass spectrometry, powerful tools for the characterization of protein complexes. Eur J Biochem 270: 570-578. » CrossRef » Pubmed

3. Blagoev B, Kratchmarova I, Ong SE , Nielsen M, Foster LJ, et al. (2003) A proteomics strategy to elucidate functional proteinprotein interactions applied to EGF signaling. Nat Biotechnol 21: 315-318. » CrossRef » Pubmed » Google Scholar

4. Dale EC, Yang X, Moore SK, Shyamala G (1997) Murine 86-KDa heat shock protein gene and promotor. Cell Stress Chaperones 2: 87-93. » Pubmed » Google Scholar

5. de Hoog CL, Foster LJ, Mann M (2004). RNA and RNA binding proteins participate in early stages of cell spreading through spreading initiation center. Cell 117: 649-662.» CrossRef » Pubmed » Google Scholar

6. Epner DE, Coffey DS (1996) There are multiple forms of glyceraldehyde-3-phosphate dehydrogenase in prostate cancer cells and normal prostate tissue. Pros- 28: 372-378. » CrossRef » Pubmed » Google Scholar

7. Foster LJ, De Hoog CL, Mann M (2003) Unbiased quantitative proteomics of lipid rafts reveals high specificity for signaling factors. Proc Natl Acad Sci USA 100: 5813-5818. » CrossRef » Pubmed » Google Scholar

8. Friesen WJ, Wyce A, Paushkin S, Abel L, Rappsilber J, et al. (2002) A novel WD repeat protein component of the methylosome binds Sm proteins. J Biol Chem 277: 8243-8247. » CrossRef » Pubmed » Google Scholar

9. Krapivinsky G, Pu W, Wickman K, Krapivinsky L, Clapham DE (1998) pICln binds to a mammalian homolog of a yeast protein involved in regulation of cell morphology. J Biol Chem 273: 10811-10814. » CrossRef » Pubmed » Google Scholar

10. Mann M (2006) Functional and quantitative proteomics using SILAC. Nat Rev Mol Cell Biol 17: 952-958. » CrossRef » Pubmed » Google Scholar

11. Ong SE, Blagoev B, Kratchmarova I, Kristensen DB, Steen H, et al. (2002) Stable isotope labeling by amino acids in cell culture, SILAC, as a simple and accurate approach to expression proteomics. Mol Cell Proteomics 1: 376-386. » CrossRef » Pubmed » Google Scholar

12. Ong SE, Mann M (2005) Mass spectrometry-based proteomics turns quantitative. Nat Chem Biol 1: 252-262. » CrossRef » Pubmed » Google Scholar

13. Everley PA, Krijgsveld J, Zetter BR, Gygi SP (2004) Quantitative cancer proteomics:stable isotope labeling with amino acids in cell culture(SILAC) as a tool for prostate cancer research. Mol Cell Proteomics 3: 729-735. » CrossRef » Pubmed » Google Scholar

14. Qi W, Liu X, Qiao D, Martinez JD (2005) Isoform-specfic expression of 14-3-3 proteins in human lung cancer tissues. Int J Cancer 113: 359-363. » CrossRef » Pubmed » Google Scholar

15. Shen DW, Lu YG, Chin KV, Pastan I, Gottesman MM (1991) Human hepatocellular carcinoma cell lines exhibit multidrug resistance unrelated to MDR1 gene expression. J Cell Science 98: 317-322. » CrossRef » Pubmed » Google Scholar

16. Shevchenko A, Wilm M, Vorm O, Mann M (1996) Mass spectrometric sequencing of proteins from silver stained polyacrylamide gels. Anal Chem 68: 850-858. » CrossRef » Pubmed » Google Scholar

17. Sugiyama A, Miyagi Y, Komiya Y, Kurabe N, Kitanaka C, et al. (2003) Forced expression of antisense 14-3-3β RNA suppresses tumor cell growth in vitro and in vivo. Carcinogenesis 24: 1549-1559. » CrossRef » Pubmed » Google Scholar

18. Takihara Y, Matsuda Y, Hara J (2000) Role of the β isoform of 14-3-3 proteins in cellular proliferation and oncogenic transformation. Carcinogenesis 21: 2073-2077. » CrossRef » Pubmed » Google Scholar

19. Tokunaga K, Nakamura Y, Sakata K, Fujimori K, Ohkubo M, et al. (1987) Enhanced expression of a glyceraldehyde-3-phosphate dehydrogenase gene in human lung cancers. Cancer Res 47: 5616-5619. » CrossRef » Pubmed » Google Scholar

20. van Heusden GP (2005) 14-3-3 proteins: regulators of numerous eukaryotic proteins. IUMBM Life 57: 623-629. » CrossRef » Pubmed » Google Scholar

21. Wang JB (1981) Establishment and some characteristics of a hepatoma cell line (QGY-7703) (authors transl). Zhonghua Zhong Liu Za Zhi 3: 241-244. » Pubmed » Google Scholar

22. Zhu HD, Wang JB (1979) The culture of human liver cell line QSG7701 and its comparison with hepatoma cells. Cancer Res. Prevention & Treatment (Chinese) 5: 7-9.