FUT family mediates the multidrug resistance of human hepatocellular carcinoma via the PI3K/Akt signaling pathway

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The fucosyltransferase (FUT) family is the key enzymes in cell-surface antigen synthesis during various biological processes such as tumor multidrug resistance (MDR). The aim of this work was to analyze the alteration of FUTs involved in MDR in human hepatocellular carcinoma (HCC) cell lines. Using mass spectrometry (MS) analysis, the composition profiling of fucosylated N-glycans differed between drug-resistant BEL7402/5-FU (BEL/FU) cells and the sensitive line BEL7402. Further analysis of the expression profiles of the FUT family in three pairs of parental and chemoresistant human HCC cell lines showed that FUT4, FUT6 and FUT8 were predominantly expressed in MDR cell lines. The altered levels of FUT4, FUT6 and FUT8 were responsible for changed drug-resistant phenotypes of BEL7402 and BEL/FU cells both in vitro and in vivo. In addition, regulating FUT4, FUT6 or FUT8 expression markedly modulated the activity of the phosphoinositide 3 kinase (PI3K)/Akt signaling pathway and MDR-related protein 1 (MRP1) expression. Inhibition of the PI3K/Akt pathway by its specific inhibitor wortmannin, or by Akt small interfering RNA (siRNA), resulted in decreased MDR of BEL/FU cells, partly through the downregulation of MRP1. Taken together, our results suggest that FUT4-, FUT6- or FUT8-mediated MDR in human HCC is associated with the activation of the PI3K/Akt pathway and the expression of MRP1, but not of P-gp, indicating a possible novel mechanism by which the FUT family regulates MDR in human HCC.

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Multidrug resistance (MDR) frequently contributes to the failure of chemotherapeutic drug treatments in patients diagnosed with cancer such as hepatic carcinoma. The mechanisms underlying the MDR of cancer cells are complex, including increase in drug efflux, reduction in drug absorption, changes in the targets of anticancer drugs, decrease in drug activity, enhancement of DNA repair following damage, changes in signaling pathway and so on. Classic MDR is the consequence of overexpression of transporter proteins belonging to the ATP-binding cassette (ABC) family such as P-glycoprotein (P-gp) and MDR-related protein (MRP). Their function is to extrude antitumor agents from the cytoplasm, thus reducing intracellular drug concentrations to sublethal levels. Furthermore, the epithelial-to-mesenchymal transition (EMT) is also associated with drug-resistant phenotypes of BEL7402 and BEL/FU cells both in vitro and in vivo. In addition, regulating FUT4, FUT6 or FUT8 expression markedly modulated the activity of the phosphoinositide 3 kinase (PI3K)/Akt signaling pathway and MDR-related protein 1 (MRP1) expression. Inhibition of the PI3K/Akt pathway by its specific inhibitor wortmannin, or by Akt small interfering RNA (siRNA), resulted in decreased MDR of BEL/FU cells, partly through the downregulation of MRP1. Taken together, our results suggest that FUT4-, FUT6- or FUT8-mediated MDR in human HCC is associated with the activation of the PI3K/Akt pathway and the expression of MRP1, but not of P-gp, indicating a possible novel mechanism by which the FUT family regulates MDR in human HCC.

Glycan biosynthesis involves multiple glycosyltransferases in a complex multistep process that builds up carbohydrate structures on proteins and lipids. Each glycosyltransferase is specific for the acceptor structure, the transferred mono-saccharide and the position the linkage it creates. The Fucosyltransferase (FUT) family is a group of fucosylation synthases. By catalyzing the transfer of fucose (Fuc) residue

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Abbreviations: FUT, fucosyltransferase gene; MDR, multidrug resistance; MS, mass spectometry; 5-FU, 5-Fluorouracil; BEL/FU, BEL7402/5-FU; HepG2/FU, HepG2/5-FU; MHCC97H/FU, MHCC97H/5-FU; PI3K, phosphoinositide 3 kinase; MRP1, multidrug resistance-related protein 1; siRNA, small interfering RNA; P-gp, P-glycoprotein; HCC, hepatocellular carcinoma; PBS, PBS containing 0.1% Tween 20; phosphate-buffered saline; MTX, methotrexate; VCR, vincristine; ADR, adriamycin; DMSO, dimethyl sulfoxide; MTT, methylthiazolyl tetrazolium; EMSA, electrophoretic mobility shift assay; IHC, immunohistochemical; EMT, epithelial-to-mesenchymal tranflation

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from the donor substrate, GDP-Fuc, to the oligosaccharide acceptor in α1-2-(FUT1 and FUT2), α1-3/4-(FUT3, FUT4, FUT5, FUT6, FUT7, FUT9, FUT10 and FUT11) and α1-6-linkage (FUT8). FUTs promote the synthesis of fucosylated oligosaccharide chain of glycoconjugates.10–12 Fucosylated oligosaccharides have been implicated in multiple cell–cell interactions in differentiation, development and malignancy.13,14 Forced FUT1 and FUT2 expression in human ovarian carcinoma-derived RMG-I cells promoted cell proliferation and resistance against anticancer drugs, such as 5-FU and carboplatin.15,16 While increasing FUT4 and FUT7 expression promoted neoplastic cell proliferation and HCC cell growth in vitro, respectively, reducing FUT3/6 expression suppressed colon carcinoma cell proliferation.17–19 FUT6 was highly expressed in HCC and was positively associated with the progression of tumor.20,21 FUT7 modified the susceptibility of apoptosis in human hepatocarcinoma cells.22 FUT8 was also upregulated in human hepatoma cell line HCCLM3 (high metastasis).23 However, the effects of the FUT family on MDR have not yet been clearly defined in human HCC cells.

Activation of the phosphoinositide 3 kinase (PI3K)/Akt pathway has a pivotal role in essential cellular functions such as survival, proliferation, migration and differentiation that underlie the biology of human cancer.24,25 In addition, several reports highlight that aberrant activation of the PI3K/Akt pathway contributes to the drug resistance of different types of human cancer cells. Activation of Akt was associated with poor prognosis and chemotherapeutic resistance in pediatric B-precursor acute lymphoblastic leukemia.26 The PI3K/Akt pathway was involved in P-gp-associated survivin transcription activity in the multidrug-resistant MCF-7 breast cancer cells.27 Inhibition of the PI3K/Akt pathway or Akt small interfering RNA (siRNA) led to the inhibition of cellular prion protein (PrP(C))-induced drug resistance in gastric cancer cells.28 However, little is known regarding the signaling pathways on FUT family-mediated HCC MDR.

Therefore, the aim of the present study was to determine fucosylated oligosaccharide alteration and differentially expressed FUT genes between the parental and chemoresistant human HCC cell lines by using mass spectrometry (MS) and real-time PCR. In addition, we wanted to investigate whether the FUT family participates in the regulation of tumor MDR via the PI3K/Akt pathway and the possible mechanisms. Our results provide further evidence that the differentially expressed FUT genes were possibly related to the MDR potential of human HCC cells.

Results

MALDI-MS analysis of N-glycan composition profiling from BEL7402 and BEL/FU cells. MALDI-TOF MS analysis was utilized to evaluate the N-glycan composition profiling of BEL7402 and BEL/FU cell lines. Figure 1 showed the MS spectra of N-glycans released from cell membranes and the observed MS signals of the N-glycans (peaks 1-33 in Figure 1a) and the assigned N-glycan signals as were summarized in Table 1. The observed signal intensities in the mass spectra were presented as a histogram (Figure 1b),...
with the estimated monosaccharide composition. High-mannose glycans (peaks 4, 8, 12, 18 and 22) were observed in both cell lines (Table 1). The N-glycans detected in BEL/FU cells showed remarkably different profiles versus those of BEL7402 cells. The peaks at 7, 25, 27 were exclusively detected only in the drug-resistant BEL/FU cell line. Peak 30 was detected exclusively in the BEL7402 cell line. BEL/FU cells showed higher incidence of additional significant peaks at 2, 3, 4, 5, 11, 20, 29, 31 and 33. Peaks 26, 28, 29 clearly showed a significant increase in the BEL7402 cell line. Surprisingly, the most significant increase peaks corresponding to fucosylated oligosaccharides were observed at peaks 2, 5, 11, 20, 27, 29 and 31 in BEL/FU cells. The fucosylated oligosaccharides observed at peaks 26 and 28 also showed significant increase in the BEL7402 sample. These data indicated that differential N-glycan composition profiling might be associated with the development of MDR in human HCC, especially fucosylated oligosaccharides.

Differential expression of the FUT gene family in three pairs of parental and chemoresistant human HCC cell lines. The MALDI-TOF MS profiles of N-glycan composition from BEL7402 and BEL/FU also showed different fucosylation levels between the drug-sensitive BEL7402 and the MDR BEL/FU cells and a higher level of fucosylated oligosaccharides in BEL/FU cells (Figure 1 and Table 1). In order to evaluate further the expression profile of FUT genes in the parental and chemoresistant human HCC cell lines, a real-time RT-PCR analysis was performed. As shown in Figure 2a, no statistically significant differences were found in the expression of FUT1, FUT2, FUT5, FUT7 and FUT11 mRNA. Only slight differences were observed in the levels of FUT3 (1.7-folds), FUT9 (1.5-folds) and FUT10 (1.8-folds) mRNA. Comparing with BEL7402 cells, BEL/FU cells showed a remarkable expression of FUT4 (3.5-folds), FUT6 (3.0-folds) and FUT8 (3.8-folds) mRNA, suggesting that BEL/FU cells displayed higher \( \alpha1,3- \) and \( \alpha1,6 \)-linked fucosylation (core fucosylation). In addition, HepG2/FU and MHCC97H/FU cells showed the same tendency. These data indicated that differential FUT gene expression might be associated with MDR of human HCC cell lines.

Silence of FUT4, FUT6 or FUT8 genes enhances the chemosensitivity of BEL/FU cells both in vitro and in vivo. Owing to the significant increase in FUT4, FUT6 or FUT8 mRNA expression in BEL/FU cells (Figure 2a), we silenced, by shRNA, FUT4, FUT6 or FUT8 in order to elucidate the direct implication FUT4, FUT6 or FUT8 in the chemosensitivity of BEL/FU cells. As shown in Figures 3a and b, the expression level of FUT4, FUT6 or FUT8 was

### Table 1 N-glycans analyzed in BEL7402 and BEL7402/5-FU cell lines by MALDI-TOF MS

| Peak no. | BEL7402 | BEL7402/5-FU | Hex | HexNAc | Man | GlcNAc | NeuAc | Deoxyhexose |
|---------|---------|-------------|-----|--------|-----|--------|-------|-------------|
| 1       | 1171.87 | 1171.89     | 0   | 0      | 3   | 2      | 0     | 0           |
| 2       | 1345.95 | 1345.92     | 0   | 0      | 3   | 2      | 0     | 0           |
| 3       | 1417.06 | 1416.99     | 0   | 1      | 3   | 2      | 0     | 0           |
| 4       | 1580.13 | 1580.09     | 0   | 0      | 5   | 2      | 0     | 0           |
| 5       | 1591.08 | 1591.10     | 0   | 1      | 3   | 2      | 0     | 0           |
| 6       | 1621.40 | 1621.07     | 0   | 1      | 3   | 2      | 0     | 0           |
| 7       | No      | 1662.06     | 0   | 0      | 6   | 2      | 0     | 0           |
| 8       | 1784.25 | 1784.22     | 1   | 1      | 3   | 2      | 0     | 0           |
| 9       | 1795.28 | 1795.22     | 2   | 1      | 3   | 2      | 0     | 0           |
| 10      | 1825.34 | 1825.20     | 0   | 2      | 3   | 2      | 0     | 0           |
| 11      | 1836.34 | 1836.24     | 2   | 2      | 3   | 2      | 0     | 0           |
| 12      | 1988.11 | 1988.28     | 2   | 2      | 3   | 2      | 0     | 0           |
| 13      | 1999.33 | 1999.24     | 2   | 1      | 3   | 2      | 0     | 0           |
| 14      | 2029.27 | 2029.29     | 3   | 1      | 3   | 2      | 0     | 0           |
| 15      | 2040.16 | 2040.35     | 1   | 2      | 3   | 2      | 0     | 0           |
| 16      | 2070.41 | 2070.29     | 2   | 2      | 3   | 2      | 0     | 0           |
| 17      | 2081.56 | 2081.32     | 0   | 3      | 3   | 2      | 0     | 0           |
| 18      | 2192.31 | 2192.29     | 0   | 0      | 8   | 2      | 0     | 0           |
| 19      | 2203.35 | 2203.30     | 3   | 1      | 3   | 2      | 0     | 0           |
| 20      | 2244.34 | 2244.29     | 2   | 2      | 3   | 2      | 0     | 0           |
| 21      | 2326.34 | 2326.35     | 4   | 4      | 3   | 2      | 0     | 0           |
| 22      | 2362.86 | 2396.27     | 0   | 0      | 9   | 2      | 0     | 0           |
| 23      | 2418.31 | 2418.27     | 2   | 2      | 3   | 2      | 0     | 0           |
| 24      | 2489.25 | 2489.29     | 2   | 3      | 3   | 2      | 0     | 0           |
| 25      | No      | 2519.18     | 3   | 3      | 3   | 2      | 0     | 0           |
| 26      | 2592.23 | 2592.20     | 2   | 2      | 3   | 2      | 0     | 0           |
| 27      | No      | 2605.17     | 2   | 2      | 3   | 2      | 0     | 0           |
| 28      | 2663.20 | 2663.18     | 2   | 3      | 3   | 2      | 0     | 0           |
| 29      | 2693.22 | 2693.16     | 3   | 3      | 3   | 2      | 0     | 0           |
| 30      | 2839.00 | 2867.06     | 4   | 2      | 3   | 2      | 0     | 0           |
| 31      | 2938.00 | 2938.15     | 3   | 4      | 3   | 2      | 0     | 0           |
| 32      | 3141.84 | 3141.86     | 4   | 4      | 3   | 2      | 0     | 0           |

Abbreviations: GlcNAc, N-acetylglucosamine; Hex, hexose; HexNAc, N-acetylhexosamine; Man, mannose; NeuAc, N-acetylneuraminic acid

The N-glycans were observed as [M + Na] +
significantly reduced in BEL/FUT-shRNA transfectants compared with control transfectants. Furthermore, the α-1, 3 fucosylation level detected by FITC-LTL lectin on the cell surface was reduced in BEL/FU-FUT4 shRNA and FUT6 shRNA cells (Figure 3c). Fluorescence intensity on FITC-LCA also revealed less α-1, 6 fucosylation in FUT8 shRNA cells than that in nontransfection cells (Figure 3c). These results clearly showed that FUT4, FUT6 or FUT8 was responsible for the overcoming tumor cells’ MDR via regulating fucosylation profile in terms of α-1, 3 or α-1, 6 branched structures in HCC cells.

After FUT4, FUT6 or FUT8 shRNA transfection, the ability of 5-5-FU, methotrexate (MTX), vincristine (VCR) and adriamycin (ADR) to inhibit the growth of BEL/FUT cells was evaluated using MTT assay. The results showed that IC50 values (drug concentration that inhibits cell growth by 50%) were significantly decreased in BEL/FU-FUT4 shRNA cells group compared with the control, suggesting that cell proliferation was inhibited by therapeutic drug when BEL/FU cells were treated with FUT4 shRNA. Similar results were obtained with BEL/FU-FUT6 or FUT8 shRNA cell group, chemosensitivity was remarkably restored when the FUT6 or FUT8 gene was suppressed (Figure 3d). In addition, MTS assay also revealed the same tendency (Supplementary Figure 1a).

To investigate the effect of knockdown of the FUT4, FUT6 or FUT8 gene on cell chemosensitivity in vivo, we used nude mice bearing BEL/FU, BEL/FU-FUT4 shRNA, BEL/FU-FUT6 shRNA and BEL/FU-FUT8 shRNA xenografts to analyze the differences of tumor volumes when therapeutic drugs were administrated. Figure 3e showed that a significant reduction in the mean tumor volume of BEL/FU-FUT4 shRNA tumor (411 ± 72 mm3) was observed, as compared with the control shRNA group (803 ± 119 mm3), and the effect of concomitant application of 5-FU. The same tendency was also seen in BEL/FU-FUT6 shRNA (405 ± 69 mm3) and FUT8 shRNA groups (420 ± 65 mm3). In the FUT4 pretreatment BEL/FU xenograft model, the percent reduction in tumor volume in the presence and absence of 5-FU upon control shRNA versus FUT4 shRNA were 48.82% and 38.22%, respectively. Similar results were obtained in the FUT6 (49.56%, 39.92%) or FUT8 (47.69%, 39.12%) pretreatment BEL/FU xenograft model. These data were consistent with the results of in vitro chemosensitivity analysis.

After the measurements of the tumor volumes, tumors were sectioned for real-time PCR and IHC (immunohistochemical) staining analysis of FUT4, FUT6 and FUT8 expression patterns; these three genes and proteins were reduced in the mice group with shRNA treatment compared with the untreated group or control group (Figures 3f and g), suggesting a positive correlation between the three gene expression and chemoresistance of BEL/FU cells. Further IHC assessment of the nuclear antigen Ki67 was used to estimate cell proliferation. The results demonstrated that the expression level of Ki67 was significantly decreased in xenograft tumors originating from FUT4-, FUT6- or FUT8-depleted cells, compared with control cells (Supplementary Figure 2a).

Overexpression of the FUT4, FUT6 or FUT8 gene enhances the chemoresistance of BEL7402 cells both in vitro and in vivo. After verifying the effect of FUT4, FUT6 and FUT8 gene suppression on tumor cell chemosensitivity,

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**Figure 2** Differential expression of the FUT gene family in three pairs of parental and chemoresistant human hepatocellular carcinoma cell lines. (a-c) The mRNA levels of FUT gene family analyzed using real-time RT-PCR. The relative amount of gene mRNA level was normalized to the GAPDH level. Three MDR cells expressed higher levels of FUT4, FUT6 and FUT8 mRNA than their parental cell types (more than threefold; *P* < 0.05). Data are the means ± S.D. of triplicate determinants.

**Figure 3** Silence of FUT4, FUT6 or FUT8 gene enhances the chemosensitivity of BEL/FU cells both in vitro and in vivo. (a) Silencing of FUT4, FUT6 or FUT8 in BEL/FU cells was analyzed with the RNAi approach. FUT4, FUT6 or FUT8 transcript was decreased apparently in BEL/FU cells by shRNA treatment. (b) After shRNA transfection, distinct reduction in FUT4, FUT6 or FUT8 was observed at protein levels using western blot analysis. (c) FITC-LTL- or FITC-LCA-binding profile of FUT4, FUT6 or FUT8 shRNA cells using flow cytometry. Histograms of fluorescence intensities of cells with specific carbohydrate expression as determined. (d) Cell chemosensitivity was assessed using cytotoxicity assays. The reported values were the IC50 (Mean ± S.D.) of three independent experiments. IC50 represents the drug concentration producing 50% decrease in cell growth. *P* < 0.05 versus BEL/FU-control shRNA cells. (e) A decrease in the mean tumor volume in mice group with BEL/FU-FUT4, FUT6 or FUT8 shRNA tumors was observed, as compared with that in the BEL/FU group and the BEL/FU-control shRNA group. Within the BEL/FU-FUT4, FUT6 or FUT8 shRNA group, an increase in tumor growth was found in group without 5-FU, compared with that with 5-FU (*P* < 0.05). Downregulation of FUT4, FUT6 or FUT8 was also shown using real-time RT-PCR (f) and IHC staining (g) in xenograft tumors derived from BEL/FU-FUT4 shRNA, BEL/FU-FUT6 shRNA or BEL/FU-FUT8 shRNA cells (400 × ). The data are means ± S.D. of three independent assays (*P* < 0.05)
we next transfected BEL7402 cells with FUT4, FUT6 or FUT8 expression vector to determine the effect of overexpression of these genes on chemoresistance of BEL7402 cells. Notably, increased levels of mRNA and protein of FUT4, FUT6 and FUT8 were detected in BEL7402 transfectants (Figures 4a and b). Figure 4c also showed that the FUT4, FUT6 or FUT8 overexpression resulted in an increase in fluorescence intensity (α-1, 3 or α-1, 6 fucosylation).
Figure 4 Overexpression of FUT4, FUT6 or FUT8 gene enhances the chemoresistance of BEL7402 cells both in vitro and in vivo. After full-length sequence transfection, FUT4, FUT6 or FUT8 mRNA (a) and protein (b) were increased notably in BEL7402 cells using real-time PCR and western blot. (c) FITC-LTL- or FITC-LCA-binding profile of BEL7402/FUT4, BEL7402/FUT6 or BEL7402/FUT8 cells using flow cytometry. Histograms of fluorescence intensities of cells with specific carbohydrate expression as determined. (d) Cell chemosensitivity was assessed using cytotoxicity assays. The reported values were the IC50 (Mean ± S.D.) of three independent experiments. IC50 represents the drug concentration producing 50% decrease in cell growth. *P < 0.05 versus BEL7402/mock cells. (e) An increase in the mean tumor in the mice group with BEL7402/FUT4, BEL7402/FUT6 or BEL7402/FUT8 tumor was observed, as compared with that in the BEL7402 group and the BEL7402/mock group. Within the BEL7402/FUT4, FUT6 or FUT8 group, an increase in tumor growth was found in the group without 5-FU, compared with that with 5-FU (*P < 0.05). Upregulation of FUT4, FUT6 or FUT8 was also shown using real-time RT-PCR (f) and IHC staining (g) in xenograft tumors derived from BEL7402/FUT4, FUT6 or FUT8 cells (400 × ). The data are means ± S.D. of three independent assays (*P < 0.05)
compared with the BEL7402/mock cells. MTT and MTS assays revealed that IC50 values of four drugs were higher in BEL7402/FUT4, BEL7402/FUT6 and BEL7402/FUT8 groups than those in the BEL7402/mock groups, suggesting a positive correlation between the three gene expression and chemoresistance of human HCC cells (Figure 4d and Supplementary Figure 1b).

Nude mice were inoculated with tumor cells BEL7402, BEL7402/mock, BEL7402/FUT4, BEL7402/FUT6 and BEL7402/FUT8. Tumor volumes were measured and compared between the groups with or without 5-FU treatment. In the group of mice bearing BEL7402 tumor, tumor volume with 5-FU treatment (353 ± 51 mm3) was lower than those without (537 ± 72 mm3). In the group of mice bearing BEL7402/FUT4 (609 ± 68 mm3), BEL7402/FUT6 (639 ± 73 mm3) or BEL7402/FUT8 (625 ± 70 mm3) tumors, tumor volumes were increased obviously even after 5-FU treatment, as compared with the BEL7402/mock group (351 ± 58 mm3) (Figure 4d).

High expression levels of FUT4, FUT6 and FUT8 in tumor cells of BEL7402/FUT4, BEL7402/FUT6 and BEL7402/FUT8 were also illustrated using real-time PCR and IHC staining, as shown in Figures 4f and g. Moreover, the protein level of FUT4, FUT6 or FUT8 was strongly related to the expression of Ki67 (Supplementary Figure 2b). Therefore, the upregulation of the FUT4, FUT6 or FUT8 gene in BEL7402 cells led to a raised resistance to chemotherapy.

**Effect of the FUT4-, FUT6- or FUT8-activated PI3K/Akt signaling pathway on the expression of MDR-associated proteins.** Given that the critical role of the PI3K/Akt pathway in controlling cell MDR, we investigated whether FUT4, FUT6 or FUT8 gene activated the PI3K/Akt pathway and whether this pathway had a central role in FUT4-, FUT6- or FUT8-mediated cell MDR. Western blot analysis showed that the levels of PI3 kinase p110α (the catalytic subunit of PI3K) and phosphorylation Akt were decreased in BEL/FU cells treated with FUT4, FUT6 or FUT8 shRNA (Figure 5a). Concomitantly, the degrees of phosphorylation of Akt at Ser473 and Thr308 and its downstream effector NF-κB were decreased markedly. By contrast, there was no change in the total amount of Akt protein, demonstrating a true decrease in the phosphorylation status. Furthermore, overexpression of FUT4, FUT6 or FUT8 in BEL7402 cells significantly enhanced protein expression of PI3K110α, Akt Ser473, Akt Thr308 and NF-κB as illustrated in Figure 5b. Interestingly, electrophoretic mobility shift assay (EMSA) also revealed the same tendency of NF-κB DNA-binding complexes on stimulation with FUT4, FUT6 or FUT8 (Supplementary Figures 3a and b).

To further investigate the effect of the FUT4-, FUT6- or FUT8-modulated PI3K/Akt pathway on the expression of P-gp and MRP1, BEL/FU cells growing exponentially were treated with FUT4, FUT6 or FUT8 shRNA, and the amounts of P-gp and MRP1 were measured using flow cytometric analysis. Lower relative expression levels of MRP1 to Na+ /K+ -ATPase α1 were detected in BEL/FU-FUT4 shRNA, BEL/FU-FUT6 shRNA and BEL/FU-FUT8 shRNA cells compared with those in control cells (Figure 5c). Moreover, BEL7402 cells expressed higher relative levels of MRP1 with FUT4, FUT6 or FUT8 overexpression (Figure 5d). Interestingly, in this study, we showed that, in BEL7402 and BEL/FU cell lines, the relative expression of MRP1, but not of P-gp (relative...
expression to Na⁺/K⁺-ATPase α1), were under the control of the FUT4-, FUT6- and FUT8-modulated PI3K/Akt axis. These data together implied that FUT4, FUT6 and FUT8 might mediate the PI3K/Akt pathway with involvement of MRP1 expression.

PI3K/Akt inhibition modulates the chemosensitivity of BEL/FU cells both in vitro and in vivo. To further determine the role of FUT4-, FUT6- or FUT8-activated PI3K/Akt signaling on cell MDR, specific inhibitor of PI3K/Akt or Akt siRNA to silence Akt was selected to treat FUT4, FUT6 and FUT8 overexpressing cells. The protein levels of PI3K110α, Akt Ser473, Akt Thr308, Akt and NF-κB were measured. As shown in Figure 6a, BEL/FU cells with the inhibitor wortmannin and Akt siRNA treatment showed significantly decreased protein levels of the main signal molecules of the PI3K/Akt pathway. EMSA assay also revealed the same tendency of NF-κB DNA-binding complexes on stimulation with inhibitor wortmannin and Akt siRNA treatment (Supplementary Figure 3b). Using MTT and MTS assays in vitro, we observed that inactivation of the PI3K/Akt pathway by wortmannin or silencing Akt tremendously made BEL/FU cells sensitive to chemotherapy (Figure 6b and Supplementary Figure 1c); the similar results were also obtained using in vivo chemosensitivity analysis. Reduced tumor volumes were observed in the mice group bearing BEL/FU tumors with impaired PI3K/Akt signaling (Figure 6c). Altered expression levels of the main signal molecules of the PI3K/Akt pathway in the mice group bearing BEL/FU tumors with wortmannin or Akt siRNA treatment were also validated using IHC staining, as shown in Figure 6d. Moreover, the inhibitor of PI3K/Akt or silencing Akt decreased the relative expression of MRP1 to Na⁺/K⁺-ATPase α1 in BEL/FU cells, whereas the level of P-gp (relative expression to Na⁺/K⁺-ATPase α1) was not changed (Figure 6e). These data implicated a role of PI3K/Akt

Figure 6  PI3K/Akt inhibition modulates the chemosensitivity of BEL/FU cells both in vitro and in vivo. (a) The BEL/FU cells were pretreated wortmannin or Akt siRNA. Expression of PI3K/Akt/NF-κB signaling molecules were then examined using western blot analysis. (b) Wortmannin or Akt siRNA treatment also alleviated chemoresistance of BEL/FU cells, revealed by in vitro and in vivo (c). (d) Downregulation of PI3K/Akt/NF-κB signaling molecules was also shown by IHC staining in xenograft tumors derived from wortmannin or Akt siRNA treatment cells (400×). (e) Flow cytometry analysis showed that the suppression of PI3K/Akt signaling resulted in reduced-level MRP1 (relative expression to Na⁺/K⁺-ATPase). The data are means ± S.D. of three independent assays. *P<0.05 versus DMSO treatment cells; #P<0.05 vs control siRNA treatment cells.
signaling in regulating MRP1 expression and modulating the chemoresistance of BEL/FU cells.

Discussion

To elucidate the mechanism of MDR, we investigated the composition of N-glycan in parental and drug-resistant human HCC cell lines. The fucosylated N-glycans were dramatically increased in resistant cells. These N-glycans were synthesized by FUTs. We further examined expression of the FUT family, which was reported to be associated with tumor MDR.

Over the past decade, extensive studies have followed the evaluation of glycan changes in proteins during disease from a variety of biological samples using different proteomic approaches, including high-sensitive MS. MS technology as a novel methodology provides high sensitivity and more rapid glycan analysis.29,30 Zhang et al. have investigated novel methodologies to identify glycogenes. The altered level of FUT4, FUT6 or FUT8 was significantly differentially expressed in BEL7402 cells, and altered the expression levels of three drug-resistance genes. The altered level of FUT4, FUT6 or FUT8 was responsible for the change of drug-resistant phenotypes of BEL7402 and BEL/FU cells with three (out of 11) glycogenes (at least threefold, Figure 2) significantly differentially expressed among the two cell lines. Comparing with BEL7402 cells, BEL/FU cells showed a higher expression of FUT4 (3.5-folds), FUT6 (3.0-folds) and FUT8 (3.8-folds) mRNA (Figure 2a). Furthermore, two pairs of resistant and sensitive HCC cell lines also showed the same results, suggesting that MDR cells displayed higher x1, 3- and x1, 6-linked fucosylation (core fucosylation). The major altering expression of FUTs in the three pairs of parental and chemoresistant HCC cell lines might be more important as indicators and functional contributors of tumor MDR.

Whether the alteration of MDR is caused by the change of the FUT family and its related proteins is an interesting problem. However, a comprehensive understanding of how FUT4, FUT6 and FUT8 correlate with the MDR of human HCC cells is not currently available. Here, we targeted FUT4, FUT6 and FUT8, which were differentially expressed in BEL7402 and BEL/FU cells, and altered the expression levels of three glycogenes. The altered level of FUT4, FUT6 or FUT8 was responsible for changed drug-resistant phenotypes of BEL7402 and BEL/FU cells both in vitro and in vivo (Figures 3 and 4). FUT4, FUT6 or FUT8 product also altered markedly the DNA synth-
We demonstrated that the resistant cell line BEL/FU presented higher PI3K/Akt activity than the sensitive one, which was in accordance with the MDR phenotype. Altered expression of FUT4, FUT6 or FUT8 markedly modulated the activity of the PI3K/Akt pathway in human HCC cell lines. In addition, inhibition of the PI3K/Akt pathway with Akt-specific inhibitor wortmannin, or Akt gene silencing by siRNA pretreatment, reversed chemoresistance of BEL/FU cells ([Figures 8b and c]. These results indicated that FUT4-, FUT6- or FUT8-modulated HCC cell ADR was, at least in part, PI3K/Akt-dependent.

Increasing evidence indicates that the PI3K/Akt pathway enhances drug efflux by ABC transporters, maintaining MDR of tumor cells.43 PI3K inhibitor, LY294002, has therapeautic potential when combined with doxorubicin in the treatment of MRPI-mediated drug resistance44 and is able to block P-gp efflux in mouse leukemic cell lines.45 However, a recent report in acute myogenous leukemia has demonstrated that MRPI but not P-gp efflux was inhibited by the PI3K inhibitor wortmannin.43 Inhibition of PI3K/Akt by LY2940002 or Akt siRNA led to the inhibition of PrPC-induced drug resistance and P-gp upregulation in gastric cancer cells.46 Here, we found that the level of MRPI had a positive relationship with the expression of FUT4, FUT6 and FUT8 and the activity of PI3K/Akt in BEL7402 and BEL/FU cell lines. No significant difference of P-gp was found between the two cell lines, which suggested activity of P-gp might have little role in the acquired MDR of BEL7402/5-FU.46 Consequently, the MDR mediated by the FUT family was also involved in the PI3K/Akt pathway activation and MRPI expression.

Taken together, analyzing the fucosylated N-glycans of BEL7402 and BEL/FU lines and detecting the quantitative changes of the FUT family, at least in this system, altered FUT4, FUT6 and FUT8 showed the unusual property of association with HCC cells’ MDR via modulating the PI3K/Akt signaling pathway and MRPI expression. Although we felt that the modification of FUT4, FUT6 and FUT8 effects remained the best explanation for the MDR phenotype, there could be other potential effects on the FUT family alteration. Therefore, the molecular bases of tumor MDR-associated phenotype remained to be further investigated.

Materials and Methods

Cell culture. Human HCC cell lines BEL7402, HepG2 and MHCC97H were obtained from the KeyGen Company (Nanjing, China). The cell line was cultured in 90% DMEM (Gibco, Grand Island, NY, USA) supplemented with antibiotics (1 × penicillin/streptomycin 100 U/mL, Gibco) and 10% heat-inactivated fetal bovine serum (Gibco). Cells were incubated at 37 °C in a humidified atmosphere containing 5% CO₂, 5-FU (Sigma, St. Louis, MO, USA) added to BEL7402, HepG2 and MHCC97H cell cultures in stepwise increasing concentrations to develop a drug-resistant cell subline BEL7402/5-FU (BEL/FU), HepG2/5-FU (HepG2/FU) and MHCC97H/5-FU (MHCC97H/FU). To maintain the MDR phenotype, the medium of the MDR cells was supplemented with 20 mg/l 5-FU. 100-mm dishes using PolyFect Transfection Reagent (QIAGEN) according to the manufacturer’s protocol. Real-time PCR was carried out on an ABI Prism 7500 fast real-time PCR system (Applied Biosystems, Foster City, CA, USA) using Quantitect SYBR Green PCR Kit (QIAGEN). The primer pairs for PCR are listed in Table 2. The relative expression level of each gene was normalized to that of the respective GAPDH. Relative fold differences were determined using the method of delta-delta CT.

Release of N-glycans from cell membrane proteins. Dried three 100 μg aliquots of cell membrane proteins were first digested with trypsin (10 μg) and chymotrypsin (10 μg) dissolved in 25 mM ammonium bicarbonate (25 μl) at 37 °C for 18 h. The digest was left in a water bath (85 °C, 5 min), and after cooling N-linked oligosaccharides were released from peptides by treatment with PNGaseF enzyme (2 μl; 8U) at 37 °C (18 h) followed by Pronase digestion (10 μg) at 37 °C (8 h). During the incubation time, the reaction sample was mixed occasionally. The released N-glycans were purified using an Oasis HLB cartridge (60 mg per 3 ml; Waters, Milford, MA, USA) and then lyophilized.

MS analysis. The mass spectra were acquired using an UltraflexIII MALDI-TOF/TOF (Bruker Daltonics, Bremen, Germany). To increase the sensitivity and provide more informative fragmentation, the released glycans were permethylated and further characterized by MALDI-TOF MS. For the type of MALDI analysis of the permethylated glycans, 2,5-DHB was used as the matrix. Values are the mean ± S.D. of three permethylated samples from N-glycan samples. All MS spectra were obtained from Na⁺ adduct ions.

Real-time PCR analysis. Real-time PCR was used to analyze gene expression. Total RNA was isolated using the RNeasy Mini Kit (QIAGEN, Valencia, CA, USA), and cDNA was synthesized using the QuantiTect Reverse Transcription Kit (QIAGEN) according to the manufacturer’s protocol. Real-time PCR was carried out on an ABI Prism 7500 fast real-time PCR system (Applied Biosystems, Foster City, CA, USA) using Quantitect SYBR Green PCR Kit (QIAGEN). The primer pairs for PCR are listed in Table 2. The relative expression level of each gene was normalized to that of the respective GAPDH. Relative fold differences were determined using the method of delta-delta CT.

Western blot analysis. Whole-cell proteins were electrophoresed under reducing conditions in 10% polyacrylamide gels. The separated proteins were transferred to a polyvinylidene fluoride membrane (Pall Corporation, Port Washington, NY, USA). After blocking with 5% skimmed milk in PBS containing 0.1% Tween 20 (PBST), the membrane was incubated with antibody (ABSENT, San Diego, CA, USA 1:500 dilution; Abcam, Cambridge, UK, 1:1000 dilution) and then with peroxidase-conjugated anti-rabbit IgG (Santa Cruz Biotechnology, Santa Cruz, CA, USA, 1:1000 dilution). GAPDH (Santa Cruz Biotechnology, 1:1000 dilution) was used as a control. All band intensities were evaluated using ECL western blotting kit (Amersham Biosciences, Little Chalfont, UK) and normalized to those of GAPDH.

Deregulation of FUT4, FUT6 or FUT8 in BEL/FU cells by RNAi. Transfection was performed in a 12-well plate at ~30% confluent cultures. BEL/FU cells were maintained in 1 ml of complete medium with 5 μg/ml Polybrene per well and were treated with 0.4 μM FUT4-, FUT6- or FUT8-specific shRNA lentiviral particles overnight; three wells were transduced with control shRNA lentiviral particles. Then, the medium in each well was replaced with 1 ml of complete medium (without Polybrene) and the cells were diluted to 1:3 for selecting stable clones expressing the shRNA by 5 μg/ml puromycin dihydrochloride. The transfection efficiency was ~80% and cell viability was 90% as demonstrated by the trypan blue dye exclusion assay. Four weeks later, several resistant colonies were picked and expanded for further study.

Overexpression of FUT4, FUT6 or FUT8 in BEL7402 cells. The human FUT4, FUT6 and FUT8 coding sequences were obtained from TaKaRa company (Dalian, China) and were inserted into the pEGFP-N2 vector (Invitrogen, Carlsbad, CA, USA), respectively, using EcoRI and XhoI sites. Cells were transfected with 5 μg of target gene expression vector or empty vector (EV) in 100-mm dishes using PolyFect Transfection Reagent (QIAGEN) according to the manufacturer’s instruction. After 4 weeks of screening, the cell lines stably expressing FUT4 (BEL7402/FUT4), FUT6 (BEL7402/FUT6), FUT8 (BEL7402/FUT8) and EV (BEL7402/mock) were established. Then cells were collected for gene expression assay for further explorations. The cell transfection efficiency was 78% and the survival rate was 90%.

In vitro drug cytotoxic assay. Cell proliferation was measured using an MTT assay to determine the chemosensitivity of the cell groups with genetic manipulation and pharmacologic inhibition of the PI3K/Akt pathway. Cells (1 × 10⁴) were plated in 96-well plates (Costar, Charlotte, NC, USA) and incubated with different anticancer drugs such as 5-FU (Sigma), MTX
was repeated three times. The drug resistance was estimated by comparing the IC50 values (drug concentration that inhibits cell growth by 50%) from growth inhibition curves.

**In vitro drug cytotoxicity assay.** The CellTiter 96 AQueous One Solution Cell Proliferation Assay (MTS) kit (Promega, Madison, WI, USA) was used to determine the chemosensitivity of cell groups with genetic manipulation and pharmacologic inhibition of the PI3K/Akt pathway. Cells (1 × 104) were seeded in a 96-well plate and incubated with 5-FU, MTX, VCR or Sigma (48 h). Then cells were stained with 10 μl MTS for 3 h at 37°C. The absorbance was measured at 490 nm using microplate reader (Model 680; Bio-Rad, Hercules, CA, USA). The drug resistance was estimated by comparing the IC50 values from growth inhibition curves.

**In vivo chemosensitivity assay.** To investigate whether FUT4, FUT6 or FUT8 is related to drug sensitivity of tumor cell in vivo, the chemosensitivity of 5-FU was examined in nude mice bearing tumor xenografts. Five-week-old male athymic nude mice were obtained from Animal Facility of Dalian Medical University and were provided with sterilized food and water. Approximately, 1 × 107 cells were injected subcutaneously into the right flank of each nude mouse, respectively. When mice bearing palpable tumors (about 1 week after tumor cell inoculation), BEL7402, BEL7402/mock, BEL7402/FUT4, FUT6 or FUT8, BEL/FU, BEL/FU-control shRNA, BEL/FU-FUT4, FUT6 or FUT8 shRNA tumor-bearing mice were randomly divided into control and treatment groups (n = 6 animals per group). The treatment groups received 30 mg/kg 5-FU i.p. three times per week for 3 weeks, and the control groups received physiological saline alone. Mice were killed and their tumors were isolated. The external caliper was used to determine the volume of xenografted tumors; the tumor volume was calculated by the following formula: tumor volume = 1/2 (length × width)3.

**Inhibition of the PI3K/Akt signaling.** Wortmannin (Sigma) was used to suppress the activity of the PI3K/Akt signaling in BEL/FU cells. Briefly, cells (1 × 106 cells per well) were incubated with DMSO (Sigma) or the PI3K inhibitor wortmannin (1 mM) dissolved in DMSO. Cells were collected after 24 h. Changes in chemosensitivity and gene expression were measured using MTT and MTS assays and western blot analysis, respectively. Each experiment was run in triplicate to determine the means ± S.D.s.

**Nuclear extractions and EMSA.** Nuclear extracts were prepared as previously described.20 DNA-binding activity of NF-κB was analyzed using EMSA, and the following sequence was used as specific oligomer for NF-κB: 5’-AGTTGAGGGGACTTTCCCAGGC-3’ (sense). Reaction mixtures (15 μl) contained, together with 10 ng of nuclear extracts, 2 μg of poly (d-dc) (Sigma) and 10 ng of biotin-labeled probes. For competition experiments, 100-fold excess of unlabeled competitor oligos were added to the extracts before the addition of labeled probes. The supershift analysis for NF-κB/DNA complex formation was determined with the NF-κB p65 antibody (sc-372, Santa Cruz Biotechnology), respectively. Biotin-labeled DNA–protein complexes were detected by the streptavidin conjugated with HRP (Panomics, Redwood, CA, USA).

**Flow cytometry analysis.** Tumor cells were washed with 1 × PBS buffer containing 20 g/l bovine serum and then were preincubated with 5% powdered skim milk for 30 min to block nonspecific binding. Cells were placed in sterile conical tubes in aliquots of 500 000 cells each and stained with one of the FITC-LTL and FITC-LCA lectin at a final concentration of 10 μg/ml for 40 min at 4°C in the dark. For surface staining of P-gp and MRPI, aliquots of cells were incubated with fluorescent isothiocyanate-anti-human P-gp, MRPI (Abcam) or a Na+/K+ -ATPase ± control antibody (Santa Cruz Biotechnology) for 40 min at 4°C at the recommended dilutions. After repeated centrifugation at 1000 rpm, labeled cells were resuspended in 0.2 ml PBS and were analyzed with FACScanCalibur (BD Biosciences, San Jose, CA, USA). Fluorescence intensity was measured using the Cell Quest software (BD Biosciences). The relative P-gp or MRPI expression level was normalized to that of the respective Na+/K+ -ATPase ±1. Each experiment was run in triplicate to determine means and S.D.s.

**IHC staining analysis.** Visible tumors were removed from the mice and immunohistochemistry was performed on paraffin-embedded sections. The slides were dried, deparaffinized, rehydrated and then were immersed in 3% hydrogen peroxide for 10 min to quench the endogenous peroxidase. After consecutive washing with PBS, the slides were labeled overnight at 4°C with FUT4, FUT6 and FUT8 antibodies (ABGENT, 1: 50 dilution), or Ki67 antibody (sc-15402, Santa Cruz Biotechnology, 1: 50 dilution), or isotype immunoglobulins (Santa Cruz Biotechnology) as negative control. The following staining was performed at room temperature for 60 min with secondary streptavidin-HRP-conjugated antibody (Santa Cruz Biotechnology). Finally, the sections were counterstained with hematoxylin and coverslipped. The Image-Pro Plus 4.5 Software (Media Cybernetics, Bethesda, MD, USA) was used to analyze the expression of proteins.

**Statistical analysis.** Each experiment was performed at least in triplicate, and the measurements were performed in three independent experiments. Data are expressed as means ± S.D. Student’s t-test was used to compare the means of two groups. P < 0.05 was considered statistically significant. All analyses were performed using SPSS 13.0 statistical packages (SPSS Inc., Chicago, IL, USA).

**Conflict of Interest**

The authors declare no conflict of interest.

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1. Tong SW, Yang YX, Hu HD, An X, Ye F, Hu P et al. Proteomic investigation of 5-fluorouracil resistance in a human hepatocellular carcinoma cell line. J Cell Biochem 2012; 113: 1671–1680.
2. Szakacs G, Paterson J. Targeting multidrug resistance in cancer. Nat Rev Drug Discovery 2006; 5: 219–234.
3. Teodori E, Dei S, Martelli C, Scapecchi S, Guaitelli F. The functions and structure of ABC transporters: implications for the design of new inhib-itors of Pgp and MRPI to control multidrug resistance (MDR). Curr Drug Targets 2006; 7: 893–909.
4. Baguley BC. Multidrug resistance in cancer. Methods Mol Biol 2010; 596: 1–14.
5. Umboir K, Kasamatsu A, Sunaga M, Yokota S, Sakurada T, Kobayashi E et al. Establishment and characterization of two 5-fluorouracil-resistant hepatocellular carcinoma cell lines. Int J Oncol 2012; 40: 1005–1010.
6. Lou B, Fan J, Wang K, Chen W, Zhou X, Zhang J et al. N1-guanylyl-1,7-diaminoheptane (GCT) enhances the therapeutic efficacy of doxorubicin by inhibiting activation of eukaryotic translation initiation factor 5A2 (eIF5A2) and preventing the epithelial- mesenchymal transition in hepatocellular carcinoma cells. Exp Cell Res 2013; 310: 604–613.
7. Kudo T, Nakagawa H, Takahashi M, Hamaguchi J, Kiyama N, Yokoo H. Nuclear lamin A/C alterations are associated with drug resistance in human hepatocellular carcinoma. Mol Cancer 2007; 6: 32–40.
8. Guo R, Cheng L, Zhao Y, Zhang J, Li C, Zhou H et al. Glycogenes mediate the invasive properties and chemosensitivity of human hepatocarcinoma cells. Int J Biochem Cell Biol 2013; 45: 347–355.
9. Brockhausen I, Mucin-type O-glycans in human colon and breast cancer: glycodynamics and functions. EMBO Rep 2006; 7: 599–604.
10. Ma B, Simala-Grant JL, Taylor DE. Fucosylation in prokaryotes and eukaryotes. Glycobiology 2006; 16: 158–184.
11. De VT, Knecht RM, Holmes EH, Macher BA. Fucosyltransferases: structure/function studies. Glycobiology 2001; 11: 119–138.
12. Chandrasekaran EV, Jain PK, Rhodes JM, Smka CA, Larsen RD, Matta KL. Expression of blood group. Lewis b determinant from Lewis a: association of this novel alpha (1, 2)-furucosylating activity with the Lewis type alpha (1, 3/4)-L-fucosyltransferase. Biochemistry 1995; 34: 4748–4756.
13. Roseman S. Reflections on glycobiology. Glycobiology 2001; 11: 158–184.
14. Liu YC, Yen HY, Chen CY. Sialylation and fucosylation of epidermal growth factor receptor (EGF-R) in gastric cancer cell lines. Int J Oncol 2012; 40: 1005–1010.
15. Lou B, Fan J, Wang K, Chen W, Zhou X, Zhang J et al. N1-guanylyl-1,7-diaminoheptane (GCT) enhances the therapeutic efficacy of doxorubicin by inhibiting activation of eukaryotic translation initiation factor 5A2 (eIF5A2) and preventing the epithelial-mesenchymal transition in hepatocellular carcinoma cells. Exp Cell Res 2013; 310: 604–613.
16. Ma B, Simala-Grant JL, Taylor DE. Fucosylation in prokaryotes and eukaryotes. Glycobiology 2006; 16: 158–184.
17. De VT, Knecht RM, Holmes EH, Macher BA. Fucosyltransferases: structure/function studies. Glycobiology 2001; 11: 119–138.
17. Yang X, Zhang Z, Jia S, Liu Y, Wang X, Yan Q. Overexpression of fucosyltransferase IV in A431 cell line increases cell proliferation. Int J Biomol Cell Biol 2007; 39: 1722–1730.

18. Wang QY, Guo P, Duan LL, Shen ZH, Chen HL, alpha-1, 3-Fucosyltransferase-VII stimulates the proliferation of colon cancer cells via the cyclin-dependent kinase inhibitor p27Kip1. Cell Mol Life Sci 2005; 62: 171–178.

19. Hiller KM, Mayben JP, Bendt KM, Manousos GA, Senker K, Cameron HS et al. Transfection of alpha (1,3) fucosyltransferase antisense sequences impairs the proliferative and tumorigenic ability of human colon carcinoma cells. Mol Cancrogn 2000; 27: 280–289.

20. Borsig L, Imbach T, Hochl C, Berger EG. a1, 3-Fucosyltransferase VI is expressed in HepG2 cells and codistributed with a1, 4-galactosyltransferase I in the Golgi apparatus and monensin-induced swollen vesicles. Glycobiology 1999; 9: 1273–1280.

21. Guo Q, Guo B, Wang Y, Wu J, Jiang W, Zhao S et al. Glycogen-related gene expression signatures in human metastatic hepatocellular carcinoma cells. Exp Ther Med 2012; 3: 415–422.

22. Katso R, Okkenhaug K, Ahmadi K, White S, Timms J, Waterfield MD. Cellular function of phosphoinositide 3-kinases: implications for development, homeostasis, and cancer. Anr Rev Cell Dev Biol 2001; 17: 615–675.

23. Dell A, Morris HR. Glycoprotein structure determination by mass spectrometry. Science 2001; 294: 2351–2356.

24. Harvey DJ. Proteomic analysis of glycosylation: structural determination of N-and O-linked glycans by mass spectrometry. Expert Rev Proteomics 2005; 2: 87–101.

25. Zhang MH, Xu XH, Wang Y, Ling QX, Bi YT, Miao XJ et al. Protein expression and fucosylated glycans of the serum haptoglobin-beta subunit in hepatitis B virus-based liver diseases. Acta Biochim Biophys Sin (Shanghai) 2011; 43: 528–534.

26. Mejías-Luque R, López-Ferrer A, Garmido M, Fabra A, de Bobis C. Changes in the invasive and metastatic capacities of 17–293/MO cells induced by the expression of fucosyltransferase-1. Cancer Sci 2007; 98: 1000–1005.

27. Vasseur JA, Goetz JA, Alley WR, Novotny MV. Smoking and lung-cancer-induced changes in N-glycosylation of blood serum proteins. Glycobiology 2012; 22: 1684–1708.

28. Lin X, Zhang X, Wang Q, Li J, Zhang P, Zhao M et al. Perifosine downregulates MDRI gene expression and reverses multidrug-resistant phenotype by inhibiting PI3K/Akt/NF-kB signaling pathway in a human breast cancer cell line. Neoplasma 2012; 59: 248–256.

29. Li L, Wei XH, Pan YP, Li HC, Yang H, He QH et al. LAPTM4B: a novel cancer-associated gene motivates multidrug resistance through efflux and activating PI3K/AKT signaling. Oncogene 2010; 29: 5785–5795.

30. Bao R, Lai CJ, Wang DG, Qu H, Yin L, Zito PK et al. Targeting heat shock protein 90 with CUDC-305 overcomes etoposide resistance in non-small cell lung cancer. Mol Cancer Ther 2009; 8: 3296–3306.

31. Yang XS, Liu S, Liu YJ, Liu JW, Liu TJ. Wang XD et al. Overexpression of fucosyltransferase IV promotes A431 cell proliferation through activating MAPK and PI3K/Akt signaling pathways. J Cell Physiol 2010; 225: 612–619.

32. Takahashi M, Yokoe S, Asahi M, Lee SH, Li W, Osumi D et al. N-glycan of ErbB family plays a crucial role in dimmer formation and tumor promotin. Biochim Biophys Acta 2008; 1780: 525–529.

33. Wang X, Gu J, Ibara H, Miyoshi E, Honke K, Tangiguchi N. Core fucosylation regulates epidermal growth factor receptor-mediated intracellular signal. J Biochem 2006; 251: 2572–2577.

34. Tazzari PL, Cappellini A, Ricci F, Evangelisti C, Papa V, Grafone T et al. Multidrug resistance-associated protein 1 expression is under the control of the phosphoinositide 3 kinase/Akt signal transduction network in human acute myelogenous leukemia blasts. Leukemia 2007; 21: 427–438.

35. Abdul-Ghani R, Serra V, Gryllo B, Ju¨rkhott K, So¨f A, Dietel M et al. The PI3K inhibitor LY294002 blocks drug export from resistant colon carcinoma cells overexpressing MRPI. Oncogene 2006; 25: 1743–1752.

36. Barancik M, Bohalcová V, Sedlák J, Sutová Z, Brewer A. LY294002, a specific inhibitor of PI3K/Akt kinase pathway, antagonizes P-glycoprotein-mediated multidrug resistance. Eur J Pharm Sci 2006; 29: 426–434.

37. Gu W, Fang FF, Li B, Cheng BS, Ling CQ. Characterization and resistance mechanisms of a 5-fluorouracil-resistant hepatocellular carcinoma cell line. Asian Pac J Cancer Prev 2012; 13: 4871–4874.

38. Kasperczyk H, La Ferla-Bruhl K, Westhoff MA, Behrend L, Zwacka RM, Debatin K-M et al. Betulinic acid as new activator of NF-kappaB: molecular mechanisms and implications for cancer therapy. Oncogene 2005; 24: 6945–6956.

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