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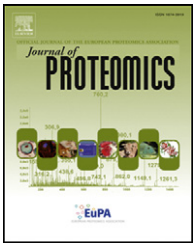

In-depth research of multidrug resistance related cell surface glycoproteome in gastric cancer

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ABSTRACT

Human gastric cancer is a big public health problem. Multidrug resistance is a main obstacle to successful chemotherapeutic treatment in gastric cancers and the underlying mechanism is not clear. Glycosylation, one of the most important post translational modifications of proteins, plays a vital role in diverse aspects of tumor progression. In the present study, we applied two multidrug resistance cell lines and their parental drug sensitive gastric cancer cell line to a modified cell surface capturing strategy with triplex labeling to characterize MDR related cell surface glycoproteome. Finally, 56 cell membrane glycoproteins were successfully identified via combination of identification by glycopeptides and quantitation by non-glycopeptides, and 11 of them were found to be differentially expressed with the same trend in both drug resistant cell lines compared with that in sensitive cell line. The further analysis by western blot and in vitro drug sensitivity assay demonstrated that our approach is reliable and accurate and suggested that these glycoproteins may represent as biomarkers for multidrug resistance in gastric cancer.

Biological significance

In this study, we performed a cell surface glycoproteomics research of multidrug resistance in gastric cancer using a modified CSC approach. Totally we identified and quantified 11 membrane N-glycoproteins which were significantly changed in MDR gastric cancer cells. These glycoproteins are quite possible to be biomarkers for predicting MDR or key regulators for targeted therapy, and are also helpful for better interpreting the sophisticated mechanisms of MDR in gastric cancer. In addition to that, this approach used in this study can be well applied to screen aberrantly glycosylated biomarkers associated with other malignant phenotypes of various kinds of cancers.

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1. Introduction

Gastric cancer is the fourth leading cause of cancer-related death in the world [1]. Chemotherapy is the primary treatment for most patients, but one severe clinical problem that often causes failure of chemotherapy is multiple drug resistance (MDR) [2]. For decades, numerous studies have been performed on MDR of gastric cancer and several systems such as ATP-binding cassette (ABC) transporters, glutathione-S transferases and topoisomerases have been found to be involved in this malignant phenotype. Though they have been intensively studied, the underlying mechanisms of MDR are still not precisely clarified, and clinically there is no satisfactory marker for predicting MDR of gastric cancer, suggesting that mechanisms responsible for MDR in gastric cancer are likely to be multifaceted and extremely intricate [3].

As reported, many cancer biomarkers are glycoproteins and get approved in clinical practice, e.g. carcinoembryonic antigen (CEA) for colon cancer, prostate specific antigen (PSA) for prostate cancer and HER2 for breast cancer [4]. Interestingly, many classical MDR related molecules, including P-glycoprotein (P-gp), multidrug resistance-associated protein 1 (MRP1), and breast cancer resistance protein (BCRP) are also glycoproteins and glycosylation could affect their activities or functions, which, together with the profound importance of glycosylation in bioprocesses, suggest that glycosylation would play an important role in MDR of gastric cancer and glycoproteins may present as potential markers [5–8]. Recently, Di Michele et al. screened the differential expressed glycoproteins between drug sensitive and resistant cell lines and found 4 glycoproteins remarkably upregulated in drug resistant cells as putative biomarkers for paclitaxel resistance in ovarian cancer [9]. Another study reported that treatment with molecule targeted drug herceptin could lead to dramatic differences of glycoproteins in breast cancer and T-lymphoblastoid cells [10]. But unfortunately, until now there is no study focusing on the correlation between the changes of glycoproteins and MDR in gastric cancer. Therefore, researches of altered MDR-related cell surface glycoproteins in gastric cancer are needed and may facilitate the discovery of potential biomarkers, and even novel targets of therapeutics.

Previously, we have successfully established two different drug resistant variants (i.e. SGC7901/VCR and SGC7901/ADR) from the parental gastric cancer cell line SGC7901 by stepwise induction with vincristine (VCR) or adriamycin (ADR), respectively. Both drug resistant cell lines showed a cross resistance to many other toxic drugs and were proven as excellent cell models for MDR research in gastric cancer by many researches [11–14]. In the present study, we subjected these three lines of cell models to a modified cell surface capturing (CSC) strategy to examine the differential expression of cell surface N-glycoproteins. Totally 56 cell membrane N-glycoproteins were identified by analyzing both non-glycosylated and glycosylated peptides derived from glycoproteins, and 35 N-glycoproteins were found to be differentially expressed between drug sensitive and resistant cell lines. We focused on 11 glycoproteins which were significantly changed with the same trend in both drug resistant cell lines compared with sensitive cell line (8 decreased and 3 increased more than 2 folds), because these proteins may present as biomarkers for MDR in gastric cancer. Our strategy was confirmed as a credible approach for MDR related N-glycoproteomic research in gastric cancer.

2. Materials and methods

2.1. Cell culture

Human gastric adenocarcinoma cell line SGC7901 was obtained from the Academy of Military Medical Science (Beijing, China) and kept in our lab. Its multidrug-resistant variant cell lines, SGC7901/VCR and SGC7901/ADR, were developed in our lab by a stepwise selection with vincristine or adriamycin, separately [15,16]. All cells were cultured in RPMI-1640 medium supplemented with 10% fetal calf serum in a humidified atmosphere containing 5% CO_2 at 37 °C. To maintain the MDR phenotype, VCR (1 μg/ml) or ADR (0.5 μg/ml) was added to the culture media for SGC7901/VCR or SGC7901/ADR cells, respectively.

2.2. Periodate oxidation and cell lysis

Cells of exponential phase were washed with 3 ml cold PBS for 3 times and then were oxidized with 5 mM $NaIO_4$ in oxidation buffer (100 mM NaAc, 150 mM NaCl, pH 5.5) in the dark at 25 °C for 1 h with gentle shaking. After removing the periodate solution, cells were harvested using a cell scraper and rinsed twice with oxidation buffer. The proteins were extracted in ice-cold lysis buffer (50 mM HEPES (PH = 7.4); 8 M urea; 65 mM Dithiothreitol (DTT); 1 mM Phenylmethanesulfonyl fluoride (PMSF); 1 mM EGTA; 1 mM EDTA; 2% (v/v) Proteinase inhibitor cocktail; 1% (v/v) Triton X-100) with sonication for 400 W × 60 s. After centrifugation at 13,000 ×*g* for 1 h, the supernatant was collected and the protein concentration was determined by Bradford assay. Samples were either immediately subjected to glycoprotein enrichment or 1 mg aliquots stored at −80 °C until needed.

2.3. Enrichment of N-linked glycoproteins

About 2 mg of extracted protein samples of each cell line underwent the following procedures. The N-linked glycoproteins in each cell lysates were captured with hydrazide chemistry as described elsewhere [17]. Firstly, 400 μl of Affi-Gel Hz hydrazide resin (slurry volume) (Bio-Rad, USA) was washed twice with coupling buffer (100 mM NaAc, 150 mM NaCl, pH 5.5). Then the extracted proteins were added to prewashed hydrazide resin. The coupling reaction was performed overnight at room temperature with adequate shaking. The resin was washed with 1.5 M NaCl, methanol and 100 mM NH_4HCO_3 sequentially three times to remove non-specifically bound proteins.

2.4. Release of non-glycopeptides and N-glycopeptides

The resin with the captured glycoproteins from the last step was diluted with 8 M urea/100 mM NH_4HCO_3. Proteins on the resin were reduced by 20 mM DTT at 37 °C for 2 h and alkylated by 40 mM iodoacetamide (IAA) at room temperature in the dark