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Original Article

Proteomics-based analysis of differentially expressed proteins in the CXCR1-knockdown gastric carcinoma MKN45 cell line and its parental cell

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C-X-C chemokine receptor types 1 (CXCR1), a cell-surface G-protein-coupled receptor has been found to be associated with tumorigenesis, development, and progression of some tumors. Previously, we have found that CXCR1 overexpression is associated with late-stage gastric adenocarcinoma. We also have demonstrated that knockdown of CXCR1 could inhibit cell proliferation *in vitro* and *in vivo*. In this study, we compared the changes of protein expression profile between gastric carcinoma MKN45 cell line and CXCR1-knockdown MKN45 cell line by 2D electrophoresis. Among the 101 quantified proteins, 29 spots were significantly different, among which 13 were down-regulated and 16 were up-regulated after CXCR1 knockdown. These proteins were further identified by mass spectrometry analysis. Among them, several up-regulated proteins such as hCG2020155, Keratin8, heterogeneous nuclear ribonucleoprotein C (C1/C2), and several down-regulated proteins such as Sorcin, heat shock protein 27, serpin B6 isoform b, and heterogeneous nuclear ribonucleoprotein K were confirmed. These proteins are related to cell cycle, the transcription regulation, cell adherence, cellular metabolism, drug resistance, and so on. These results provide an additional support to the hypothesis that CXCR1 might play an important role in proliferation, invasion, metastasis, and prognosis, and drug resistance of gastric carcinoma.

Keywords CXCR1; gastric carcinoma; proteomics

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Introduction

Gastric carcinoma is one of the most deadly types of cancer worldwide, especially in China [1,2]. Despite gaining great advances in treatment and putting in more effort in research over the past few decades, the outcome of gastric cancer remains unsatisfactory, and the overall 5-year survival rate of advanced gastric adenocarcinoma patients is low. Therefore, improvement in the therapy of gastric cancer now depends on improving our understanding of the complex molecular mechanisms governing the progression and aggressiveness of the disease. Uncontrolled proliferation, invasion, and metastasis as a whole are major poor prognostic factors for advanced gastric cancer [3]. Non-resolving inflammation plays a critical role in the development and progression of gastric cancer [4,5]; and in the dialectical relationship between inflammation and tumor dynamic network, chemokine receptors and their ligands, an important class of non-resolving inflammatory factors, involved in carcinogenesis, proliferation, growth, invasion, metastasis, and drug-resistance process [6–10].

C-X-C chemokine receptor types 1 (CXCR1) belong to chemokine receptor family which is G-protein-coupled receptors containing seven transmembrane domains, and also is a kind of receptor for interleukin-8 (IL-8) and transduces the signal through a G-protein activating second messenger system. CXCR1 expresses mainly on neutrophils and is originally characterized by their ability to induce chemotaxis of leukocytes. Recently, it has been found that CXCR1 overexpresses in numerous solid tumors, which shows a close correlation with proliferation, angiogenesis, invasion, and metastasis, and drug resistance of tumor [11–15]. Even though there have been some studies on CXCR1 in several cancer types as well as in gastric carcinoma [16], they are all from the single aspect rather than the overall level. Proteomics is from the perspective of the overall to analyze intracellular changes in the protein profile, the expression levels and modified state, to understand protein–protein interactions and connections, and to reveal the protein function and cell life law. Hence, with the help of it, we can have a good knowledge of the proteins composition as well as screening differential proteins in human gastric cancer cell lines with CXCR1 knockdown on a whole level.

Our current knowledge of the functional role and mechanism of CXCR1 in the progression of gastric carcinoma is based on the literature accumulation and our earlier experiment [17,18]. In this study, we investigated the changes of

protein expression profile after CXCR1 knockdown to identify the related proteins in response to the down-regulation of CXCR1 protein in gastric carcinoma MKN45 cell line.

Materials and Methods

Cell culture

The gastric carcinoma cell line MKN45 was provided by Shanghai Bogoo BioScience (Shanghai, China). Then, the green fluorescence plasmid DNA of expressing CXCR1 short hairpin RNAs and the empty green fluorescent protein plasmid were transfected into MKN45 cells. The stably transfected cells were obtained by antibiotic G418 screening which both emit green fluorescence. The two types of cells were cultured at 37°C with 5% CO_2 in Dulbecco's modified Eagle's medium (Gibco, Carlsbad, USA) media supplemented with 10% fetal bovine serum (Gibco), 1% penicillin and streptomycin (Sigma, St Louis, USA).

Sample preparation

Cells (1×10^7) were harvested, and then washed three times with phosphate-buffered saline (PBS, pH 7.4), subsequently lysed with lysis buffer containing 1 mM phenylmethylsulfonylfluoride. The lysed cells were sonicated with a probe sonicator for 5 min followed by centrifuging at 15,000 *g* for 20 min. The supernatant was collected and loaded into four tubes (250 μl each), then 1 ml acetone was added and stored at −20°C overnight for precipitation. The precipitated proteins were centrifuged at 4°C, 15,000 *g* for 20 min, and the supernatant was removed, followed by natural drying to obtain protein clumps. The rehydration buffer (RB) stock (200 μl) was then added to redissolve the clumps followed by sonicating for 5 min and centrifuging at 4°C 15,000 *g* for 20 min. Proteins in the supernatant were quantified by the Bradford method, and stored at −80°C until use for electrophoresis.

Two-dimensional electrophoresis and image analysis

Isoelectric focusing electrophoresis was conducted on 22 cm (pH 3–10) immobilized pH gradient (IPG) strips at 20°C according to the manufacturer's instructions (GE, Bethesda, USA). Approximately 1200 μg protein was loaded onto each gel, and triplicate gels for each sample were run to achieve reproducible two-dimensional electrophoresis (2-DE) results. The sample solution ingredients are as follows: the appropriate amount of protein, 1% DL-dithiothreitol (DTT), 1% IPG buffer, 1× black pigmented bacteroides, and adding RB to a total volume of 460 μl. Briefly, the strips were rehydrated without voltage for 10–12 h, then subject to electrophoresis at 300 V for 30 min, 700 V for 90 min, 1500 V for 90 min, 9000 V for 180 min, 9000 V for 240 min until a total of 52 kVh. The focused strips were equilibrated in sodium dodecyl sulfate (SDS) electrophoresis buffer with 6 M urea, 50 mM Tris-HCl, 30% glycerol, 2% SDS, and trace bromophenol blue, and were subsequently treated by the reduction of DTT and alkylation of iodoacetamide. The treated strips were transferred onto 12.5% uniform SDS–polyacrylamide gels running at 2 W per each gel for 45 min and 17 W per each gel until the bromophenol blue dye reached the bottom of the gel. After that, the gels were subjected to silver staining. Then, the stained gels were scanned by Imagescanner (UMAX Powerlook1100) and the image analysis was conducted with ImageMaster 2D platinum 5.0 (GE). The threshold of the difference was defined as 1.5 folds of change in a spot volume upon comparison of gels between the CXCR1-knockdown MKN45 cell and its parental cell.

Mass spectrometry for protein identification

The protein spots identified as significant changes in spot volume were excised and transferred into Eppendorf tubes individually. The gel pieces were treated by reduction of DTT and alkylation of iodoacetamide followed by a thorough process of washing with Milli-Q water and drying. Finally, the treated gel pieces were incubated with 50 mM NH_4HCO_3 containing 0.05 mg/ml trypsin at 37°C shaker for 30 min. After centrifuge, the resultant supernatants were mixed with 50% acrylonitrile and 0.1% trifluoroacetic acid (Promega, Madison, USA) and then delivered to mass spectrometry. Peptide mass fingerprinting (PMF) was performed in an Autoflex speed^{TM} MALDI-TOF-TOF instrument (Bruker Dalton, Billerica, USA) under 20 kV accelerating voltage in the reflectron mode with UV wavelength of 355 nm and the *m*/*z* range was from 700 to 3200 Da. All PMFs were externally calibrated using standard peptide mixtures and internally calibrated using the masses of trypsin autolysis products. Then, flex analysis software (Bruker Dalton) was used to filter the signal baseline peak and recognize the signal peak. The peptide mass spectra were searched against the National Center for Biotechnology Information (NCBI) non-redundant database by BioTools (Bruker Dalton) software using the Mascot search engine, in which one incomplete cleavage was allowed in order to find matching protein, inquire about its function, and clearly identify the protein. The searching parameter was set up as follows: the taxonomy was selected as Homo sapiens; the mass tolerance was ± 50 ppm; the missed cleavage sites were allowed up to one; the Global modification was selected as carbamidomethy; and the variable modification was selected as oxidation.

Western blot analysis

The cells were collected from flasks, washed with cold PBS three times, and then lysed at 4°C for 30 min in a lysis buffer (50 mM Tris, pH 7.4, 100 mM $NaCl_2$, 1 mM $MgCl_2$, 2.5 mM Na_3VO_4, 1 mM PMSF, 2.5 mM ethylenediaminetetraacetic acid, 0.5% Triton X-100, 0.5% NP-40, 5 mg/ml of

aprotinin, pepstatin A, and leupeptin). The lysates were centrifuged at 10,000 *g* for 15 min at 4°C. Protein concentration was determined using a bicinchoninic acid protein assay reagent kit (Pierce, Rockford, USA) according to the manufacturer's protocol. Forty micrograms of total proteins were electrophoresed on a 12% denaturing SDS gel and transferred onto a polyvinylidene difluoride (PVDF) membrane. The PVDF membrane was then incubated with blocking buffer (PBS containing 5% non-fat milk) for 2 h at room temperature, followed by incubating with rabbit polyclonal antibodies against keratin8, HSP27, NADPK, hnRNPK (Anbo Biotechnology, San Francisco, USA), and mouse monoclonal antibody against sorcin (Santa Cruz Biotechnology, Santa Cruz, USA) diluted at 1 : 200 overnight with gentle shaking. The membrane was washed twice with PBS for 5 min, then incubated with horseradish peroxidase-conjugated goat anti-rabbit/mouse immunoglobulin G (Santa Cruz Biotechnology) as secondary antibody diluted at 1 : 2000 for 2 h at room temperature. As a loading control, GAPDH was detected using a rabbit polyclonal antibody (Santa Cruz Biotechnology). Experiments were repeated three times.

Gene ontology analysis

To search identified proteins by using a local blast program (version 2.2.23+) against 'UniProt Knowledgebase Release 2013_01' consisting of Swiss-Prot and TrEMBL databases. A text file which consisted of each identified protein's accessions number and the corresponding protein's accessions was contained from blast output of Swiss-Prot and TrEMBL database with alignment $\geq$30% and *E* value $<1 \times 10^{-10}$. Then, based on this text file and gene association file from gene ontology (GO) ftp, each identified protein's accessions and the corresponding GO terms were confirmed using an in-house Perl script.

Statistical analysis

SPSS 13.0 software (SPSS Inc., Chicago, USA) was used for two sample *t*-test analysis. $P < 0.05$ was considered statistically significant.

Results

Proteomic comparison of the CXCR1-knockdown cells with the control cells

The protein expression modulations in MKN45 cell after CXCR1 knockdown were analyzed by 2-DE. Protein lysates from each cell lines with and without CXCR1 knockdown were characterized in triplicate. In three gels, we choose the one with the most spots, both in high quality and definition. As illustrated in **Fig. 1**, the proteins were well resolved on 2-DE. The proteins from MKN45 cell with and without CXCR1 knockdown showed similar migration pattern related to molecular mass and pI. Further analysis by combination of ImageMaster and manual check revealed that total spots from the cell lines with CXCR1-knockdown cells and control cells were 2120 and 1982, respectively. One point 5 folds of change in the spot volume, gel by gel, were set as the threshold to define the statistical differences among these images. A total of 101 spots were identified to have change in response to CXCR1 knockdown, among which 57 spots were down-regulated, 6 spots were completely knocked out, 30 spots were up-regulated, and an additional 8 spots emerged after CXCR1 knockdown. Part of the comparative enlargement picture of protein spots with statistical significance were shown in **Fig. 2**.

Sequence identification of the differential spots in 2-DE

Twenty-nine of 101 proteins with over 2-folds difference were chosen for further analysis. They were digested in-gel by trypsin and analyzed by matrix-assisted laser desorption

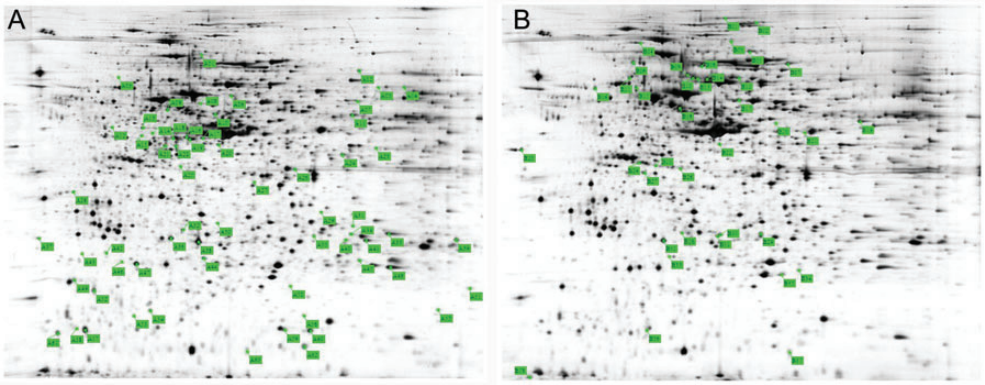

Figure 1 Two-dimensional electrophoresis pattern of proteins from MKN45 cell with and without CXCR1 knockdown (A) Cell with CXCR1 knockdown; (B) Cell without CXCR1 knockdown. One hundred and one differential expression protein spots marked with arrows were identified using MALDI-TOF-MS. PI, pH index.

ionization-time of flight mass spectrometry (MALDI-TOF-MS). Mass spectra were internally calibrated with the masses of two trypsin autolysis products with a typical mass measurement accuracy of 50 ppm. Stringent criteria were adopted to ensure the accuracy of protein identification: (i) the identified protein must rank at the top two hits with at least five matched sequences and (ii) the total coverage must be >10%. On the basis of the data from mass spectrometry, the Mascot search identified 29 proteins (listed in Supplementary Table S1). The protein peptide quality fingerprint, MS/MS fragmentation, database matching results, and the Mascot score of heat shock protein 27 (HSP27) are shown in **Fig. 3**.

The cellular component, molecular function, and biological process of the selected proteins were annotated by the GO database

In terms of the GO database, the differentially expressed proteins were divided into three categories: cellular component (CC), molecular function (MF), and biological process (BP). The top five components for the CC were organelle, extracellular region, cytoplasm, cytosol, and nucleus. The top five components for MF were binding, protein binding, catalytic activity, nucleotide binding, and hydrolase activity. The top five components for BP were metabolic process, primary metabolic process, regulation of biological process, biosynthetic process, and signal transduction (**Fig. 4**).

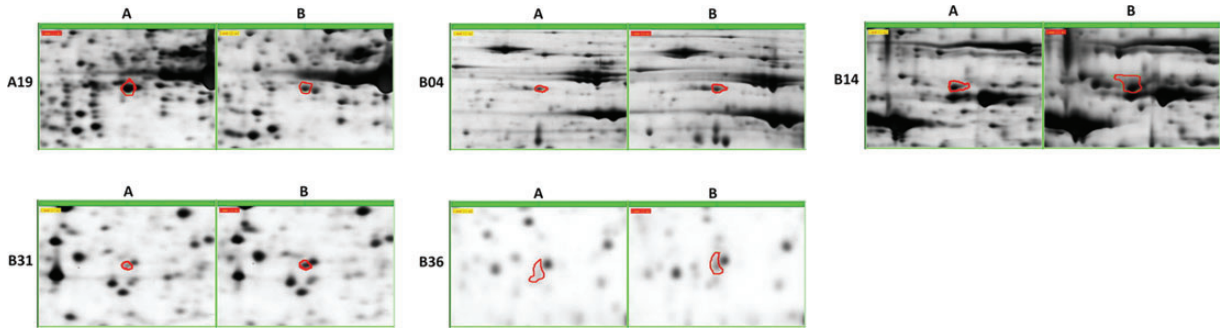

Figure 2 Close-up image of partial differentially expressed protein spots (A) Cell with CXCR1 knockdown; (B) Cell without CXCR1 knockdown.

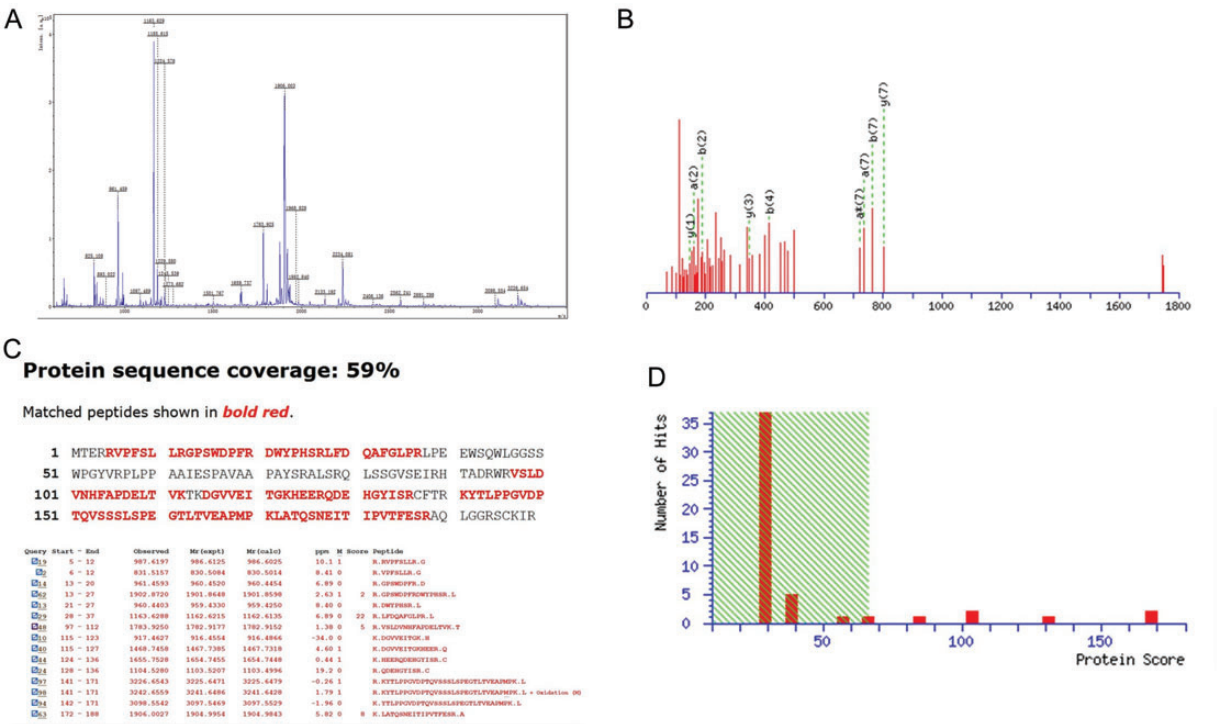

Figure 3 The identification results of B31 protein spot (A) The peptide mass fingerprint; (B) MS/MS fragmentation; (C) Database matching results; and (D) Probability-based Mascot score picture. Protein score is $-10*\mathrm{Log}(P)$, where P is the probability that the observed match is a random event. Protein scores >66 are significant ($P < 0.05$). Protein scores are derived from ions scores as a non-probabilistic basis for ranking protein hits. The NCBI database was searched according to the number of matching fragments, coverage, and Mascot scores. The protein was identified as HSP27.

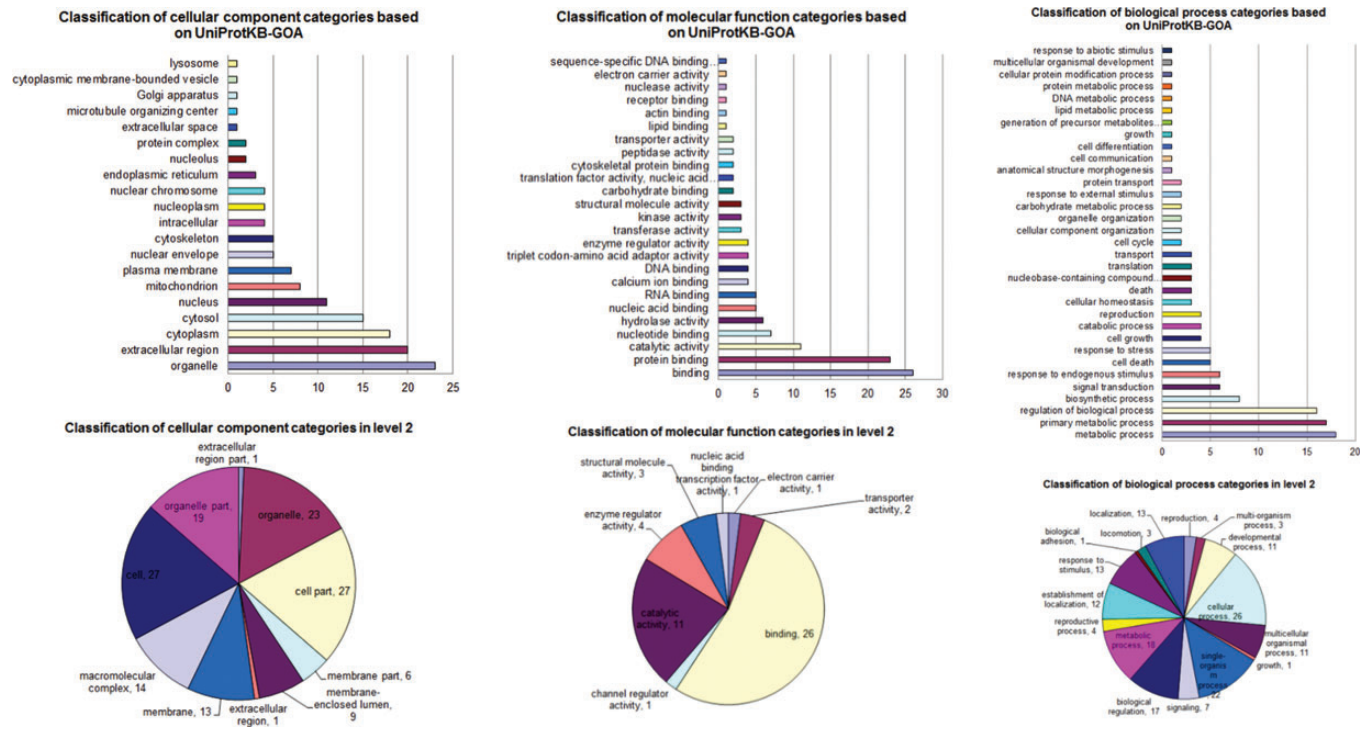

Figure 4 GO annotation of differentially expressed proteins The differentially expressed proteins were divided into three categories: CC, MF, and BP.

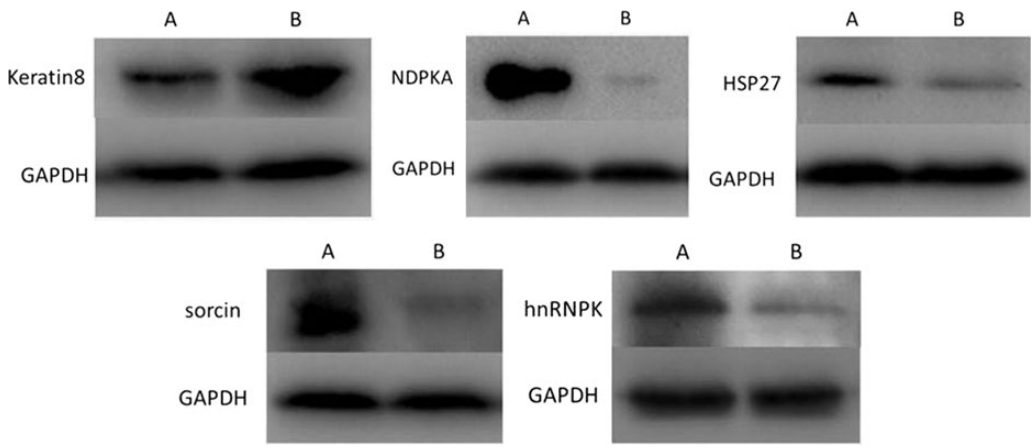

Figure 5 Western blot analysis of keratin8, NDPKA, HSP27, sorcin, and hnRNPK (A) Cell without CXCR1 knockdown; (B) Cell with CXCR1 knockdown.

The protein expression in response to the CXCR1 knockdown was measured by western blot

To confirm the differential expression levels of the proteins identified by the comparative proteomics technique, the expression levels of the partial proteins in both CXCR1-knockdown MKN45 cell and control MKN45 cell were measured by western blot analysis. **Figure 5** shows the representative result of keratin8, NDPKA, HSP27, sorcin, and hnRNPK expression in both CXCR1-knockdown MKN45 cell and control MKN45 cell detected by western blot analysis. Compared with control MKN45, CXCR1-knockdown MKN45 revealed an obvious down-regulation of NDPKA, HSP27, sorcin, and hnRNPK, and up-regulation of keratin 8, which were identical with the results of the proteome analysis.

Discussion

CXCR1, one member of the chemokine receptor family, has been studied in several cancers, showing a close correlation with drug resistance, survival, growth, invasion, and metastasis in breast cancer [13], melanoma [14], prostate cancer [19], colon cancer [15], etc. In melanoma, CXCR1 knockdown or the use of antagonists or neutralizing antibodies affects tumor cell proliferation, growth, and migration, strongly indicating the involvement of this receptor in

melanoma progression [20]. Previously, we found that CXCR1 overexpression is associated with late-stage gastric adenocarcinoma, and CXCR1/2 might be a useful marker for progression of the tumors and a promising target for gastric carcinoma therapy [17,18]. We subsequently found that knockdown of CXCR1 could block cell proliferation *in vitro* and *in vivo*, induce gastric cancer cell cycle arrest and apoptosis, retard cell growth, inhibit gastric cancer cell migration and invasion, and decrease the phosphorylation level of serine/threonine protein kinase (AKT) and extracellular signal-regulated kinase (ERK)1/2, indicating that CXCR1 might trigger AKT and ERK signal pathway. Li *et al*. [21] also found that CXCR1 up-regulated proliferation, growth, and restricted apoptosis in esophagus carcinoma cell line by activating the PTEN/Akt pathway and mediating the expression of survivin and Bcl-2, while another study argued that IL-8/CXCR1/2 signaling was partly mediated via a novel SRC and epidermal growth factor receptor/human epithelial factor receptor2 (EGFR/HER2)-dependent pathway [22]. In this study, we used a proteomic approach to identify proteins that were differentially expressed in the human gastric cancer cell line MKN45 with or without CXCR1 knockdown to identify the affected proteins in response to the down-regulation of CXCR1 protein. We totally observed 101 protein spots that showed different expression in the two cell lines at the default 1.5 folds. Then, 29 proteins with most significant difference (>2 folds) were analyzed by MALDI-TOF-MS.

According to different protein function, these differentially expressed proteins can be divided into six categories: molecular chaperones, protein synthesis, signal transduction, DNA repairs, metabolism-related protein, and transcription, which were involved in cell cycle, the transcription regulation, cell adherence, cellular metabolism, drug resistance, and so on. Heat shock protein is a kind of molecular partner. They can regulate cell intermediate activity in multiple signaling pathways, such as cell apoptosis signaling pathway. They can be divided into two kinds: apoptosis-promoting and apoptosis-inhibiting. HSP27 is an important small molecule heat shock protein which could inhibit apoptosis [23,24]. The phosphorylated form of HSP27 inhibits death domain associated protein (Daxx) apoptotic protein and prevents the association of Daxx with factor associated suicide (Fas) and apoptosis signal regulating kinase (Ask)1 [25]. Besides, HSP27 can promote the activation of the proteasome by speeding up the degradation of irreversibly denatured proteins and junk proteins by binding to ubiquitinated proteins and to the 26S proteasome. It also enhances the activation of the nuclear transcription factor (NF-κB) pathway that regulates many processes, such as cell growth, inflammatory, and stress responses. Since CXCR1 is an inflammation receptor [26], this may explain why we observe the significant change of HSP27 level after CXCR1 knockdown. The research in gastric cancer shows that the level of HSP27 protein in gastric cancer tissues is much higher than that in para-cancerous tissues, gastritis tissues, and normal gastric tissues [27], which is also related to tumor cell differentiation and lymph node metastases. HSP27 expression in highly differentiated gastric cancer is higher than that in poorly differentiated gastric cancer, and the serum level of HSP27 in patients with lymph node metastasis was higher than those in patients without lymph node metastasis [28]. What is more, it is extremely correlated with carcinoma and associated with carcinoma cell drug resistance [29], radiosensitivity [30], and prognosis [31] of tumors. We found that HSP27 is a highly down-regulated protein in CXCR1-knockdown gastric carcinoma cell lines, which indicates that CXCR1 expression positively correlates with HSP27 expression, and CXCR1 may work by regulating HSP27.

Sorcin, a sytosolic protein of 22 kDa, belongs to the penta-EF-hand (PEF) family which also includes calpains, grancalcin, ALG-2, and peflin [32]. The binding of Ca^{2+} triggers the reversible translocation of all PEF proteins from the cytoplasm to the cell membranes, where they interact with specific target proteins and participate in a variety of physiological processes. Sorcin was first identified in a vincristine-resistant Chinese hamster lung cell line, and was later demonstrated to be overexpressed in several tumor cell types [33–35] and many multidrug resistance (MDR) cell lines [36,37]. As to the role of sorcin overexpression in human gastric cancer and the underlying mechanisms of sorcin in drug resistance, Deng *et al*. [38] argued that the overexpression of sorcin was related to the depth of invasion, TNM stage, and lymph node metastasis, which suggested that sorcin may contribute to progression and metastasis of gastric carcinoma and sorcin plays an important role in the development of gastric carcinoma probably by regulating the apoptotic pathways in gastric carcinoma cells, which was further elucidated in another paper [39]. It was reported that sorcin was identified as a new binding partner of TRAP1 (a mitochondrial chaperone HSP75) in MDR of human colorectal carcinoma cells, indicating that the cytoprotective function of sorcin may be involved in TRAP1-associated signaling pathway, which was coincident with the result of Maddalena *et al*. [36] They found that RNAi-mediated silencing of sorcin activated caspase-3, caspase-12, and GRP78/BiP, and then triggered apoptosis through the mitochondrial pathway, which established that human colorectal cancer cells overexpressing sorcin as an adaptive mechanism to escape apoptosis triggered by chemotherapeutic agents, while He *et al*. [40] argued that regulation of P-glycoprotein (P-gp) might be one of the mechanisms of sorcin-mediated MDR, for they found that overexpression of sorcin up-regulated the expression of P-gp, and P-gp inhibitor verapamil partially reversed the sorcin-mediated MDR in SGC7901 cell. In our previous study, we found that

CXCR1-knockdown cell lines were more vulnerable to 5-fluorouracil. In this study, we observed that almost no sorcin protein was detected in CXCR1-knockdown MKN45 cell line, which indicated that CXCR1 and sorcin may be involved in MDR of gastric cancer cells. With further investigation of the function and underlying mechanism, CXCR1 combining with sorcin may be a useful therapeutic strategy for reversal of gastric MDR.

The heterogeneous nuclear ribonucleoproteins (hnRNPs) are RNA-binding proteins with important roles in multiple aspects of nucleic acid metabolism, including the packaging of nascent transcripts, alternative splicing, and translational regulation [41,42]. According to different physiological functions, hnRNPs can be divided into two kinds: one is localized in cell nuclei, which cannot freely shuttle between nuclear and cytoplasm, such as hnRNPB, C, etc, and the other is free to move back and forth in the nucleus and cytoplasm, such as hnRNPA, D, E, I, and K. The hnRNPK is a 65 kDa nuclear phosphor-protein that acts as a docking platform to integrate signals from a variety of kinase-mediated signal transduction pathways controlling nucleic acid-directed processes [43]. It has a wide range of nuclear and cytoplasmic functions, including mRNA silencing transcription, splicing, and regulation of mRNA stability and translation [44,45]. The hnRNPC, including C1 and C2 (41 and 43 kDa, respectively), are major constituents of hnRNP complex in vertebrates. In our study, we identified that hnRNPK was down-regulated, but hnRNPC (C1/C2) was up-regulated after CXCR1 knockdown. According to a large number of research reports, only hnRNPK was closely associated with tumors. One mechanism is that hnRNPK may regulate tumor progression by controlling the expression of proteins that play roles in proliferation. The hnRNPK binds to the promoter element of the *4IF4E* gene resulting in increasing transcriptional activation. 4IF4E plays an important role in cellular proliferation, and is overexpressed in several tumors including head and neck, breast cancers, colon, lung, lymphoma, and bladder [46]. Interestingly, *4IF4E* transcription is up-regulated when both c-myc and hnRNPK are co-overexpressed [46], suggesting that hnRNPK may co-operate with additional oncoproteins which are permissive toward cancerous growth. In breast cancer cells [47], prostate carcinoma [48], and pancreatic cancer [49], plenty of researches demonstrated that hnRNPK significantly enhances the proliferative activity of tumor cells. Besides that, several research findings have confirmed that there is a close relation between hnRNPK and tumorigenesis. In hepatocellular carcinoma (HCC) [50], hnRNPK is a potential biomarker, either alone or in combination with serum alpha fetal protein, for the detection of early HCC. High expression of hnRNPK could be helpful to discriminate early HCC from a non-malignant nodule, especially for patients with liver cirrhosis. In colon carcinoma, hnRNPK is shown to be overexpressed and its subcellular localization is aberrant. In normal colon cells, hnRNPK expresses exclusively in nucleus, however, in tumor cells hnRNPK is present both in the cytoplasm and the nucleus. In addition, Dukes C patients who present tumors with strong hnRNPK nuclear expression have a better survival outcome [51], and overexpression of HSP27 and hnRNPK are independent markers of poor prognosis, and their combination definitively predicts adverse outcomes in colorectal cancer patients [52]. As for gastric carcinoma [53], there is a higher expression of hnRNPK in gastric carcinoma, and Helicobacter pylori L-form infection may be associated with the up-regulated hnRNPK expression, and the two factors may play a synergetic role in gastric carcinogenesis. In another study [54], researchers found that there were differential expression proteins including hnRNPK between normal gastric mucosa and gastric carcinoma using comparative proteomic technology. Xiao *et al*. [55] and Zhang *et al*. [56] also found that hnRNPK were highly expressed in gastric intraepithelial neoplasia tissues, which is related to the occurrence of gastric intraepithelial neoplasia. Further studies showed that the positive rates of hnRNPK and c-myc in the lymph node metastasis group were significantly higher than those in the non-metastases gastric carcinoma group, suggesting that hnRNPK may play an important role in the process of gastric adenocarcinoma by c-myc gene activation. All these results showed that hnRNPK is closely associated with tumors and one of the mechanisms in promoting tumor growth is to increase the proliferation of cancer cells. In previous experiments, we found that MKN45 cell with high CXCR1 expression had strong self-proliferation ability while the proliferation rate of MKN45 cell decreased significantly after CXCR1 silence, which also indicates CXCR1 is likely to play an important role in promoting cell proliferation and induce the occurrence and development of cancer by interacting with hnRNPK.

Nucleoside diphosphate kinase A (NDPKA), also being called nm23-H1, is an enzyme that is encoded by the *NME1* gene in humans, which belongs to the human NDP kinases Group I including NDP kinases A–D [57]. In normal physiological conditions, NDPKA is involved in cell proliferation, development, and differentiation [58]. However, in human tumors, NDPKA is thought to be a metastasis suppressor, especially in melanoma, nasopharyngeal carcinoma, breast cancer, HCC, lung carcinomas, and so on [59–64]. However, in other cancer types such as prostate carcinoma, neuroblastoma, malignant lymphoma, and some kind of colon carcinoma, and gastric carcinoma [65–69], the expression loss of NDPKA is not a significant metastatic factor, in which high NDPKA expression might be correlated with more aggressive disease. In gastric carcinoma, Wang *et al*. [69] found that NDPKA immunoreactivity was not different in primary and metastatic gastric cancer, and Liu *et al*. [70]

found that the expression of NDPKA did not decrease the metastasis to distant organ in gastric cancer, but all specimens were positive expressions. This is in good agreement with the results of our experiments, because we observed significant NDPKA down-regulation in CXCR1-knockdown MKN45 cell.

Keratin 8 (cytoskeletal intermediate filament keratin 8) is identified as a physiological PRL-3-interacting protein. One study has reported that colorectal carcinoma cells with high PRL-3 expression showed reduction or loss of phosphorylated keratin 8 expression, especially at the invasive front and in the liver metastases, indicating that it may be a metastasis suppressor [71]. In our study, we found that Keratin 8 was up-regulated after CXCR1 kockdown, which implied that CXCR1 may induce gastric tumor metastasis and invasion by reducing Keratin 8 protein. While Kim *et al*. [72] identified Keratin 8 as one of the gastrointestinal tract cancer cell-related genes, but until now there was no related report exploring its true function in gastric carcinoma.

In addition, we found that the expression of many metabolic enzymes was changed in CXCR1-knockdown gastric carcinoma MKN45 cell including galactokinase, chymotrypsinogen, KIAA0088, peptidase mitochondrial processing beta (PMPCB) protein, and so on, indicating that CXCR1 is involved in tumor metabolism.

In conclusion, we identified 29 differentially expressed proteins associated with gastric cancer in CXCR1-knockdown gastric carcinoma MKN45 cell line and its parental cell by proteomic approach. The functions of these proteins are associated with cancer cells proliferation, differentiation, invasion, metastasis, cellular signaling transduction, apoptosis, and drug resistance. Our results suggest that CXCR1 plays an important role in gastric carcinoma progression activating certain key factors (such as HSP27, sorcin, and so on) in some signaling pathways. Although whether CXCR1 directly binds to the promoters of these identified proteins and how CXCR1 interacts with these proteins needs to be further elucidated, our study paved a way to determine the role of CXCR1 in the progression of gastric carcinoma.

Supplementary Data

Supplementary data are available at *ABBS* online.

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