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Research Article

Multidrug-resistance-associated protein MGr1-Ag is identical to the human 37-kDa laminin receptor precursor

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Abstract. We report the isolation and functional characterization of the gene encoding MGr1-Ag, a multidrug-resistance-associated protein. A λgt11 cDNA library derived from colorectal carcinoma SW480 cells was screened with monoclonal antibody MGr1. DNA homology analysis of 22 positive clones (designated R1–R22) suggested human 37-kDa laminin receptor precursor (37LRP, R7/R9/R15/R16/R19/R20) and a novel gene (R22) as candidate genes encoding MGr1-Ag. Western blot analysis showed that anti-R20 serum reacted with a unique protein band that was consistent with MGr1-Ag, while anti-R22 serum could not react with MGr1-Ag. The coding gene for MGr1-Ag was amplified using reverse transcription-PCR. Sequence analysis revealed that the MGr1-Ag and 37LRP genes shared the same coding sequence. An in vitro drug sensitivity assay indicated that down-regulation of 37LRP by an antisense technique could significantly enhance the cytotoxicity of anticancer drugs to gastric cancer cells. Thus we draw the conclusion that MGr1-Ag is identical to 37LRP.

Key words. Stomach neoplasm; multidrug resistance; MGr1; library screening; human 37-kDa laminin receptor precursor.

A key obstacle in chemotherapy of gastric cancer is the development of multidrug resistance (MDR) in cancer cells [1]. MGr1-Ag was initially found highly expressed in vincristine (VCR)-resistant gastric cancer SGC7901/VCR cells [2]. Further studies revealed that its specific monoclonal antibody MGr1 could significantly enhance sensitivity of SGC7901/VCR cells to VCR, adriamycin (ADR) and 5-fluorouracil (5-Fu), and that MGr1 could increase intracellular accumulation and retention of ADR in SGC7901/VCR cells [3]. Through immunohistochemical staining, Fan et al. [4] found that the positive rate of MGr1-Ag in 50 gastric cancer cases was 18%. All these data suggested an association between MGr1-Ag and MDR phenotypes of gastric cancer cells.

Previous studies had characterized MGr1-Ag as a glycoprotein with an apparent molecular weight of 42 kDa, which could be secreted or shed from gastric cancer cells [5]. Immunoelectromicroscopy showed that MGr1-Ag was localized not only on the membrane but also in the cytoplasm of gastric cancer cells [2]. However, the structure and MDR mechanisms of MGr1-Ag remained to be elucidated. The present study describes the isolation and functional characterization of the gene encoding MGr1-Ag. The results reveal that MGr1-Ag is identical to the human 37-kDa laminin receptor precursor.

Materials and methods

Detection of MGr1-Ag using Western blot analysis

Gastric cancer SGC7901/VCR and SGC7901 cells and colorectal carcinoma SW480 cells were cultured in RPMI1640 (Gibco, UK) supplemented with 10% fetal calf serum (Gibco). Cells in log phase were harvested, and

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total cellular proteins were prepared with lysis buffer (pH 8.0) containing 1% NP-40, 50 mmol/l Tris base, 150 mmol/l sodium chloride, 0.1 mmol/l PMSF and 1 mg/l aprotinin. SDS-PAGE was performed according to a standard protocol. Separated proteins were electrotransferred to nitrocellulose filters and stained with ponceau S. The bands of protein standards were marked with a marker pen corresponding to their positions. After being washed with deionized water to remove ponceau S, the filter was blocked and incubated in turn with MGr1 ascites (prepared from BALB/c mice bearing MGr1 hybridoma cells and characterized by Li et al. [2] and Shi et al. [3]) overnight at 4 °C and horseradish-peroxidase-conjugated goat anti-mouse IgG (1 : 1500; DAKO) for 45 min at room temperature. Finally, the filter was subjected to diaminobenzidine (DAB) solution for 5–30 min to visualize positive signals.

Immunoscreening of cDNA library

A λgt11 cDNA library (Clontech, USA), generated from mRNA from colorectal carcinoma SW480 cells, was used to isolate the gene encoding MGr1-Ag. Immunoscreening was done according to the manufacturer's protocol. The reactive clones were subcloned and purified three times. DNA from plate lysates of the positive plaques was isolated using the Lambda DNA extraction kit (Qiagen, Germany) according to the manufacturer's guidelines. The inserts were amplified by PCR using insert screening primers according to the instructions of the cDNA library. PCR products were cloned into pUCm-T vector (Sangon, Shanghai, China) using the T-A cloning technique. The cDNA inserts were sequenced on a 377 model automatic DNA sequencer (Perkin Elmer, USA). Insert sequences were used for an on-line homology search with FASTA against all submitted sequences in GenBank.

Preparation and characterization of antisera

The candidate inserts were subcloned from pUCm-T vectors into pQE30 vector (Qiagen) and expressed as fusion proteins in *Escherichia coli* strain M15 (Qiagen). These recombinant fusion proteins were used as immunogens to raise mouse antisera according to D'Souza et al. [6] with some modification. In brief, recombinant proteins were separated by SDS-PAGE. After staining with 0.25 mol/l potassium chloride, the portion of the polyacrylamide gel corresponding to recombinant proteins was cut out and crushed in phosphate-buffered saline (PBS). BALB/c mice were injected subcutaneously with gel particles containing about 10 μg recombinant proteins emulsified with complete Freund's adjuvant (Sigma). Three booster injections with gel particles emulsified with incomplete Freund's adjuvant (Sigma) were given at 2-week intervals. Animals were bled 1 week after the last immunization and sera were evaluated with Western blot as described above.

Reverse transcription – PCR

Reverse transcription (RT)-PCR reaction was performed with an Access Quick RT-PCR System (Promega) according to the manufacturer's protocol. Five micrograms of total RNA isolated from SGC7901/VCR cells was used as template. Two oligonucleotides, 5′-GCTACCTGCAGAGGGGTCCATAC-3′ and 5′-TTTTTTTTTTTTTTTTTTGCAAC-3′, served as upstream and downstream primer, respectively. The reaction tubes were incubated at 48 °C for 45 min, and then proceeded with PCR cycling. The cycling was performed starting with a 5-min denaturation step at 94 °C followed by 30 cycles of 50 s at 94 °C, 50 s at 55 °C, 90 s at 72 °C and a final extension step at 72 °C for 10 min. PCR products were cloned into pUCm-T vector by the T-A cloning technique and sequenced.

Down-regulation of candidate gene expression in gastric cancer cells

Candidate gene expression in gastric cancer cells was down-regulated by an antisense technique. To prepare antisense RNA expression vector, the candidate cDNA fragment was released from pUCm-T vector and inversely inserted into pcDNA3.1(+) (Invitrogen). Recombinant pcDNA3.1(+) vector was introduced into SGC7901/VCR cells by mediation of Lipofectamine 2000 (Gibco) according to the manufacturer's instruction. After selection with G418 (Gibco) and evaluation of gene expression by Western blot analysis, the candidate clones were identified and isolated.

In vitro drug sensitivity assay

The sensitivity of gastric cancer cells to anticancer drugs was evaluated using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay as described previously [7]. Dose-effect curves of anticancer drugs were drawn on semi-logarithm coordinate paper and IC_{50} values were determined.

Fluorescence intensity assay of intracellular ADR

The fluorescence intensity of intracellular ADR was determined by flow cytometry as described previously [7]. Briefly, gastric cancer cells in log phase were plated into six-well plates (1×10^6 cells/well) and cultured overnight at 37 °C. After addition of ADR to a final concentration of 5 μg/ml, cells continued to be cultured for 1 h. Cells were then trypsinized and harvested (for detection of ADR accumulation) or, alternatively, cultured in drug-free RPMI1640 for another 30 min followed by trypsinization and harvesting (for detection of ADR retention). Cells were washed twice with cold PBS and the fluorescence intensity of intracellular ADR was determined using flow cytometry with an exciting wavelength of 488 nm and receiving wavelength of 575 nm. Finally, the ADR releasing index of gastric cancer cells was calculated according to the formula: releasing index = (accumulation value – retention value)/accumulation value.

Statistical analysis

Analysis of variance and Student-Newman-Keuls test were performed to evaluate the changes of IC_{50} values and ADR fluorescence intensity. Statistical significance was determined at $p < 0.05$.

Results

Detection of MGr1-Ag using Western blot analysis

As figure 1 shows, even when diluted to 1:1000, MGr1 antibody retained its ability to detect MGr1-Ag. The figure also demonstrates that MGr1-Ag could be detected in colorectal carcinoma SW480 cells indicating that screening of a cDNA library derived from SW480 cells with MGr1 antibody was suitable for gene cloning of MGr1-Ag.

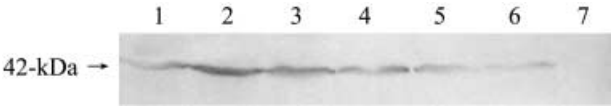

Figure 1. Detection of MGr1-Ag using Western blot analysis. Total proteins from SGC7901 cells (lane 1), SGC7901/VCR cells (lanes 2, 4, 5, 6 and 7) and SW480 cells (lane 3) were separated by SDS-PAGE and blotted to nitrocellulose filter. MGr1 ascites diluted at 1:100 (lanes 1, 2 and 3), 1:200 (lane 4), 1:500 (lane 5) or 1:1000 (lane 6) were used as primary antibody. Normal mouse serum diluted at 1:200 served as negative control (lane 7).

Library screening and analysis

The library vector phage λgt11 contains one copy of the *lacZ* gene. There is a unique *Eco*RI site in *lacZ* gene sequence, which provides a cloning site for exogenous cDNA fragments. Thus the exogenous cDNA fragment can be expressed by λgt11 as fusion protein, which contains a galactosidase fragment at the N terminal and exogenous polypeptide at the C terminal. Sometimes, the fusion protein can be recognized by specific antibody. In the present study, MGr1 antibody diluted to 1:1000 was used to screen a cDNA library derived from SW480 cells. Among 2.1×10^6 phage clones in the primary library, 22 clones (designated R1–R22) showed reactivity to MGr1. On-line homology analysis revealed that these 22 inserts belonged to 12 genes (table 1). Inserts R1, R2, R9 and R14 were identical to R11, R6, R16 and R17, respectively. R10, R12 and R13 shared the same size and sequence. Because inserts R4, R5, R7, R9/R16, R20, R10/R12/R13 and R18 contained unreported coding sequences, they were submitted to an EST database. Their accession numbers in GenBank were BQ103848, BQ103849, BQ103850, BQ103851, BQ103852, BQ103853 and BQ103854, respectively. Insert R22 also contained unreported coding sequence. There was a complete open reading frame of 263 amino acid residues in R22, which was subsequently confirmed by in vitro transcription and translation [unpublished observations]. R22 was therefore submitted as a novel gene to GenBank. Its accession number was AF326778.

From the results of DNA sequence analysis, these 22 inserts could be divided into four classes: the first class consisted of inserts R1, R3, R5, R8, R11, R14, R17, R18 and R21. Inserts R1/R11, R3, R8, R14/R17 and R21 corresponded to the coding region of their homologous gene. The molecular weights of human sorting nexin 9 (R1/R11), occludin (R3), peroxisomal targeting signal receptor 1 (R8), KIAA0792 (R14/R17) and KIAA0784 (R21) protein products were much higher than 42 kDa. Similarly, the theoretical molecular weights of polypeptides coded by inserts R5 and R18 were also higher than 42 kDa. Given that the apparent molecular weight of MGr1-Ag is about 42 kDa, these seven genes could be excluded as the gene encoding MGr1-Ag.

Table 1. On-line homology analysis of 22 inserts selected from a cDNA library by monoclonal antibody MGr1.

Inserts	Sizes (bp)	Identities	Homologous genes (accession number in GenBank)
R1/R11	762	762 bp (100%)	human sorting nexin 9 (AF121859)
R2/R6	312	312 bp (99%)	HT019 (AF225424)
R3	1418	1418 bp (99%)	human occludin (NM×002538)
R4	1161	1161 bp (100%)	human genome DNA sequence localized in chromosome 12
R5	1686	1686 bp (100%)	human genome DNA sequence localized in chromosome 22
R7/R9/R15/R16/ R19/R20	1208/452/436/ 452/338bp/1019	329 bp (99%)/366 bp (100%)/ 436 bp (99%)/366 bp (100%)/ 324 bp (99%)/755 bp (98%)	human 37-kDa laminin receptor precursor (NM×002295)
R8	1601	1601 bp (100%)	peroxisomal targeting signal receptor 1 (U19721)
R10/R12/R13	522	no	no homology in GenBank
R14/R17	1597	1597 bp (97%)	KIAA0792 (AB018335)
R18	1434	no	no homology in GenBank
R21	1244	1244 bp (96%)	KIAA0784 (AB018327)
R22	1098	825 bp (98%)	FLJ11391 fis (AK021453)

The second class consisted of inserts R2 and R6. Inserts R2/R6 corresponded to nucleotides 1072–1385 of the HT019 gene. However, the coding region of the HT019 gene has been localized at nucleotides 210–1022. Obviously, HT019 was not the gene encoding MGr1-Ag.

The third class consisted of inserts R4, R10, R12 and R13. When expressed by the λgt11 vector, the translation reading encountered a stop codon at nucleotide 16 in R4 or at nucleotide 6 in R10/R12/R13. In other words, only five and two amino acid residues could be translated from inserts R4 and R10/R12/R13, respectively. To our knowledge, antigen epitopes consist of no less than seven amino acid residues. The MGr1 antibody might bind to the joint site of galactosidase-R4 or galactosidase-R10/R12/R13 fusion protein. These two genes, therefore, could be excluded as the coding gene of MGr1-Ag.

The fourth class consisted of inserts R7, R9, R15, R16, R19, R20 and R22. Although R7, R9/R16, R15, R19 and R20 were not completely identical to each other, these six inserts shared the same homologous gene, human 37-kDa laminin receptor precursor (37LRP). The reported sequence of 37LRP consisted of 1039 bp. The coding region of 37LRP has been localized at nucleotides 86–973, encoding 295 amino acid residues. The nucleotides 24–251 of insert R7 corresponded to nucleotides 712–1039 of 37LRP. Nucleotides 1–366 of insert R9/R16 corresponded to nucleotides 674–1039 of 37LRP. Nucleotides 1–436 of insert R15 corresponded to nucleotides 563–998 of 37LRP. Nucleotides 1–324 of insert R19 corresponded to nucleotides 716–1039 of 37LRP. Nucleotides 1–756 of insert R20 corresponded to nucleotides 284–1039 of 37LRP. When expressed by λgt11 vector, all polypeptides encoded by these six inserts corresponded to the C-terminal fragment of 37LRP. These results strongly suggested that 37LRP could be recognized by the MGr1 antibody. In addition, insert R22 encoded a polypeptide of 263 amino acid residues, which could also be recognized by the MGr1 antibody.

Taking these results of sequence analysis together, we drew the conclusion that 37LRP (R7/R9/R15/R16/R19/R20) and R22 were candidate coding genes of MGr1-Ag.

Preparation and characterization of antiserum

The R20 cDNA fragment was chosen as representative of 37LRP for further characterization because it had a longer coding sequence than the other five inserts. R20 and R22 cDNA fragments were expressed by pQE30 vector in *E. coli* M15. The protein products were isolated by polyacrylamide gel electrophoresis and used as immunogens to elicit antibody in BALB/c mice. Mouse anti-R20 and anti-R22 sera were harvested and characterized with Western blot. As shown in figure 2, anti-R20 serum reacted with a unique protein band that was consistent with MGr1-Ag, while anti-R22 serum reacted with a unique protein band of about 60 kDa. These data strongly suggested that R20 (37LRP) but not R22 was likely to be the gene encoding MGr1-Ag.

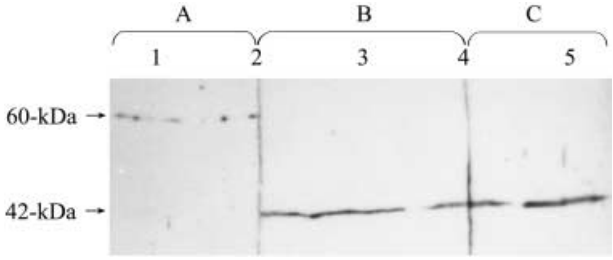

Figure 2. Characterization of antisera by Western blot analysis. Total cellular proteins from SGC7901/VCR cells were separated by SDS-PAGE. After electrotransferring and staining with ponceau S, the nitrocellulose filter was divided into three sheets. Both lane 2 and lane 4 were divided equally into two separate sheets. Sheets A, B and C were stained with anti-R22 serum (1:1000), MGr1 (1:1000) and anti-R20 serum (1:1000), respectively.

RT-PCR

RT-PCR was performed to clone the coding gene of MGr1-Ag. Based on the presumption that R20 (37LRP) was the coding gene of MGr1-Ag, an upstream primer corresponding to nucleotides 17–39 of 37LRP and a downstream primer corresponding to nucleotides 783–761 of R20 were used as primer pair in the PCR reaction. The known sequence of 37LRP had no binding site for the downstream primer, while the known sequence of R20 had no binding site for the upstream primer. The PCR reaction yielded a product of about 1000 bp. DNA sequence analysis revealed that this product consisted of 1050 bp. Nucleotides 1–1023 of this product were completely identical to nucleotides 17–1039 of 37LRP, and the nucleotides 1024–1050 of this product were completely identical to nucleotides 757–783 of R20. Obviously, this PCR product and 37LRP shared the same coding sequence, but their 3′-untranslated regions differed somewhat. This PCR product was identified as the coding gene of MGr1-Ag and submitted to GenBank. Its accession number was AF503367.

Down-regulation of MGr1-Ag/37LRP in gastric cancer cells

The R20 cDNA fragment was subcloned from pUCm-T vector into pcDNA3.1(+) to construct an antisense RNA expression vector. The recombinant vector pcDNA3.1-anR20 and empty vector pcDNA3.1(+) were then introduced into SGC7901/VCR cells and the G418-resistant cell clones were designated as SGC7901/VCR-anR20 and SGC7901/VCR-pcDNA3.1, respectively. Expression of MGr1-Ag in gastric cancer cells was determined by Western blot analysis using MGr1 as primary antibody. As shown in figure 3, the expression level of MGr1-Ag in SGC7901/VCR-anR20 cells was significantly lower than

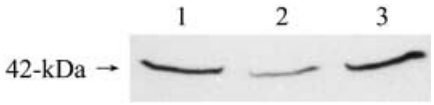

Figure 3. Expression of MGr1-Ag in gastric cancer cells. The same amount of total cellular proteins extracted from SGC7901/VCR cells (lane 1), SGC7901/VCR-anR20 cells (lane 2) and SGC7901/VCR-pcDNA3.1 cells (lane 3) was separated by SDS-PAGE and transferred to a nitrocellulose filter. MGr1 antibody (1:1000) served as primary antibody to detect MGr1-Ag.

that in SGC7901/VCR cells and SGC7901/VCR-pcDNA3.1 cells. When using anti-R20 serum as primary antibody to detect 37LRP, Western blot analysis yielded similar results. These data indicated that expression of MGr1-Ag/37LRP was down-regulated in SGC7901/VCR-anR20 cells.

In vitro drug sensitivity assay

To explore the effects of down-regulation of MGr1-Ag/37LRP on MDR phenotypes of gastric cancer cells, the sensitivities of gastric cancer cells to anticancer drugs were evaluated using the MTT assay. As shown in table 2, compared to SGC7901/VCR cells, the IC_{50} values of VCR and ADR on SGC7901/VCR-pcDNA3.1 cells decreased slightly, while the IC_{50} values of 5-Fu, mitomycin (MMC) and cisplatin (DDP) on SGC7901/VCR-pcDNA3.1 cells increased slightly. However, none of these changes were statistically significant ($p>0.05$). In comparison with SGC7901/VCR cells and SGC7901/VCR-pcDNA3.1 cells, SGC7901/VCR-anR20 cells showed significantly decreased IC_{50} values for VCR, ADR, 5-Fu and DDP ($p<0.01$). But the IC_{50} values for MMC on these three types of cells showed no significant differences ($p>0.05$). All these data suggested that down-regulation of MGr1-Ag/37LRP could partly reverse MDR phenotypes of gastric cancer cells.

Fluorescence intensity assay of intracellular ADR

The effects of down-regulation of MGr1-Ag/37LRP on ADR accumulation and retention in gastric cancer cells were explored further. As shown in figure 4, compared to

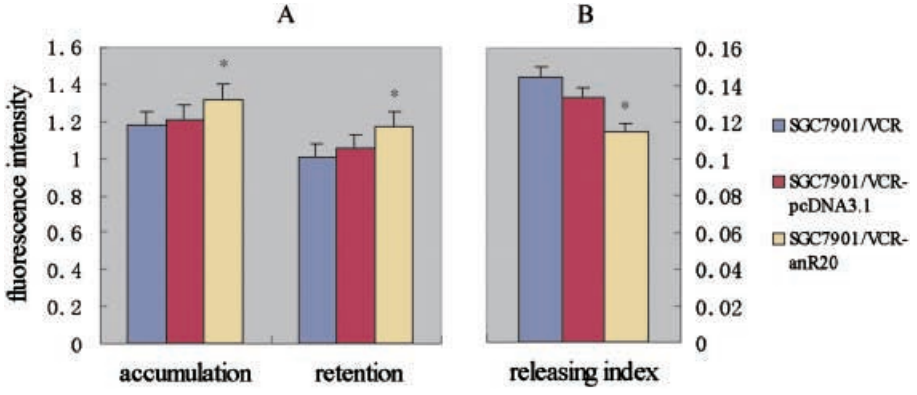

Figure 4. Fluorescence intensity of intracellular adriamycin in gastric cancer cells. Gastric cancer cells in log phase were plated into six-well plates (1×10^6 cells/well) and cultured overnight at 37 °C. After addition of adriamycin to a final concentration of 5 μg/ml, cells were cultured for a further 1 h. Cells were then trypsinized and harvested (for detection of adriamycin accumulation), or alternatively cultured in drug-free RPMI1640 for another 30 min followed by trypsinization and harvesting (for detection of adriamycin retention). Finally, cells were washed twice with cold PBS, and the fluorescence intensity of intracellular adriamycin was determined using flow cytometry with an exciting wavelength of 488 nm and a receiving wavelength of 575 nm. * $p<0.05$ vs SGC7901/VCR cells and SGC7901/VCR-pcDNA3.1 cells. (*A*) Fluorescence intensity of intracellular adriamycin. (*B*) Adriamycin releasing index of gastric cancer cells. Releasing index = (accumulation value – retention value)/accumulation value.

Table 2. The IC_{50} values (mean ± SD μg/ml) of anticancer drugs on different gastric cancer cells in an MTT assay.

	VCR	ADR	5-Fu	MMC	DDP
SGC7901	0.44±0.09	0.15±0.04	0.60±0.11	0.57±0.12	0.42±0.07
SGC7901/VCR	19.41±2.08[a]	0.51±0.11[a]	4.00±0.86[a]	1.62±0.38[a]	0.84±0.15[a]
SGC7901/VCR-pcDNA3.1	16.14±1.54	0.46±0.13	5.10±0.83	2.22±0.49	0.87±0.13
SGC7901/VCR-anR20	1.58±0.27[b]	0.11±0.01[b]	2.80±0.44[b]	1.71±0.32	0.51±0.09[b]

VCR, vincristine; ADR, adriamycin; 5-Fu, 5-fluorouracil; MMC, mitomycin; DDP, cisplatin.
[a] $p<0.01$ vs SGC7901 cells; [b] $p<0.01$ vs SGC7901/VCR cells and SGC7901/VCR-pcDNA3.1 cells.

SGC7901/VCR and SGC7901/VCR-pcDNA3.1 cells, SGC7901/VCR-anR20 cells showed slightly increased ADR accumulation and retention as well as a decreased releasing index. Statistical analysis revealed that these changes were significant ($p<0.05$). These data suggested that down-regulation of MGr1-Ag/37LRP could enhance ADR accumulation and retention in SGC7901/VCR cells.

Discussion

The present study characterized MGr1-Ag as 37LRP based on following results. First, among 22 positive inserts selected from the primary library by MGr1 antibody, six different inserts shared high homology with 37LRP. Second, antisera against 37LRP bound to a protein band that was consistent with MGr1-Ag. Third, the coding genes of MGr1-Ag and 37LRP share the same coding sequence and protein product. Fourth, down-regulation of 37LRP could partially reverse the MDR phenotype of SGC7901/VCR cells, consistent with functions of MGr1-Ag. Finally, the basal lateral distribution of human 67-kDa laminin receptor (67LR) and shedding of 67LR from cancer cells [8] are consistent with the known characteristics of MGr1-Ag.

However, the apparent molecular weight of MGr1-Ag was not consistent with the reported molecular weight of 37LRP. Mafune et al. [9] named 37LRP the 32-kDa laminin-binding protein because its deduced molecular weight was about 32 kDa. Rao et al. [10] found by in vitro translation that 37LRP mRNA encoded a 37-kDa protein in a rabbit reticulocyte cell-free translation system. Auth and Brawerman [11] and Ardini et al. [12] found that 37LRP was identical to the 40-kDa ribosomal protein p40 that lacked any laminin-binding activity. Coggin et al. [13] identified a 32- to 44-kDa oncofetal antigen as 37LRP. Our suspicion is that the difference in molecular weight of 37LRP results from different experimental conditions or a different functional status of 37LRP in cells.

37LRP has been confirmed to have diverse functions. Besides serving as a precursor to 67LR [14, 15], it has been reported to take part in the translation machinery [11, 12]. Considering these two functions of 37LRP, we speculate there are two possible ways for 37LRP to intervene with MDR in gastric cancer cells.

The first is related to interaction between cells and extracellular matrix. Teicher et al. [16] found that drug-resistant tumor cells induced in vivo became sensitized when they were removed from their host animal. Grigorieva et al. [17] demonstrated that elements of bone marrow might be a contributing factor to the resistance of myeloma cells to steroid drugs such as dexamethasone. Damiano et al. [18] believed that components within the bone marrow microenvironment are capable of modulating the tumor cell response to DNA-damaging agents, and they named this drug-resistant phenotype as cell-adhesion-mediated drug resistance (CAM-DR). Further studies revealed that cell adhesion to extracellular matrix could protect tumor cells from drug-induced apoptosis, which was associated with up-regulation of p27kip1 and Bcl-2 [19–21]. Up-regulation of 37LRP might increase the quantity of 67LR molecules, promote cell adhesion to extracellular matrix and thus enhance survival of tumor cells during drug attack.

The second way is related to the translation machinery. Bertram et al. [22] conducted comparative analysis of the gene expression profile of a doxorubicin-resistant and its corresponding parental colon carcinoma cell line (LoVo H67P) by differential display-PCR and subtractive suppressive hybridization. They found overexpression of ribosomal protein L4 and L5 and putative alternative elongation factor PTI-1 in the doxorubicin-resistant cell line. In another study, we demonstrated that up-regulation of ribosomal protein L23 and S13 was associated with the MDR phenotype of gastric cancer cells [unpublished observations]. All these data indicate that alteration of ribosomal proteins (including 37LRP) might influence the protein translation process, and then affect the response of tumor cells to anticancer drugs.

In conclusion, we have isolated the coding gene for MGr1-Ag by immunoscreening of a cDNA library. DNA homology analysis and functional characterization revealed that MGr1-Ag is identical to 37LRP. Two possible MDR mechanisms of 37LRP have been speculated, and need further elucidation.

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