EXPRESSION OF MDR1, MRP AND LRP GENES IN GASTRIC CARCINOMA AND THEIR CLINICAL SIGNIFICANCE

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ABSTRACT

Objective: To explore the expression of mdr1, multidrug resistance-associated protein (MRP) and lung resistance protein (LRP) genes in human gastric cancer and their clinical significance. Methods: The mdr1 mRNA was assayed by RT-PCR, the MRP and LRP were detected by flow cytometry. Results: The positive rate of mdr1 mRNA was 44.4% (12/27), and the mean MRP and LRP expression were independent upon patient histologic type, nodal involvement, and TNM stage. The mdr1 mRNA expression in patients with serosa invasion was 30.0% (6/20), much lower than that without serosa invasion (85.7%). Conclusion: The multidrug resistance cells are present in primary gastric carcinomas prior to chemotherapy, and analysis of mdr1 gene, MRP, LRP may have guiding significance in the treatment of gastric carcinoma.

Key words: Stomach cancer, Adenocarcinoma, Drug resistance, Multidrug resistance-associated protein, Lung resistance protein

Multidrug resistance (MDR) of tumor cells to anticancer drugs is a major problem in chemotherapy, and the chief mechanism responsible for MDR is the overexpression of P-gp which is encoded by mdr1 gene.[1] In addition to P-gp, two other MDR-related proteins MRP and LRP have been identified.[2,3] In this paper, the expression of mdr1 gene, MRP and LRP in human gastric carcinoma (GC) and their significance were studied.

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MATERIALS AND METHODS

Specimens

GC tissues were obtained from 27 consecutive patients (17 men and 10 women) who underwent gastrectomy in the Department of General Surgery, the First Affiliated Hospital of Shandong Medical University from September 1997 to January 1998. All GC tissues resected were diagnosed as adenocarcinomas. No patient in this study had undergone chemotherapy before surgery.

RNA Extraction and RT-PCR

Total cellular RNA was extracted by the acid guanidine isothiocyanate method.[4] Using RT-PCR Kit, the fragments of mdr1 and β_2-microglobulin (β_2-MG, interal control) genes were amplified as described in literature.[5] The tissue in which coexists a 157-bp mdr1 fragment and a 114-bp β_2-MG fragment is mdr1 positive, and the one which has a single 114-bp fragment is mdr1 negative. Whenever the tissues were examined, a positive and negative specimen, which the Kit contained, was set up as control.

Flow Cytometry (FCM)

The MRP and LRP were measured in 14 specimens by FCM as described by Yin, et al.[6] the peripheral blood monocytes of healthy blood donors were measured as negative controls, and the adjacent mucosa as the normal controls. The specific MRP and LRP monoclonal antibodies QCRL-1, LRP-56 were kindly supplied by Dr. SPC Cole (Queen's University, Ontario, Canada) and Dr. RJ Scheper (Free University Hospital, Amsterdam, the Netherlands).

RESULTS

Mdr1 mRNA Expression

The mdr1 mRNA was positive in 12 (44.4%) and negative in 15(55.6%) GC. The clinical data of the patients are summarized in Table 1. The histologic type, lymph node involvement and TNM classification were not different between patients with mdr1 mRNA-negative tumors and those with mdr1 mRNA-positive tumors. With respect to tumor invasion, the percentage of mdr1 mRNA positive expression was lower in patients with serosa invasion than those without serosa invasion.

Table 1. mdr1 mRNA expression and clinicopathological variables

	mdr1 mRNA(+)	mdr1 mRNA(−)
Histologic type		
Adenocarcinoma	9	11
Mucoid carcinoma	3	4
Nodal involvement		
LN(+)	4	9
LN(−)	8	6
Serosa invasion		
−	6	1*
+	6	14
TNM stage		
I	3	1
II	5	4
III	4	6
IV	0	4

*$P<0.05$, *vs* nodal involvement(+).

MRP and LRP Expression

The mean MRP positive rate of negative controls was 1.89% ±0.11%, of normal controls was 11.9% ± 2.5%, of the tumor specimens was 39.1% ± 13.4%, and the mean LRP positive rate was 1.72% ± 0.23%, 16.9% ± 7.5%, and 29.9% ± 9.8%, respectively. The distribution of positive tumor cells for MRP or LRP are shown in Table 2, and the difference between the MRP or LRP expression and pathologic parameters were not significant.

Table 2. The distribution of MRP and LRP positive cells

	0-20%	21%-40%	41-60%	>70%
MRP	1	6	6	1
LRP	2	10	2	0

DISCUSSION

MDR, which is characterized by cross-resistance to a number of structurally and functionally unrelated compounds, is a major obstacle to successful cancer chemotherapy and has been closely associated with treatment failure. MDR arises through a variety of mechanisms. The best characterized mechanism responsible for MDR is the overexpression of the mdr1 gene, which encodes a 170-kD transmembrance P-glycoprotein (P-gp) that acts as an ATP-dependent drug efflux pump, increasing transport of various anticancer compounds out of cells and decreasing cellular accumulation of drugs and, thus, their efficacy. Like P-gp, MRP is a member of the ATP-binding cassette family transporter proteins, and confers resistance by decreasing the intracellular concentration of drugs. LRP is the human major vault protein;[7] its mechanism conferring MDR is still unknown. But concerning the reduced nuclear accumulation of daunorubicin in the LRP-overexpressing MDR cell line 2R120 and the evidence supporting a role of vaults as transporter unit of the nuclear pore complexes, it is tempting to hypothesise that LRP can mediate drug resistance by regulating both the cytoplasmic redistribution and the nucleocytoplasmic transport of drugs.[8]

Our study suggested that the GC has a high intrinsic drug resistance. This may explain why the chemotherapy has an unsatisfactory effect on GC. So, if the tumor express the mdr1, MRP or LRP gene, the patient should avoid using the MDR-associated drugs. Analysis of these products can be helpful to determine a reasonable treatment program. Meanwhile, the analysis of them maybe acts as indexes for using the MDR reversing agents, which overcome the MDR by increasing the intracellular drug accumulation of cancer cells. The coexpression of them demonstrated that the MDR can be mediated by them simultaneously, and combined administration of different MDR reversing agents could achieve a better effect.

In our study, we also investigated the relationship between the mdr1 mRNA, MRP and LRP expression and the clinicopathological variables. The percentage of mdr1 mRNA positive expression in patients with serosa invasion was 30.0%, much lower than those without serosa invasion (85.7%), and with respect to TNM stages, the percentage of mdr1 mRNA expression exhibited a descending tendency: I 75%, II 55.6%, III 40.0% and IV 0. The data indicated that the absence of mdr1 mRNA expression seems to correlate with advanced disease, although we did not observe an association of mdr1 gene expression with lymph node involvement. The similar results were obtained in a recent study, which demonstrated that GC xenografts in nude mice expressed lower levels of mdr1 RNA than the primary tumors from patients. One explanation for this finding would be that MDR cells display reduced tumorigenicity.[9] Therefore, further study should be done to confirm whether the mdr1 gene expression could act as an independent

prognosis factor.

REFERENCES

[1] Fujii H, Tanigawa N, Muraoka R, et al. Clinical significance of multidrug resistance and P-glycoprotein expression in patients with gastric carcinoma. J Surg Oncol 1995; 58:63.

[2] Cole SPC, Bhardwaj G, Gerlach JH, et al. Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. Science 1992; 258: 1650.

[3] Scheper RJ, Broxterman HL, Scheffer GL, et al. Overexpression of a Mr. 110,000 vescular protein in non-P-glycoprotein-mediated multidrug resistance. Cancer Res 1993; 53: 1475.

[4] Chomezynski P, Sacchi N. single-step method of RNA isolation by acid guanidinium thiocyanate-phenal-chloroform extraction. Anal Biochem 1987; 162: 156.

[5] Wang S, Jin XQ, Yang CZ, et al. The mdr1 gene expression in children with solid malignant tumors. Chin J Pediatr Surg 1997; 18: 259.

[6] Yin GP, Sun XM, Chen SF. Clinical significance of getermination of multidrug resistant gene expression by flow cytometry in malignant solid tumors. Chin J Clin Oncol 1998; 25: 489.

[7] Scheffer GL, Wijingaard PLJ. Flens MJ, et al. The drug resistance-related protein LRP is the human major vault protein. Natl Med 1995; 1: 578.

[8] Izquierdo MA, scheffer GL, flens MJ, et al. Major vault protein LRP-related multidrug resistance. Eur J Cancer 1996; 32A: 979.

[9] Mizoguchi T, Yamada K, Furukawa T, et al. Expression of MDR1 gene in human gastric and colorectal carcinomas. J Natl Cancer Inst 1990; 82: 1679.