Multidrug resistance 1 gene expression in Indian patients with gastric carcinoma

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Background: Gastric carcinoma is frequently refractory to chemotherapy. The multidrug resistance 1 gene (MDR1) encodes for a protein (p-glycoprotein) that functions as a drug efflux pump and thus contributes to resistance to chemotherapeutic agents. Methods: We studied gastric tissues from 28 patients with gastric cancer for MDR1 expression, using immunohistochemistry. Results: Sixteen (57%) of 28 cases showed MDR1 expression. Sections of normal mucosa away from the tumor showed perinuclear staining for MDR1 in surface epithelial cells, whereas tumor cells showed diffuse cytoplasmic positivity. Conclusions: Over one half of gastric carcinoma specimens at our center show MDR1 gene expression.

Key words: Cancer chemotherapy, MDR1

Gastric carcinoma has proven to be refractory to chemotherapy. Three genes are known to be related to drug resistance: the multidrug resistance genes (MDR), multidrug resistance protein (MRP) and lung resistance gene (LRP). Of these the best characterized gene is the multidrug resistance 1 (MDR1) gene. This gene encodes for a 170 kD glycoprotein called p-glycoprotein, which can be expressed in both normal and tumor tissues. This protein functions as a drug efflux pump and promotes efflux of certain chemotherapeutic agents. MDR1 expression in tissue can be detected by immunohistochemistry and by molecular methods. We studied MDR1 expression in 28 cases of gastric carcinoma using an immunohistochemical method.

Methods
Tissue specimens obtained from 28 patients with gastric carcinoma (aged 47-72 years, mean 57.9; 22 men) admitted to the Government General Hospital and Government Royapettah Hospital, Chennai were studied. Of these, 19 were formalin-fixed paraffin-embedded surgical resection specimens and 9 were endoscopic biopsies. Hematoxylin and eosin-stained sections were examined and the tumors were classified as per the WHO criteria and graded according to the criteria of Compton et al. Immunohistochemistry was performed using a highly-sensitive avidin-biotin-peroxidase complex (ABC) technique in conjunction with primary monoclonal antibody to MDR1. In nine cases, sections of normal mucosa away from the tumor were also available and were examined similarly.

Briefly, the sections were dewaxed and antigen retrieval was done by pressure cooking of sections in citrate buffer, pH 6.0 for 2 min. Sections were then incubated with primary antibody (clone JSB1; Zymed, San Francisco, USA), followed by biotinylated secondary antibody (diluted 1:100 dilution; Dako, Copenhagen, Denmark) and then ABC (Dako). Finally, the sections were immersed in a substrate solution containing aminomethane carbazole (Sigma, USA) as chromogen, counterstained with hematoxylin (Qualigens, Mumbai) and mounted in aqueous mount (glycerol gelatin). Extensive washing in phosphate buffer solution was carried out between steps. Endogenous peroxidase activity was blocked before addition of primary antibody, using 3% H2O2 methanol. A specimen was considered positive if more than 5% of cells showed reddish-brown staining.

Sections from a block of normal renal tissue, obtained at autopsy, were used as positive controls, one section being incorporated into every batch of slides being immunostained; the luminal surface of the tubules showed strong positivity for MDR1 protein. Negative controls were also included in every batch of slides being stained. Each section was examined and scored independently by two observers (SR and PS); in case of discordance, a mutual consensus was reached by discussion.

Results
Of the 28 tissue specimens examined, 16 (57%) stained positive for MDR1; the proportion of cells that stained positive was 10% to 60%. MDR1 positivity was observed in 5 of 7 grade I tumors, 4 of 4 grade II tumors, 1 of 2 grade III lesions and 6 of 15 grade IV tumors. The Table shows the relationship of MDR1 positivity rate with histological subtype of the tumor.

The positivity was cytoplasmic and was diffuse and granular (Figs. 1 and 2). In nine specimens in whom normal mucosa was also examined, surface epithelial cells showed positivity localized to the perinuclear cytoplasm.

In 25 of 28 patients, the two observers reached a concordant conclusion about positivity or negativity of the MDR1 staining.
Table: MDR1 positivity in histological subtypes of gastric carcinoma

<table>
<thead>
<tr>
<th>Histological subtype</th>
<th>MDR1 positive cases (n)</th>
<th>Total no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubular</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Tubulopapillary</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Mucinous</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Small cell</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td>Unclassified</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Discussion

In patients with gastric carcinoma, chemotherapy provides complete response in fewer than 15% of patients. Many chemotherapeutic agents, such as doxorubicin and etoposide, are rendered less effective by the presence of p-glycoprotein, the product of MDR1 gene. Expression of MDR1 gene has thus been widely studied in gastric tumors. However, there are little data on MDR1 in Indian patients with gastric carcinoma.

In our study, 16 of 28 (57%) cases showed MDR1 expression. In previous studies, positivity rates of 10% to 80% have been reported. The reasons for these wide differences are not clear. The difference may be related to geographical factors or may be due to technical differences, like variations in the efficiency of antigen-retrieval techniques used to unmask antigenic epitopes that get masked during formalin fixation. Studies reporting lower positivity rates did not use antigen-retrieval methods. The differences could also be due to differences in sensitivity rates of various molecular techniques used, like in situ hybridization and polymerase chain reaction.

MDR1 protein or p-glycoprotein is expressed in a wide range of normal tissues. There is evidence that this protein is involved in secretion of metabolites and toxic substances into various excretory paths, including bile, urine and gastrointestinal tract. The pattern of MDR1 positivity in tumor cells was different from that in normal gastric tissue. Normal surface epithelial cells showed perinuclear positivity whereas tumor cells showed diffuse cytoplasmic staining. The pattern of staining observed by us was similar to that reported by others. All four patients with grade II carcinoma were positive for the MDR1 gene product. This finding has also been reported by other authors; however, all tissues from grades III and IV tumors did not express the gene. MDR1 expression has been found to be an independent prognostic factor in gastric carcinoma and the expression of p-glycoprotein was found to be associated with poorer cumulative survival. We do not have follow-up data on response to chemotherapy or survival in our patients since these patients were transferred to regional cancer centers or discontinued treatment for other reasons.

In conclusion, over half the gastric carcinoma specimens show MDR1 gene expression. Larger prospective studies with detailed follow up of treatment response are required to establish the relationship of MDR1 gene expression with response to chemotherapy in Indian patients with gastric carcinoma.

References

Fig 1: MDR1 positivity in tumor cell cytoplasm in moderately differentiated gastric carcinoma (ABC, 250X).

Fig 2: Poorly differentiated gastric carcinoma: cytoplasmic positivity for MDR1 in individual tumor cells (arrows) (ABC, 630X).

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