Allele polymorphisms of tumor integrins correlate with peritoneal carcinosis capability of gastric cancer cells in radically resected patients

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Background: Preclinical studies suggested that integrins are relevant for gastric cancer diffusion. We investigated integrins polymorphisms role in determining peritoneal carcinosis or hematogenous metastases in radically resected gastric cancer.

Patients and methods: Integrins genotyping was carried out on pT3 radically resected gastric tumors recurring with either peritoneal-only carcinosis or hematogenous metastases.

Results: The following factors resulted independently associated with peritoneal carcinosis or hematogenous metastases: the A genotype of rs2269772 (ITGA3) [odds ratio (OR) for peritoneal carcinosis: 22.2, 95% confidence interval 1.2–40, \( P = 0.03 \), the G genotype of rs2269772 (ITGA3) (OR for hematogenous metastases: 5.5, 95% confidence interval 2.2–14.15, \( P = 0.0003 \), the C genotype of rs11902171 (ITGV) (OR for peritoneal carcinosis: 6.8, 95% confidence interval 1.3–33.4, \( P = 0.01 \), the G genotype of rs11902171 (ITGV) (OR for hematogenous metastases: 2.5, 95% confidence interval 1.1–5.7, \( P = 0.02 \), diffuse histology (OR for peritoneal carcinosis: 4.7, 95% confidence interval 1.9–11.3, \( P = 0.0005 \)) and intestinal histology (OR for hematogenous metastases: 4.2, 95% confidence interval 1.9–9.9, \( P = 0.0008 \)).

Conclusions: Tumor histology represents a crucial issue conditioning tumoral behavior; genotyping of rs2269772 (ITGA3) and rs11902171 (ITGV) may be a further asset in the definition of high-risk patients for peritoneal carcinosis among those relapsing after curative resection. The selection tool deriving from this analysis may allow an optimal use of innovative treatment strategies.

Key words: gastric cancer, genotyping, peritoneal carcinosis, tumor integrins

introduction

Although many advances have been made in the diagnosis and treatment of gastric cancer, the global outcome even for radically resected patients is still disappointing with a 5-year survival rate ranging between 15% and 35% of all cases in Western Countries [1, 2].

Peritoneal carcinosis may be the first site of relapse in ~40% to 50% of patients undergoing radical resection and in virtually all patients, this clinical condition also represents a crucial event irreparably affecting prognosis [3]. As a consequence, median overall survival for these patients does not usually exceed 6 months with no survival at 5 years [3–5]. Based on these data in the last few years, a major role for i.p. chemotherapy (with or without hyperthermia) for the prevention of subsequent peritoneal carcinomatosis has been hypothesized. In fact, a recently published meta-analysis of clinical trials investigating i.p. chemotherapy suggested that this treatment strategy may be able to improve overall survival of radically resected gastric cancer patients [4]. However, many shortcomings related to this procedure prevented it from becoming widely accepted, particularly when we consider that a further critical spreading route for gastric cancer is represented by hematogenous dissemination [4–6]. Although many clinical determinants potentially able to predict the risk of peritoneal dissemination have been analyzed in the past, only few of them proved to be effectively relevant. Among the others, a prominent role is ascribable to factors such as tumor serosal involvement, tumor histology (diffuse versus intestinal) and the presence of tumor cell in peritoneal lavage during laparotomy [3, 7, 8]. However, none of these factors seemed to
possess the necessary potential for an accurate prediction of peritoneal-limited tumor diffusion, leaving the critical need for patients selection in this setting partially unanswered. At the molecular level, preclinical studies suggested that integrins may play a critical role in the biological process leading to gastric cancer diffusion both local and distant [9–11]. Integrins belong to the family of transmembrane glycoprotein heterodimer which mediates adhesion of neighboring cells and participate in the growth and repair of cells as important receptors of extracellular matrix proteins [9–11]. Biological findings from molecular studies have demonstrated that the initial steps for cancer invasive growth and metastasis is mainly determined by the interaction between tumor integrins on tumor cell surface to extracellular matrix [9, 12].

Preclinical analyses also suggested that different expression of cancer integrins may be correlated to a different likelihood of developing peritoneal carcinosis [12–17]. Nishimori et al. [13] selected a gastric tumor cell line showing high peritoneal potential and found out that these tumor cells preferentially overexpressed α1 to α6 integrins in contrast to the parental cell line with a low peritoneal diffusion capability. These data not only confirmed that tumor integrins are relevant in the cancer metastatic process but more importantly they suggested that integrins expression pattern in cancer cells is crucial in determining the metastatic site itself.

Further in vitro studies in gastric cancer cells also showed that treatment with functional blocking antibody to tumor integrins was able to elicit a decline in the peritoneal dissemination [18]. Analogously, a subsequent study demonstrated that the transforming growth factor β receptor inhibitor A-77 was able to decrease the adhesion ability of scirrhous gastric cells to the peritoneum by decreasing the expression of α2, α3 and α5 tumor integrins [19]. Globally, these latter analyses confirmed the possibility that tumor integrins may be important for the subsequent peritoneal diffusion of cancer.

Our study investigated the role of tumor integrins polymorphisms in determining either peritoneal carcinosis or hematogenous metastases in radically resected gastric cancer with the aim to suggest a biological integrin-based profile to be employed as a tool for the appropriate treatment selection in the appropriate patient.

patients and methods

patients selection

The patients study population was selected from a central database including patients affected by gastric cancer, operated in four different institutions. Classification of the T, N and M factors was made according to the numeric system introduced by the 5th tumor nodes metastasis.

Only patients who had recurred with either peritoneal-only carcinosis or hematogenous metastases after curative gastrectomy for a PT3 gastric adenocarcinoma and whose tumor specimen was available were selected for integrins polymorphisms analysis. Patients with either distant metastases at the time of diagnosis or presence of exfoliated tumor cells in peritoneal lavage obtained during laparotomy were excluded from analysis.

This analysis was approved by our local ethical committee.

tumor integrins genotyping

Integrins genotyping was carried out on formalin-fixed paraffin-embedded tissue block (~30 mg) of primary gastric cancer samples.

Paraffin wax was removed with xylene and the samples were washed twice with 100% ethanol. DNA was isolated from the deparaffinized tissue using the RecoverAll™ Total Nucleic Acid Isolation Kit for FFPE Tissues (Applied Biosystems, Foster City, CA), according to the manufacturer’s instructions. DNA from each sample was then eluted in 120 μl of eluting solution.

Single nucleotide polymorphisms (SNPs) within each gene were selected using the Pupasuite software (http://pupasuite.bioinfo.cifp.es/index.jsf—version 2.0.0, bioinfo 2008), the CIPF Single Nucleotide Polymorphism database generated by the National Center for Biototechnology Information (http://www.ncbi.nlm.nih.gov/SNP) and by review of the medical literature, using the following criteria:

- the polymorphism had some degree of likelihood to alter the structure or the expression of the gene in a biologically relevant manner (i.e. affecting base sequences, 3' untranslated region (3' UTR) or promoter region);
- the minor allele frequency was above 10% (with the only exception of rs2269772);
- the genetic polymorphism was established and well-documented.

Selected SNPs were as follows: two polymorphisms in the ITGA2 gene (rs1128643, C>T and rs1109526, A>G), three in ITGA3 (rs199147, C>G; rs2301628, C>T and rs2269772, A>G), two in ITGA6 (rs17664, G>A and rs2293649, G>A); four in ITGB1 (rs1902171, G>C; rs2595389, A>G; rs1448424, A>G and rs3738919, C>A) and two in ITGB5 (rs2291079, G>C and rs26767, T>C).

Further considerations drove the selection of SNPs for our study. A correlation between the presence of a specific allele on a polymorphic site and the expression of the respective protein has been previously documented for integrins genes [20, 21]. SNPs in regulatory sequences, such as introns and 5' and 3' UTRs, have been shown to alter messenger RNA (mRNA) stability [22, 23] processing efficiency [24], isoform expression [25, 26] and localization. They have also been shown to induce epigenetic changes [22, 24]. Moreover, regulatory motif sequences within the 3' UTR of mRNAs have been shown to affect the stability of the message and/or its translational efficiency [26]. Thus, it can be argued that SNPs in these sequences may influence integrins gene expression. Also on these bases, we selected the SNPs known to affect integrin expression and those located in regulatory sequences, for which a putative role in protein regulation can be assumed.

Chromosomal locations and positions of investigated gene SNPs are depicted in Table 1.

SNP genotyping was carried out by TaqMan technology, using SNP genotyping products (Applied Biosystems). Polymerase chain reaction (PCR) was carried out and genotypes were analyzed on the 7300 Real-Time PCR System (Applied Biosystems) using an ABI Prism 7300 Sequence Detection System software (version 1.3.1; Applied Biosystems). Each reaction contained 0.2 μl of total genomic DNA. Genotyping was carried out by laboratory personnel blinded to patient status, and a random 10% of the samples were repeated to validate genotyping procedures.

statistical analysis

Statistical analysis was carried out with the MedCalc software version 10.4.8 for Windows.

The association between categorical variables was estimated by chi-square test.

Logistic regression analysis was used to assess the independent role of variables resulted significant at univariate analysis.
Table 1. Chromosomal locations, positions and biological effects of investigated gene SNPs

<table>
<thead>
<tr>
<th>SNP ID</th>
<th>Gene</th>
<th>Chromosome no.</th>
<th>Chromosomal position</th>
<th>Position in the gene/effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1126643</td>
<td>ITGA2</td>
<td>5</td>
<td>52347369</td>
<td>Syn; ESE</td>
</tr>
<tr>
<td>rs1109526</td>
<td>ITGA2</td>
<td>5</td>
<td>52387323</td>
<td>3’ UTR</td>
</tr>
<tr>
<td>rs2269772</td>
<td>ITGA3</td>
<td>17</td>
<td>48149386</td>
<td>Syn; ESE</td>
</tr>
<tr>
<td>rs199147</td>
<td>ITGA3</td>
<td>17</td>
<td>48135567</td>
<td>Intronic</td>
</tr>
<tr>
<td>rs2301628</td>
<td>ITGA3</td>
<td>17</td>
<td>48148413</td>
<td>Intronic</td>
</tr>
<tr>
<td>rs17666</td>
<td>ITGA6</td>
<td>2</td>
<td>173369231</td>
<td>3’ UTR</td>
</tr>
<tr>
<td>rs2293649</td>
<td>ITGA6</td>
<td>2</td>
<td>173352103</td>
<td>Syn; ESE</td>
</tr>
<tr>
<td>rs3738919</td>
<td>ITGAV</td>
<td>2</td>
<td>187521260</td>
<td>Intronic</td>
</tr>
<tr>
<td>rs11902171</td>
<td>ITGAV</td>
<td>2</td>
<td>187543227</td>
<td>3’ UTR</td>
</tr>
<tr>
<td>rs1448424</td>
<td>ITGAV</td>
<td>2</td>
<td>187507972</td>
<td>Intronic</td>
</tr>
<tr>
<td>rs2595389</td>
<td>ITGAV</td>
<td>2</td>
<td>187534183</td>
<td>Intronic</td>
</tr>
<tr>
<td>rs2676</td>
<td>ITGB5</td>
<td>3</td>
<td>124481987</td>
<td>3’ UTR</td>
</tr>
<tr>
<td>rs2291079</td>
<td>ITGB5</td>
<td>3</td>
<td>124483089</td>
<td>Intronic</td>
</tr>
</tbody>
</table>

tested variables included sex (male versus female), age (<65 versus ≥65 years), absence or presence of lymph node metastases (pN0 versus pN+), type of lymphadenectomy (D1 versus D2), tumor histology according to Lauren’s classification (intestinal versus diffuse), lymphatic/blood vessels invasion (presence versus absence) and each integrins polymorphism.

The ratio of the odds of the outcome [odds ratio (OR)] in the two groups (peritoneal carcinosis only and hematomagenous metastases) was calculated with a 95% confidence interval. A significant level of 0.05 was chosen to assess the statistical significance.

All polymorphisms were examined for deviation from Hardy–Weinberg equilibrium using the Powermarker v. 3.25 package (http://statgen.ncsu.edu/powermarker/).

Linkage disequilibrium (LD) analysis was also carried out using the Powermarker v. 3.25 package (www.statgen.ncsu.edu/powermarker). LD was estimated using r2, with r2 <0.33 suggesting absence of LD.

results

patients characteristics

Ninety-nine patients were available for our analysis: 64 males and 35 females with a median age at diagnosis of 65 years (range 36–80). Follow-up data were available for all patients included. Follow-up consisted of physical examination, a complete blood count, chest radiography and US of the abdomen or computed tomography/magnetic resonance imaging scanning as clinically indicated. Only patients who exclusively developed either peritoneal carcinosis or hematogenous metastases during the entire course of their disease were considered.

All patients underwent radical surgery (R0) for pT3 gastric cancer; regional lymphnodes were negative for metastases (pN0) in 22 patients (22%). Most of the patients underwent a D2 lymphadenectomy (91 cases, 92%). Pathology report showed intestinal histology in 55 cases (55%) and diffuse histology in 37 patients (37%). Other histology subtypes were diagnosed in the remaining seven cases (8%) (Table 2). Forty-four patients (45%) developed peritoneal carcinosis-only tumor diffusion, whereas hematogenous metastases were diagnosed in the remaining 55 patients (55%).

Clinicopathological variables of both groups (carcinosis-only and hematogenous metastases) were comparable with the exception of tumor histology. All major clinical and pathological characteristics are summarized in Table 1.

tumor integrins genotyping

Patients tumors with the G genotype of rs2269772 (ITGA3) resulted less prone to peritoneal carcinosis (peritoneal carcinosis versus hematogenous metastases: 48% and 84% of patients, respectively, P = 0.0003), whereas the A genotype of rs2269772 (ITGA3) was more frequently associated to peritoneal carcinosis (peritoneal carcinosis versus hematogenous metastases: 16% and 0% of patients, respectively, P = 0.007). The C genotype of the ITGAV SNP rs2291079 was significantly less present in tumor of patients with peritoneal carcinosis (peritoneal carcinosis versus hematogenous metastases: 43% and 71% of patients, respectively, P = 0.009), whereas the G/C genotype of the ITGB5 SNP rs2291079 was significantly less present in tumor of patients with peritoneal carcinosis (peritoneal carcinosis versus hematogenous metastases: 43% and 22% of patients, respectively, P = 0.003).

The T genotype of rs22676 (ITGB5) was significantly linked to hematogenous metastases (peritoneal carcinosis versus hematogenous metastases: 53% and 80% of patients, respectively, P = 0.006). The C genotype of rs11902171 (ITGV) was more frequently associated to peritoneal carcinosis (peritoneal carcinosis versus hematogenous metastases: 20% and 4% of patients, respectively, P = 0.002). On the contrary, patients with the G genotype of rs11902171 in the ITGV gene

Table 2. Patients characteristics

<table>
<thead>
<tr>
<th>Patients</th>
<th>Peritoneal carcinosis</th>
<th>Hematogenous metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>44 (45%)</td>
<td>55 (55%)</td>
</tr>
<tr>
<td>Age (range)</td>
<td>67 (36–80)</td>
<td>65 (38–78)</td>
</tr>
<tr>
<td>Sex, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>29 (66%)</td>
<td>35 (64%)</td>
</tr>
<tr>
<td>Females</td>
<td>15 (34%)</td>
<td>20 (36%)</td>
</tr>
<tr>
<td>Stage at diagnosis, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pT3 pN0 M0</td>
<td>10 (23%)</td>
<td>12 (22%)</td>
</tr>
<tr>
<td>pT3pN1 M0</td>
<td>18 (41%)</td>
<td>24 (44%)</td>
</tr>
<tr>
<td>pT3pN2 M0</td>
<td>12 (27%)</td>
<td>14 (25%)</td>
</tr>
<tr>
<td>pT3pN3 M0</td>
<td>4 (9%)</td>
<td>5 (9%)</td>
</tr>
<tr>
<td>Tumor histology, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestinal</td>
<td>16 (36%)*</td>
<td>39 (71%)*</td>
</tr>
<tr>
<td>Diffuse</td>
<td>25 (57%)*</td>
<td>12 (22%)*</td>
</tr>
<tr>
<td>Others</td>
<td>3 (7%)</td>
<td>4 (7%)</td>
</tr>
<tr>
<td>Lymphatic/blood vessels invasion, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>6 (14%)</td>
<td>10 (18%)</td>
</tr>
<tr>
<td>Absent</td>
<td>38 (86%)</td>
<td>45 (82%)</td>
</tr>
</tbody>
</table>

*P = 0.00008; †P = 0.001.
developed peritoneal carcinosis less frequently than hematogenous metastases (peritoneal carcinosis versus hematogenous metastases: 41% and 64% of patients, respectively, \( P = 0.04 \)) (Table 3). Analysis of the remaining integrins polymorphisms did not show any correlation with either peritoneal carcinosis or hematogenous metastases.

**other clinical/pathological factors**

Among the other tested variables, only tumor diffuse histology showed a correlation with peritoneal carcinosis (peritoneal carcinosis versus hematogenous metastases: 57% and 22% of patients, respectively, \( P = 0.001 \)), whereas tumor intestinal histology was linked to hematogenous metastases (peritoneal carcinosis versus hematogenous metastases: 36% and 71% of patients, respectively, \( P = 0.001 \)) (Table 2). OR for all factors analyzed have been summarized in Table 4.

**multivariate analysis**

At multivariate analysis, A and G genotypes of rs2269772 (ITGA3), G and C genotypes of rs11902171 (ITGV) and tumor histology showed to independently correlate with either peritoneal or hematogenous metastases (\( P = 0.001 \)). OR results for these latter factors confirmed their role in guiding tumor cells through the metastatic process toward the peritoneum or hematogenous sites (Table 3). Among the factors resulted independently correlated with metastatic diffusion site, interestingly the odd ratio (OR) of genotype A of ITGA3 for peritoneal carcinosis was 22.2, considerably superior to OR for peritoneal carcinosis deriving from diffuse histology.

**Hardy–Weinberg equilibrium and LD**

The frequencies of the tumor integrins genotypes resulted comparable with those reported in Caucasians, with no significant deviation from the Hardy–Weinberg equilibrium.

LD was not observed for the tumor integrins genotypes resulted independently correlated with either peritoneal or hematogenous metastases (rs2269772 and rs11902171).

**conclusions**

The (next) introduction of innovative therapeutic strategy (i.e., chemotherapy and the anti-epithelial cell adhesion molecule monoclonal antibody catumaxomab) for the treatment of peritoneal carcinosis from gastric cancer has reopened the question of optimal patients selection for such approaches [3–6]. A critical limiting factor for the putative potential of these novel treatment options is represented by the occurrence of hematogenous metastases. The containment of disease with a locoregional treatment may be in fact inadequate in patients at high risk for hematogenous metastases and should be better

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**Table 3.** Tumor integrins polymorphisms associated with either peritoneal-only diffusion or hematogenous metastases at univariate analysis

<table>
<thead>
<tr>
<th>Tumor integrins polymorphisms</th>
<th>G</th>
<th>A</th>
<th>A/G</th>
<th>ND</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peritoneal carcinosis, ( n ) (%)</strong></td>
<td>21 (48%)</td>
<td>7 (16%)</td>
<td>13 (29%)</td>
<td>5 (7%)</td>
</tr>
<tr>
<td><strong>Hematogenous metastases, ( n ) (%)</strong></td>
<td>46 (84%)</td>
<td>0 (0%)</td>
<td>7 (12%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>( P )</td>
<td>0.0003</td>
<td>0.007</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tumor integrins polymorphisms</th>
<th>C</th>
<th>G</th>
<th>G/C</th>
<th>ND</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peritoneal carcinosis, ( n ) (%)</strong></td>
<td>19 (43%)</td>
<td>2 (5%)</td>
<td>19 (43%)</td>
<td>4 (9%)</td>
</tr>
<tr>
<td><strong>Hematogenous metastases, ( n ) (%)</strong></td>
<td>39 (71%)</td>
<td>2 (4%)</td>
<td>12 (22%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>( P )</td>
<td>0.009</td>
<td>ns</td>
<td>0.003</td>
<td>ns</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tumor integrins polymorphisms</th>
<th>C</th>
<th>T</th>
<th>C/T</th>
<th>ND</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peritoneal carcinosis, ( n ) (%)</strong></td>
<td>4 (9%)</td>
<td>23 (53%)</td>
<td>13 (29%)</td>
<td>4 (9%)</td>
</tr>
<tr>
<td><strong>Hematogenous metastases, ( n ) (%)</strong></td>
<td>1 (2%)</td>
<td>44 (80%)</td>
<td>8 (14%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>( P )</td>
<td>ns</td>
<td>0.006</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tumor integrins polymorphisms</th>
<th>C</th>
<th>G</th>
<th>G/C</th>
<th>ND</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peritoneal carcinosis, ( n ) (%)</strong></td>
<td>9 (20%)</td>
<td>18 (41%)</td>
<td>14 (32%)</td>
<td>3 (7%)</td>
</tr>
<tr>
<td><strong>Hematogenous metastases, ( n ) (%)</strong></td>
<td>2 (4%)</td>
<td>35 (64%)</td>
<td>13 (23%)</td>
<td>5 (9%)</td>
</tr>
<tr>
<td>( P )</td>
<td>0.002</td>
<td>0.04</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

The two groups of patients with either peritoneal or hematogenous metastases have been compared for each tumor integrins polymorphism. ns, non significant.

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**Table 4.** Odds ratio results for all variables analyzed for a possible association with either peritoneal-only diffusion or hematogenous metastases

<table>
<thead>
<tr>
<th>Factors</th>
<th>OR</th>
<th>95% CI</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>1.1</td>
<td>0.8–2.5</td>
<td></td>
</tr>
<tr>
<td>Stage at diagnosis</td>
<td>1</td>
<td>0.9–2.7</td>
<td></td>
</tr>
<tr>
<td>Diffuse histology(^*)</td>
<td>4.7</td>
<td>0.0005–19.11</td>
<td></td>
</tr>
<tr>
<td>rs2269772 (ITGA3, A)(^*)</td>
<td>22.2</td>
<td>0.03–12.40</td>
<td></td>
</tr>
<tr>
<td>rs2269772 (ITGA3, A/G)</td>
<td>2.5</td>
<td>0.06–7.8</td>
<td></td>
</tr>
<tr>
<td>rs2291079 (ITGB5, G)</td>
<td>1.2</td>
<td>0.8–9.3</td>
<td></td>
</tr>
<tr>
<td>rs2291079 (ITGB5, G/C)</td>
<td>2.7</td>
<td>0.02–13.61</td>
<td></td>
</tr>
<tr>
<td>rs2676 (ITGB5, C)</td>
<td>5.4</td>
<td>0.1–50</td>
<td></td>
</tr>
<tr>
<td>rs2676 (ITGB5, C/T)</td>
<td>2.4</td>
<td>0.07–6.6</td>
<td></td>
</tr>
<tr>
<td>rs11902171 (ITGV, C)(^*)</td>
<td>6.8</td>
<td>0.01–33.4</td>
<td></td>
</tr>
<tr>
<td>rs11902171 (ITGV, C/G)</td>
<td>1.5</td>
<td>0.3–6.6</td>
<td></td>
</tr>
</tbody>
</table>

Factors resulted independently correlated with either peritoneal or hematogenous metastases at multivariate analysis have been indicated with an asterisk (\(^*\)).

CI, confidence interval.
reserved to patients most likely to recur with peritoneal-only diffusion. The possibility to individuate this subgroup of patients would then represent a major advance in this setting. In our analysis, A and G genotypes of rs2269772 (ITGA3), G and C genotypes of rs11902171 (ITGV) and tumor histology have been showed to independently correlate with either peritoneal or hematogenous metastases. These findings seem to confirm data deriving from preclinical studies that suggested a different gastric tumor integrins profile for different metastatic sites [12–17]. Moreover, according to our observations, a different metastatic site may also be linked to different polymorphisms within the same tumor integrin gene. At the molecular level, we know from previously published analyses that integrins play a key role in cell migration and in the regulation of angiogenesis. It has been demonstrated that up-regulation of integrins subclasses could in fact enhance neoplastic cells motility and vascular endothelial growth factor expression [9–11, 27–29]. Integrins may consequently provide a switch to activate either tumor cells capability to local invasion and diffusion leading to peritoneal carcinosis or a program of angiogenesis-driven tumor progression leading to hematogenous metastases.

Different integrins polymorphisms determining different level of protein expression may then interfere with the metastatic potential of cancer cells through activation of different tumor progression pathways. It is also possible that integrins polymorphisms may have an influence in the interaction process between different integrins subtypes with their ligands, thus determining a tumor predisposition to local infiltration [30]. Tumor histology is a further factor able to identify a gastric tumor potential for peritoneal carcinosis. Nonetheless, a diffuse tumor histology is not exclusively linked to peritoneal carcinosis. In fact, in our experience, similarly to other previous reports, a not negligible proportion of patients with peritoneal carcinosis (43%) did not show a diffuse histology. The presence of tumor serosal involvement and/or exfoliated cancer cells in peritoneal lavage is a further example of clinical indicators of possible peritoneal carcinosis. However, not all gastric tumors with serosal infiltration will eventually develop carcinosis and a negative peritoneal lavage unfortunately does not exclude a future peritoneal diffusion [3–5, 7, 8]. In our study, we then decided to include only pT3 primary gastric tumors with negative peritoneal lavage in order to avoid any confounding factor and possibly better identify the role of tumor integrins genotyping in this clinically relevant group of patients. The need for individuating patients with peritoneal-only recurrence excluding those with both metastatic sites (i.e. peritoneum and hematogenous sites) is another crucial issue. Our results seem to indicate that combining information from genotyping of rs2269772 (ITGA3), rs11902171 (ITGV) and tumor histology could allow clinicians to individuate gastric cancer at high risk for exclusive recurrence in the peritoneum among patients undergoing apparent radical surgery for pT3 cancer without exfoliated cancer cells in peritoneal lavage. However, it is also important to note that given the retrospective nature of the present study and the sample size analyzed, unwanted methodological biases may have influenced results that should then be considered exploratory.

Globally, we believe that genotyping of rs2269772 (ITGA3) and rs11902171 (ITGV) may represent a critical asset in identifying patients developing peritoneal carcinosis among those relapsing after curative resection. The selection tool deriving from this analysis may allow an optimal use of innovative treatment strategies in gastric cancer patients. Further analysis in a wider series will be crucial for a definitive translation into clinical practice.

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disclosure
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references


