SHORT COMMUNICATION

hMLH1 and hMSH2 expression and BAX frameshift mutations in ovarian cancer cell lines and tumors

G.Colella1, F.Vikhanskaya2, A.M.Codegoni1, C.Bonazzi3, M.D’Incalci1 and M.Broggini1,4
1Molecular Pharmacology Unit, Department of Oncology, Istituto di Ricerche Farmacologiche ‘Mario Negri’ via Eritrea, 62 20157 Milan, 2Department Experimental Oncology, Istituto Nazionale per la Ricerca sul Cancro, Genova and 3Ospedale San Gerardo, Università di Milano, Monza, Italy
4To whom correspondence should be addressed
Email: broggini@irfn.mnegr.it

The expression of mismatch repair proteins hMSH2 and hMLH1 was investigated in human ovarian cancer cell lines and in biopsies of ovarian carcinomas obtained from 20 patients undergoing surgical operation. By Western blotting analysis hMSH2 protein was detected in all the tumor samples analyzed and in eight out of nine human ovarian cancer cell lines, while hMLH1 was undetectable in four out of 20 ovarian tumors and in five out of nine human ovarian cancer cell lines analyzed. The possible presence of frameshift mutations in the BAX gene, which contains a sequence of eight contiguous guanines in its third exon, was tested in all the samples. All the cell lines presented the normal alleles for the BAX gene while only in one of the tumor samples a heterozygous frameshift mutation was found. The frameshift mutation was associated to a low, almost undetectable, level of BAX protein which was instead present at much higher levels in all the other samples investigated. The results indicate that frameshift mutations in the BAX gene, possibly arising as a consequence of microsatellite instability (detectable in these tumors), is detectable in human ovarian cancer although quantitatively it does not appear to be a major determinant of the low apoptotic response to chemotherapy observed in ovarian cancer cells.

Inactivation of the mismatch repair (MMR*) system by mutations or deletions has been found in different tumor types (1–5). Mutations of the MMR genes are also responsible for hereditary predisposition to nonpolyposis colorectal cancer (HNPPC). The lack of functional hMLH1 or hMSH2 has been associated to the presence of microsatellite instability (MIN) found in some human tumors. Frameshift mutations, associated to alterations in the normal MMR function, have been reported among others genes, in the TGF-β gene and more recently in the BAX gene, which presents a tract of eight consecutive guanines representing a possible mutation site in MMR defective cells (6).

We first analyzed the expression of hMSH2 and hMLH1 in the cultured human ovarian cancer cell lines (Figure 1A) by Western blot analysis (24) using polyclonal antibodies against hMSH2 and hMLH1 (Santa Cruz Biotechnology, CA). Detection was with the enhanced chemiluminescence (ECL) system after addition of antirabbit IgG (Santa Cruz Biotechnology, CA). On the same blots antibodies against actin were used to verify equal loading of proteins in the different samples. Nine different human ovarian cancer cell lines (OVCAR-3, OVCAR-5, OVCAR-8, SKOV3, SW626, A2780, A2774, IGROV-1 and PA-1) were used. They were maintained as described (8) in RPMI 1640 supplemented with 10% FCS. Four out of nine cell lines expressed both hMSH2 and hMLH1, while only 1 (A2774) expressed neither of the two proteins. Apart from A2774, all the cell lines did express hMSH2, while SW626, OVCAR-3, OVCAR-5 and SKOV-3 did not express hMLH1 having a normal level of hMSH2. The results obtained are in good agreement with the data reported in the literature for some of these cell lines (3,20).

Figure 1B reports the expression of the two MMR proteins in the 20 tumor samples obtained at first laparotomy from ovarian cancer patients before any other treatment. The stage and the histological grading of the primary tumors were defined according to the FIGO criteria and were: Eight stage I-II and 12 stage III-IV, 12 were serous type, three Endometroid, three undifferentiated and two mucinous. The tissues were freed from necrotic, hemorrhagic and connective tissue, minced and stored at –80°C in cryotubes (Nunc) until processed.

hMSH2 was detected in all the analyzed samples while in two out of 20 samples (patients 2 and 4) we could not detect

*Abbreviations: MMR, mismatch repair; HNPPC, hereditary predisposition to nonpolyposis colorectal cancer; MIN, microsatellite instability; ECL, enhanced chemiluminescence.
hMLH1. The presence of frameshift mutations in the BAX gene, which contains a sequence of 8 G in the third exon, was assessed by PCR using specific primers amplifying a 94 bp fragment in the wt BAX gene.

Genomic DNA was isolated from cell lines and tumor specimens according to standard procedures (24). PCR amplifications of the 94 bp BAX fragment were conducted using primers and conditions as described (6) in the presence of 0.3 µCi of a [33P]dATP. Amplified fragments were separated on 8% sequencing gel and subjected to autoradiography.

Figure 2 reports the results obtained in the nine cell lines and in the tumor specimens. As a control we amplified DNA from SW620 and LoVo cells which contain a normal G8 sequence (SW620) and a G7 and G9 sequence (LoVo). In all the nine ovarian cancer cell lines studied no frameshift mutations were observed and the expected fragment of 94 bp, corresponding to the presence of a normal G8 sequence was amplified. In the 20 ovarian tumors analyzed (Figure 2), we observed a frameshift mutation in only one sample in which the presence of both a G8 and G7 sequence was found. In all the other samples the normal, G8-containing BAX fragment was amplified.

By Western blotting analysis we then evaluated the expression of BAX in the different tumors (Figure 3). We could detect a normal BAX expression in 17 out of 20 tumor samples while in one case the protein recognized by the antibody had an apparent lower molecular wt and in two others the protein was hardly detectable. Interestingly the tumor sample presenting in at least one allele of the frameshift mutation in the BAX gene, was one of the two in which the protein was almost undetectable. The tumor sample presenting the faster migrating BAX had a normal G8 sequence in the BAX gene indicative of the absence of a frameshift mutation, while it presents microsatellite instability and does not express detectable levels of hLMH1.

In ovarian cancer cell lines growing in culture the apoptotic process is quantitatively modest and studies have demonstrated that, even in the presence of exogenous p53, these cells tend to arrest in G1 or G2 phases of the cell cycle rather than undergoing apoptosis (25). In addition, recent studies have indicated that in ovarian cancer cells, treatment with cytotoxic agents did not induce apoptosis, independently on the presence of a wild-type p53 (8,9).

The low induction of apoptosis after chemotherapeutic agents treatment of ovarian cancer cells has been associated with the lack of correlation between p53 status and sensitivity
to DNA damaging agents reported by some authors (8,9,26). On the other hand, there is evidence showing that the degree of apoptotic response of ovarian cancer cells to in vitro anticancer drug treatment is a determinant of cell susceptibility (27–29 and F.Vikhanskaya, submitted).

One of the players in the complex process of apoptosis activation is BAX, and it might be hypothesized that the frameshift mutation of this protein, consequent to a MMR defect, might be responsible for the low level of apoptotic induction observed in these tumors after exposure to chemotherapeutic agents. This hypothesis is also supported by the recent report showing that defects in the expression of hMLH1 were associated to a poor response to chemotherapy of patients with ovarian cancer (30). The results of the present study indicate that BAX frameshift mutations are not likely to be a major determinant of resistance of ovarian cancer to chemotherapy. In 10 patients for which we had availability of normal lymphocytes, we analyzed microsatellite instability. Using three different loci, reported to be among the most altered in ovarian cancer (23), we could detect microsatellite instability in four out of 10 samples (Table I) but nevertheless in none of these the BAX gene was altered. For patient 4 we could not detect alterations at the three tested loci.

The data reported here do not confirm the high incidence of BAX frameshift mutations observed in colon cancer. It is to note that in gastrointestinal tumors, while microsatellite instability was associated with alterations in the E2F-4 gene, no changes were observed for two other genes containing instability was associated with alterations in the E2F-4 gene, in four out of 10 samples (Table I) but nevertheless in none of these the BAX gene was altered. For patient 4 we could not detect alterations at the three tested loci.

Acknowledgements

This work was partially supported by ‘Fondazione Nerina e Mario Mattioli’. The generous contribution of the Italian Association for Cancer Research is gratefully acknowledged.

References

3. Boyer,J.C., Umar,A., Risinger,J.L., Lipford,J.R., Kane,M., Yin,S., Gratefully acknowledged. The generous contribution of the Italian Association for Cancer Reserch is

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Locus</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>7</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>14</th>
<th>15</th>
<th>18</th>
</tr>
</thead>
<tbody>
<tr>
<td>DXS981</td>
<td>X</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D13S175</td>
<td>0</td>
<td>X</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>D3S1611</td>
<td>X</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

0 = normal.
X = abnormal.

Table I. Microsatellite analysis at three different loci

Mismatch repair genes and BAX alterations in ovarian cancer


693
effects of doxorubicin in a human ovarian cancer-cell line expressing wild-type p53 and WAF1/CIP1 genes. Int. J. Cancer, 61, 397–401.

Received on September 16, 1997; revised on November 12, 1997; accepted on December 12, 1997