ABCD Arq Bras Cir Dig 2012;25(4):240-244

Original Article

MICROSATELLITE INSTABILITY – MSI MARKERS (BAT26, BAT25, D2S123, D5S346, D17S250) IN RECTAL CANCER

Instabilidade de microsatélite – msi nos marcadores (BAT26, BAT25, D2S123, D5S346, D17S250) no câncer de reto

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ABSTRACT – Background - Colorectal cancer has an important genetic component. Microsatellites are considered phenotypic markers of prognosis, therapeutic response and identify patients with mutations in DNA repair genes. Aim – To evaluate the molecular profile of tumors underwent to transanal endoscopic microsurgery – TEM in surgical treatment of rectal cancer. Method – Thirty eight surgical specimens were evaluated according to pathological staging and the region of the tumor were dissected and submitted to DNA extraction. The colorectal tumors were tested for microsatellite instability – MSI using a panel of five markers (BAT25, BAT26, D2S123, D5S346, and D17S2720) technique of Polymerase Chain Reaction (PCR). Result – From total 63% were male and 47% female, with mean age of 58.4 years. In relation to tumor type adenomas were 58%, 24% low-grade adenomas and 76% high grade; 42% were carcinomas. The depth of resection 80% included the rectal perirenal fat and 20% the muscularis propia. The most frequent microsatellite amplification was BAT26 (100%) and lowest D17S2720 (85.4%). Sixteen patients (42%) were MSI, ten were carcinomas, two low grade adenomas and four high grade. Twenty-two cases (68%) showed microsatellite stable – MSS. The allelic loss of microsatellite markers was statistically significant in cases of carcinoma in relation to adenomas. The most frequent microsatellite amplification was BAT26 (100%) and lower D17S2720 (85.4%). Sixteen patients (42%) had microsatellite instability – MSI thereof ten were carcinomas, two low grade adenomas, four high-grade adenomas and 22 cases (58%) were microsatellite stable – MSS. Conclusion - Microsatellite instability (MSI-H) was significantly associated with rectal carcinomas, confirming its use as a prognostic marker in colorectal carcinogenesis.

RESUMO – Racional - O câncer colorretal tem importante componente genético. Os microsatélites são considerados marcadores fenotípicos de prognóstico, resposta terapêutica e de identificação de pacientes com mutação nos genes de reparo do DNA. Objetivos – Avaliar o perfil molecular dos tumores submetidos à microcirurgia endoscópica transanal (TEM) para tratamento do câncer de reto. Método – Foram selecionados 38 espécimes avaliados segundo o estadiamento patológico. Foram escolhidas amostras da região tumoral e realizada dissecação e extração do DNA. Os tumores colorretais foram testados para instabilidade de microsatélite – MSI utilizando um painel composto de cinco marcadores (BAT25, BAT26, D2S123, D5S346 e D17S2720), técnica da reação em cadeia da polimerase (PCR). Resultados – Nos 38 casos observou-se que 63% eram do sexo masculino e 47% feminino com média de idade de 58,4 anos. Em relação ao tipo tumoral 58% eram adenomas, sendo 24% adenomas de baixo grau e 76% de alto grau; 42% eram carcinomas. Quanto à profundidade de ressecção, verificou-se que 80% dos casos incluíam a gordura perirenal e 20% até a muscular própria. O microsatélite com maior frequência de amplificação foi o BAT26 (100%) e o menor D17S2720 (85,4%). Dezesseis casos (42%) apresentaram MSI; eram dez carcinomas, dois adenomas de baixo grau e quatro de alto grau. Vinte e dois casos (68%) tinham microsatélite estáveis – MSS. A perda alélica dos marcadores de microsatélites foi estatisticamente significante nos casos de carcinoma em relação a adenomas. O microsatélite com maior frequência de amplificação foi o BAT26 (100%) e o menor D17S2720 (85,4%); 16 casos (42%) apresentaram instabilidade de microsatélite – MSI. Desses, dez eram carcinomas, dois adenomas de baixo grau e quatro de alto grau; 22 casos (58%) apresentaram microsatélite estáveis – MSS. Conclusão - A instabilidade de microsatélite (MSI-H) foi significativamente associada com carcinomas reais, confirmando sua utilização como marcador prognóstico na carcinogênese retal.
INTRODUCTION

The mapping of the human genome, together with the development of new molecular techniques, has allowed not only the gene discovery of genetic predisposition to cancer, but also the possibility of large scale of genomic analysis. The development of cancer is the result of a series of steps involving gene mutations, chromosomal breaks and losses, gene amplifications and genomic instability, and epigenetic mechanisms. Among the multiple genes involved in this process, is highlighted the proto-oncogenes, tumor suppressor genes, and the DNA repair genes act ensuring that each gene and the hMSH2 on chromosome 2p21, in the repair of DNA. Observation of the microsatellite instability - MSI results from inactivation of the repair genes (mismatch repair genes - MMR), and its function is exerted continuously, preserving cellular tissues and variations in the process of cell division. To correct such changes exist some proteins with the function of repairing and maintaining the integrity of DNA. These proteins are produced from repair genes (mismatch repair genes - MMR), and its function is exerted continuously, preserving cellular tissues. The microsatellite instability - MSI and variations in the process of cell division. To correct such changes exist some proteins with the function of repairing and maintaining the integrity of DNA. These proteins are produced from repair genes (mismatch repair genes - MMR), and its function is exerted continuously, preserving cellular tissues. The microsatellite instability - MSI results from inactivation of the repair genes (mismatch repair genes - MMR), and its function is exerted continuously, preserving cellular tissues.

The DNA is a molecule that frequently suffers alterations through changes of segments and variations in the process of cell division. To correct such changes exist some proteins with the function of repairing and maintaining the integrity of DNA. These proteins are produced from repair genes (mismatch repair genes - MMR), and its function is exerted continuously, preserving cellular tissues. The microsatellite instability - MSI results from inactivation of the repair genes (mismatch repair genes - MMR), and its function is exerted continuously, preserving cellular tissues. The microsatellite instability - MSI results from inactivation of the repair genes (mismatch repair genes - MMR), and its function is exerted continuously, preserving cellular tissues. The microsatellite instability - MSI results from inactivation of the repair genes (mismatch repair genes - MMR), and its function is exerted continuously, preserving cellular tissues. The microsatellite instability - MSI results from inactivation of the repair genes (mismatch repair genes - MMR), and its function is exerted continuously, preserving cellular tissues.

From the point of view of genetic, colorectal cancer (CRC) can be divided into two groups: 1) sporadic, occurring without family liaison and correspond about 85% of cases, and 2) hereditary, based on genetic defect in the family, generation after generation. Hereditary cases occurs at earlier ages - from age 20 - and may be present on several people within the same family. The results of treatment of CRC is directly related to early diagnosis, i.e., the sooner you make the diagnosis, the higher the cure rate, reaching over 90% in early cases. In recent years, with the rapid and growing development of molecular techniques, are identified specific genetic defects in at least two major hereditary syndromes on large intestine, the familiar adenomatous polyposis (FAP) and hereditary colorectal cancer without polyposis (HNPCC). Both are vertically transmitted from one generation to another, through Mendelian inheritance pattern, in general type autosomal dominant. The proto-oncogene (K-ras), tumor suppressor genes (APC, DCC and TP53) and DNA repair genes or MMR (English, mismatch repair - genes called MSH2, MLH1, PMS1, PMS2 and MSH6) have central participation in the development of CRC. Approximately 25% to 40% of DNA molecule is formed by repetitive nucleotide sequences, which might be seen several times throughout the genome, and subdivided into repetitive dispersion and repetitive tandem (or satellite). Such repetitions can be classified according to the extent of repetitive sequences: satellite, minisatellite and microsatellite depending on the number of nucleotides. The number of minisatellite and microsatellite varies among species.
individuals, that’s why they are considered DNA fingerprinting and used in paternity tests\(^1\). The microsatellite instability - MSI is the observation that the DNA extracted from cells of certain tumors show changes in the number of repeating units in one or more microsatellites compared to the same existing microsatellite DNA samples from normal tissue of the same individual - blood cells, for example. Therefore, tumor cells have “fingerprints” defective in their DNA compared to other body tissues.

The promoter hypermethylation of hMLH1 gene appears to be a major genetic alteration present in CCR. Molecular analysis of mutator via carcinogenesis by detection of MSI has application in clinical practice, since tumors called “mutator” exhibit distinct biological characteristics\(^3\). There is evidence that these tumors are different in several aspects: location (proximal colon), age (less advanced), histological appearance (higher incidence of mucinous tumors) and prognosis (better survival compared to microsatellite stable CCR)\(^26\). However, MSI tumors are associated with resistance to chemotherapy with 5-fluorouracil (5FU) and shorter survival of patients after treatment with the drug\(^5\). MSI CCR marker can also be inherited or acquired susceptibility to other cancers such as gastric cancer, uterus and ovary\(^21\). Several theories have been proposed to explain why MSI-H tumors (High-instability) have a better prognosis in sporadic colorectal carcinomas, despite the unfavorable characteristics.

This study aims to assess the profile of molecular tumors underwent to transanal endoscopic microsurgery (TEM) for the treatment of rectal cancer.

**METHODS**

Were selected 38 surgical specimens resected by transanal endoscopic microsurgery (TEM) in the period from 2003 to 2006, evaluated according to pathological staging\(^27\). Thereafter, the samples were chosen from region tumor embedded in paraffin, submitted to dissection and extraction of DNA using commercial kit (QIAmp® DNA FFPE Tissue Handbook – Qiagen, Califórnia, USA) Losso, 2010. Colorectal tumors were tested for microsatellite instability - MSI using a panel of five primer pairs (BAT25, BAT26, D2S123, D5S346, D17S2720) (Table 1). The method used for detection of microsatellite was VNTRs (Variable Number of Tandem Repeats) Kashyap et al. 2,004. Were used 100 ng / ul purified DNA in a final volume of 25μl PCR reaction. The PCR conditions were: initial denaturation at 95° C for five minutes, followed by 10 continuous cycles of denaturation at 95° C for one minute annealing starting at 60 ° C and lowered 1° C every cycle until reaching the temperature of 51° C in the last cycle extension at 72° C for one minute and at 72° C for one minute. Completing the 30 cycles, the material remained five minutes at 72° C to provide a longer extension for all 40 cycles. Subsequently, the PCR products was analyzed by electrophoresis gel on 10% polyacrylamide (16 hours, 450 V, room temperature) stained with silver nitrate 0.1%.

**RESULTS**

From the 38 cases of rectal cancer operated by TEM (Figure 1), 63% were male and 47% female with a mean age of 58.4 years. Regarding tumor type, adenomas were 58% - 24% lower and 76% - high grade – and 42% were carcinomas (Table 1). In relationship to the resection depth was found that 80% included the perirectal fat and 20% the muscularis propria.

**TABLE 1 - Panel with five microsatellite markers**

<table>
<thead>
<tr>
<th>Microsatellite</th>
<th>Location</th>
<th>Size</th>
<th>Forward primer</th>
<th>Reverse primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAT25</td>
<td>gene c-Kit cr. 4q12</td>
<td>110 - 130 pb</td>
<td>5’-TGG CTC CCA AGA ARG TAA GT-3’</td>
<td>5’-TCG CCA TTG TAA CTA TGG CTC-3’</td>
</tr>
<tr>
<td>BAT26</td>
<td>gene hMSH2 cr. 2p</td>
<td>100 - 120 pb</td>
<td>5’-TGA CTA CTT TTG ACT TCA GCC-3’</td>
<td>5’-AAC CAT TCA ACG TTT TTA ACC-3’</td>
</tr>
<tr>
<td>D2S123</td>
<td>gene hMSH2 cr. 2p</td>
<td>200 - 230 pb</td>
<td>5’-AAA CAG GAT GCC TGC CTT TA-3’</td>
<td>5’-GGA CTT CAC ACC TAG GGG AC-3’</td>
</tr>
<tr>
<td>D5S346</td>
<td>gene APC cr. 5q21q22</td>
<td>100 - 130 pb</td>
<td>5’-AGC AGA TTA GAC AGT ATT ACT AGT-3’</td>
<td>5’-ACT CAC TCT ACT GAT AAG TGG G-3’</td>
</tr>
<tr>
<td>D17S2720</td>
<td>gene BRC1 cr17q11.2-q12</td>
<td>140 - 170 pb</td>
<td>5’-GGAGA ATG AAA TAG ACA AT-3’</td>
<td>5’-GCT GGC CAT AAT ATT TAA ACC-3’</td>
</tr>
</tbody>
</table>

**FIGURE 1 - Transanal endoscopic microsurgery (TEM): A) the endoscopic system positioned; B) low rectal adenoma (prolapsed).**
The microsatellite with more frequent amplification was BAT26 (100%) and the lowest D17S2720 (85.4%). Sixteen cases (42%) showed MSI. Of these, ten were carcinomas, two adenomas with low-grade and four high. Twenty-two (68%) cases showed stable microsatellite (MSS). The allelic loss of microsatellite markers was statistically significant in the cases of carcinoma in relation to the adenomas (p = 0.0003, OR 16.7; 95% CI: 2.8-98.0) (Table 2 and Figure 2).

**DISCUSSION**

The identification of parameters that reflect the biological behavior of rectal cancer is determining in the prognosis and cancer therapy. The results of this study showed the importance of molecular analysis in the appearance of rectal tumors, and may imply therapeutic design. The presence of MSI may be significantly corroborated by evaluating the number of concurrent intramural active cytotoxic lymphocytes. This observation supported the hypothesis that MSI-H tumors are associated with the production of new immunogenic epitopes due to defective repair system. This hypothesis attempts to explain why patients with sporadic CRC MSI-H exhibit antitumor immune response more effective than MSS patients with more favorable clinical outcome. In this study it was observed that the presence of MSI-H was significantly higher in carcinomas than in adenomas, confirming the prognostic value of MSI in CRC. It should also be noted that the articles published in CCR about MSI are mainly from Eastern and European populations; few studies were conducted in Brazil. Considering that our country has great genetic heterogeneity - the result of five centuries of miscegenation -, are necessary studies to establish the frequency of MSI in different regions of Brazil.

The survival of patients with metastatic CRC (mCRC) progressively improved over the past decades. Was due primarily to new chemotherapeutic combinations (5-fluorouracil, irinotecan, oxaliplatin), and the introduction of new therapies. Among them are two monoclonal antibodies against the receptor of epidermal growth factor receptor (EGFR) - cetuximab and panitumumab - which have demonstrated efficacy in the treatment of mCRC. However, due to toxicity and cost, it is essential to use tools to select patients most likely to have benefit with the treatment.

Therefore, the identification of cases of rectal cancer with MSI may allow proper neoadjuvant chemotherapy specific to each patient, thus sparing the toxicity of therapy and enabling the implementation of a new therapeutic modality.

**CONCLUSIONS**

The microsatellite instability (MSI-H) was significantly associated with rectal carcinomas, confirming its use as a prognostic marker in colorectal carcinogenesis.

**REFERENCES**


