BAX as a susceptibility factor in hereditary non polyposis colorectal cancer

Key words: Hereditary non polyposis colorectal cancer. Genetic inheritance. Youth. Genetic study. BAX. Bethesda. hMLH1. hMSH2.

Dear Editor,

BAX belongs to the Bcl-2 protein family and plays a vital role in the regulation of mitochondrial apoptotic pathway regulation in epithelial cells (1,2). It is frequently altered in tumors with a micro satellite instability phenotype (MSI) (3). Tumors with MSI are characterized by the low detection rate of mutations in apoptosis p53 gene promoters compared to those with MSS (micro satellite stability), which allows us to speculate about whether tumors with MSI could be related to BAX, instead of p53 (the gene involved in the change from adenoma to carcinoma). The proapoptotic activity of BAX seems to be in balance with its activity as a survival factor exercised by Bcl-2, which is why it has been assumed that an alteration in only one of the BAX alleles would be enough to sway the balance towards cell proliferation (1,4). The loss of the terminal region of the long arm of chromosome 19, which contains the BAX gene, has been previously described in colorectal cancer (CRC) (1-4). The objective of this study was to investigate whether the somatic loss of chromosomal region 19q13.1-13.4, which affects the lack of BAX expression, is a common occurrence in CRC.

Forty-four patients diagnosed with CRC before the age of 51 years of age were selected. DNA extraction was performed in the peripheral blood (germinal line) and tumor tissue (tumor line). The presence of germinal mutations was established in all exons from the hMLH1 and hMSH2 genes by using denaturing gradient gel electrophoresis. In order to ascertain the existence and grade of instability of the repetitive sequences in DNA, we used the criteria set out under the “international guidelines for the evaluation of MSI in colorectal cancer”, which establish the MSI phenotype by applying the criteria of 5 microsatellites. To analyze the loss of this 19q13.1-13 chromosomal region of chromosome 19, microsatellite polymorphic sequences were used (D19S112, D19S223, D19S425, ErCC1 and the DMPK triplet), together with single nucleotide polymorphisms (SNP) located in positions 8092 and 8106 of the ERCC1 gene (GenBank access number M63796). In order to do this, we selected areas of the tumor piece that showed a minimal amount of stromal infiltration and inflammatory cells, given that healthy tissue can hide the losses that may arise in cancer cells. The loss of the 19q13.1-13.4 chromosomal region was analyzed by comparing the allele patterns in the tumor and germinal lines from polymorphic sequences located in this same region. Statistical analysis: The $\chi^2$ test where possible and Fisher’s exact test in the rest of the cases.

In 30 of the 44 cases analyzed, necessary information was gathered in order to be able to analyze the chromosomal loss. Of these, 17 (57%) were deletion carriers according to the DNA taken from the tumor. There were no differences in the frequency with which one or more allele was lost in the SNPs. Of the 12 cases in which the deletion was detected using SNP8092, on 6 occasions the allele with the adenine nucleotide was lost (A) and many others times the allele with cytosine was lost (C). In the two deletions detected using SNP8106 the fragment carrying adenine was lost, making the SNP8092 uninformative in these cases. Depending on the phenotype, 24 out of the 25 MSS tissues were informative, and in 12 of these (50%) deletion of the analyzed chromosomal region was confirmed. In the MSI tissues (n = 19) 6 cases were informative, and the existence of deletion was indicated in 5 of these (83%).

The inactivation of the apoptosis p53 gene promoter is believed to be crucial in the malignant process of the tumor. In CRC, the frequency with which this gene appears to be mutated is a differential feature between tumors with a phenotype of MSS (50% have this mutation) and MSI (< 30%) (5,6). BAX expression is induced by the action of p53, and it is estimated...
that approximately half of the action brought about by p53 during apoptosis requires BAX mediation (7). The high frequency with which inactivating mutations of the BAX gene are identified in CRC with the MSI phenotype, allows us to speculate that it may be the inactivation of the BAX gene that having an effect on the evolution of adenoma to carcinoma in this type of tumor.

In the study of the loss of chromosomal fragments through the analysis of polymorphic microsatellite sequences, the presence of normal tissue should be taken into account, mixed together with tumor tissue. This is especially true for CRC, given the high level of lymphocytic infiltration that is a feature of this kind of tumor. What is more, if as generally accepted, the chromosomal losses are events that occur in an advanced phase of tumor development, not all the cells present in a primary tumor will have lost the analyzed fragment. In the case of the MSI tissues, the analysis of PCR-amplified microsatellite sequences leads to a “conflictive” method when it is time to identify the loss of chromosomal regions, given that the appearance of “new” alleles in the tumor tissue, the fruit of instability, makes it difficult to interpret the results. For this reason, in order to identify the deletion in MSI tissues, only the results obtained with SNPs have been taken into account.

Six of the cases analyzed with tumors classified as MSI were informative for the SNPs used. 83% (n = 5) revealed a loss of this chromosomal region in the tissue tumor. It is notable that in the only case in which it was not possible to identify the deletion, the tissue sample obtained was fresh and therefore the selection of the region to be analyzed could not be so precise as in the paraffin-embedded tissues. The results of the analysis of microsatellites in these unstable tissues revealed that many of them had an almost complete loss of some of the alleles observed in the germline line. These disappearances are, generally speaking, difficult to pass off as a result of genetic instability, given that they would lead to a clear bias in the selection of certain alleles during clonal proliferation. In some MSI tissues, the study of tumor regions that had a greater cell density proved the existence of a heterogeneous pattern in the distribution of these microsatellite sequences in the tumor mass. By accepting that the most densely populated tumor regions cover areas of faster cell proliferation, the results obtained here would suggest that the deletion of this chromosomal region has a highly adaptive value, and the altered cell in which it occurs has some kind of selective advantage which allows it to enter into a clonal expansion phase.

In MSS tissues, the combined analysis of the microsatellites and the SNPs made it possible to obtain information from the region next to the BAX in 24 cases of which 50% (n = 12) had a loss of this chromosomal region in the tumor tissues.

Due to the poor quality of the DNA extracted from the paraffin-embedded pieces, it has not been possible to clearly establish the limits of the minimal deleted region, although with the data currently available, it could be stated that this region includes the loci corresponding to D19S425 and the BAX gene, the equivalent to 2 and 3 megabases of genomic DNA. There are several genes located in this fragment of DNA and therefore these may be possible targets of the identified deletion. Even so, BAX meets the necessary characteristics for being considered a tumor suppressor gene (6,8). In MSI tumors, this gene frequently appears altered in a series of 8 guanines found in exon 3, although other types of mutations that specifically alter the genetic sequence of this exon have been identified together with tumor lines with MSI and MSS phenotypes (9). It would be necessary to increase the number of cases in the series to be able to confirm these findings.

To conclude, the deletion of chromosomal region 19q13.1-13.4 is common in CRC, especially in those patients who have a microsatellite instability phenotype. The deletion apparently occurs in an advanced stage of the tumor and consequently it would have cell entry in an expansive phase of clonal development. This deletion could be the cause of the reduction in the expression of the BAX gene observed in CRC, regardless of whether its phenotype is MSI or MSS. Furthermore, the fact that the BAX allele conserved in the tumor could be a carrier of mutations in a fundamental dominion for protein activity, would be proof of the importance of the inactivation of this gene in the evolution of CRC.

REFERENCES