Somatic frameshift mutations in the Bloom syndrome BLM gene are frequent in sporadic gastric carcinomas with microsatellite mutator phenotype

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Abstract

Background: Genomic instability has been reported at microsatellite tracts in few coding sequences. We have shown that the Bloom syndrome BLM gene may be a target of microsatellite instability (MSI) in a short poly-adenine repeat located in its coding region. To further characterize the involvement of BLM in tumorigenesis, we have investigated mutations in nine genes containing coding microsatellites in microsatellite mutator phenotype (MMP) positive and negative gastric carcinomas (GCs).

Methods: We analyzed 50 gastric carcinomas (GCs) for mutations in the BLM poly(A) tract as well as in the coding microsatellites of the TGF\textit{\beta}1-RII, IGFIIIR, hMSH3, hMSH6, BAX, WRN, RECQL and CBL genes.

Results: BLM mutations were found in 27% of MMP+ GCs (4/15 cases) but not in any of the MMP negative GCs (0/35 cases). The frequency of mutations in the other eight coding regions microsatellite was the following: TGF\textit{\beta}1-RII (60 %), BAX (27%), hMSH6 (20%), hMSH3 (13%), CBL (13%), IGFIIIR (7%), RECQL (0%) and WRN (0%). Mutations in BLM appear to be more frequently associated with frameshifts in BAX and in hMSH6 and/or hMSH3. Tumors with BLM alterations present a higher frequency of unstable mono- and trinucleotide repeats located in coding regions as compared with mutator phenotype tumors without BLM frameshifts.

Conclusions: BLM frameshifts are frequent alterations in GCs specifically associated with MMP+ tumors. We suggest that BLM loss of function by MSI may increase the genetic instability of a pre-existent unstable genotype in gastric tumors.
Background

Cancer is a progressive genetic disease characterized by progressive accumulation of mutations in both coding and non-coding sequences [1]. Simple sequence repeats or microsatellites are highly unstable in some human neoplasms, identifying a class of tumors with the microsatellite mutator phenotype (MMP+) [2]. MMP++ve status is defined by instability in more than 30% of analysed microsatellites, including at least one mononucleotide (such as BAT26 or BAT25) [3]. Microsatellite instability (MSI) has been described as a frequent genetic alteration in various human solid tumors including sporadic and familial gastrointestinal carcinomas (GCs). In almost all patients with hereditary nonpolyposis colorectal cancer (HNPPC), molecular studies have shown that MSI is caused by germline mutations in genes encoding proteins required for DNA mismatch repair (MMR) [4]. Genes such as hMSH2, hMLH1, PMS1, PMS2 and hMSH6/GTBP[5–8], were defined as "caretakers" because, when disrupted, they favor the acquisition of mutations at high frequency in several genes including oncogenes and tumor suppressor genes [9]. However, in a significant fraction of sporadic MMP+ tumors, mutation analysis has not allowed the identification of the genes responsible for MSI [2,10], suggesting that, in such cases, MSI might be caused by epigenetic mechanisms. In fact, gene silencing by promoter hypermethylation was recently shown for the hMLH1 gene [11–13].

The significance of MSI in tumorigenesis has been supported by the demonstration that the genetic instability characterizing MMP+ gastrointestinal cancers may target short repetitive tracts within the coding region of genes important for cell survival and proliferation. Insertion/deletion mutations were found in the genes for the transforming growth factor-beta type II receptor (TGFβRII), the insulin-like growth factor type II receptor (IGFIIR), the DNA mismatch-repair genes hMSH3 and hMSH6, the proapoptotic gene BAX and the transcription factor E2F-4[14–18]. In all the cases, with the exception of the E2F-4, the nucleotides insertion/deletion resulted in frameshift mutations and protein truncation. Although the biological significance of these alterations is not fully established, it was proposed that the absence of TGFβ1-RII receptors from the surface of gastrointestinal epithelial cells could eliminate the negative growth control of TGF-β, leading to uncontrolled cell growth [19]. IGFIIR has a role in growth suppression by binding and activating the TGFβ1 receptor complex and by mediating internalization, with subsequent degradation, of the growth factor IGF-II [20,21]. BAX promotes apoptosis and it was reported that BAX inactivation leads to rapid tumor growth [22]. The human genes hMSH3 and hMSH6 are homologous to the bacterial MutS gene whose products bind DNA mismatches to initiate strand-specific repair of DNA replication errors [23]. Somatic and germline mutations in hMSH6 were associated with sporadic MMP+ colorectal carcinomas and HNPPC [6].

We have found previously that the BLM gene may also be a target of MSI [24]. The frameshifts, found in a short poly-adenine repeat, were predicted to generate a truncated and non-functional BLM protein. Homozygous or compound heterozygous mutations at the BLM gene were shown to cause the Bloom syndrome (BS; OMIM 210900), a pre-malignant condition characterized by chromosomal instability and increased mutational rate. BS patients are predisposed to a wide variety of neoplasms diagnosed at early age. Noteworthy, among 100 neoplasms recorded in the BS registry, 19 were gastrointestinal (esophagus, stomach and colon) and only two were diagnosed after the age of 40 [25]. These data indicate that mutations in the BLM gene can promote tumorigenesis.

To better define the role of BLM in gastrointestinal tumorigenesis we screened a panel of MMP+ and MMP- gastric carcinomas (GCs) for: a) frameshifts in the BLM coding region microsatellite and b) for possible associations with other intragenic frameshifts.

Results

MSI status

For the present study, our panel of 50 GCs contains 15 MMP+ tumors with MSI at two or more loci (Table 2) and 35 GCs MMP- with alteration at one microsatellite (4 tumors) or without MSI (31 tumors). All the MMP+ cases were BAT 26 positive, and BAT 25 instability was found in 13 out of 15 cases (except cases 67R and 27P). The clinicopathological features of MMP+ tumors are described in Table 1. The MMP+ phenotype was closely associated with a low pTNM stage and with a less prevalent nodal metastases (p = 0.02 and p = 0.001 at Fisher exact test, respectively, vs. MMP-tumors). Within the MMP+ group, an excess of tumors in early stages and without lymph node metastasis was found (10/15 cases vs. 10/32 cases of MMP- cases, p = 0.02 and 11/15 cases vs. 7/32 cases of MMP- cases, p = 0.001, respectively), data that support the proposed better prognostic of GCs MMP+ [33,34]. Of note, no excess of intestinal type among MMP+ tumors was found (12/15 cases vs. 20/32 of MMP- cases) (Table 1), data in accordance with recent studies showing no significant difference in the frequency of MSI in intestinal-type and diffuse-type carcinomas [26,35].

BLM frameshifts

PCR amplification of the BLM microsatellite revealed abnormal bands, absent in the normal matched DNAs, in 4
Table 1: Histotype and staging parameters associated with the MMP phenotype in our GCs

| Variable          | Total (%) | MMP+ (%) | MMP- (%) |
|-------------------|-----------|----------|----------|
| **Histotype**     |           |          |          |
| Intestinal        | 32 (68.0) | 12 (37.5)| 20 (62.5)|
| Diffuse + Mixed   | 15 (32.0) | 3 (20.0) | 12 (80.0)| NS<sup>c</sup> |
| **T (depth of wall infiltration)** | | | |
| T1 + T2           | 12 (25.5) | 6 (50.0)| 6 (50.0)| NS |
| T3 + T4           | 35 (74.5)| 9 (25.7)| 26 (74.3)| NS |
| **N (lymph node status)** | | | |
| N0                | 18 (38.3)| 11 (78.5)| 7 (21.5)| |
| N+                | 29 (61.7)| 4 (12.1)| 25 (87.9)| 0.001 |
| **M (metastases)** | | | |
| M0                | 40 (85.1)| 14 (35.0)| 26 (65.0)| |
| M+                | 7 (14.9)| 1 (14.2)| 6 (85.8)| NS |
| **Staging**       | | | |
| I + II            | 20 (42.5)| 10 (58.8)| 10 (41.2)| |
| III + IV          | 27 (57.5)| 5 (16.6)| 22 (83.4)| 0.02 |
| **Total**         | 47 | 15 | 32 |

<sup>a</sup> – data were available for 47 out of 50 cases  <sup>b</sup> – at Fisher exact test  <sup>c</sup> – NS = not statistically significant

Table 2: Status of the coding microsatellites within BLM, WRN, RECQL, CBL, hMSH6, hMSH3, IGFII, TGF-βRII and BAX genes in the series of gastric carcinomas with microsatellite mutator phenotype.

| Case No. | Histotype<sup>a</sup> | Staging<sup>b</sup> | BLM<sup>c</sup> | WRN<sup>c</sup> | RECQL<sup>c</sup> | CBL<sup>c</sup> | hMSH6<sup>c</sup> | hMSH3<sup>c</sup> | IGFII<sup>c</sup> | TGF-βRII<sup>c</sup> | BAX<sup>c</sup> |
|----------|------------------------|---------------------|-----------------|-----------------|-----------------|-----------------|----------------|----------------|-----------------|-----------------|-------------|
| 31R      | I (T2N0M0)             | 2/5                 | -               | -               | -               | -               | -              | -              | -               | -               | -           |
| 57R      | I (T2N1M1)             | 5/5                 | -               | -               | -               | -               | -              | -              | +               | +               | +           |
| 63R      | I (T2N0M0)             | 5/5                 | -               | -               | -               | +               | -              | -              | +               | +               | +           |
| 67R      | I (T2N0M0)             | 4/5                 | -               | -               | -               | -               | -              | -              | +               | +               | +           |
| 69R      | D (T3N0M0)             | 4/5                 | +               | -               | -               | +               | +              | -              | +               | +               | +           |
| 79R      | I (T2N0M0)             | 5/5                 | -               | -               | -               | -               | -              | -              | -               | -               | -           |
| 3P       | I (T3N0M0)             | 4/7                 | +               | -               | -               | -               | -              | -              | +               | +               | +           |
| 11P      | I (T3N1M0)             | 3/7                 | +               | -               | -               | -              | +              | +              | +               | +               | +           |
| 14P      | M (T3N0M0)             | 6/7                 | +               | -               | -               | +              | -              | -              | +               | +               | +           |
| 17P      | I (T3N0M0)             | 3/7                 | -               | -               | -               | -              | -              | -              | +               | +               | +           |
| 19P      | D (T3N1M0)             | 3/7                 | -               | -               | -               | -              | -              | -              | -               | -               | -           |
| 27P      | I (T4N0M0)             | 2/5                 | -               | -               | -               | -              | -              | -              | +               | -               | -           |
| 30P      | I (T2N0M0)             | 3/6                 | -               | -               | -               | -              | -              | -              | -               | -               | -           |
| 37P      | I (T2N0M0)             | 3/7                 | -               | -               | -               | -              | -              | -              | -               | -               | +           |
| 38P      | I (T3N2M0)             | 3/7                 | -               | -               | -               | -              | -              | +              | +               | +               | -           |

| % present study (number of cases) | 27% (15/15) | 0% (0/15) | 0% (0/15) | 13% (2/15) | 20% (3/15) | 13% (2/15) | 7% (1/15) | 60% (9/15) | 27% (4/15) |

<sup>a</sup> – I = intestinal type; D = diffuse type; M = mixed type  <sup>b</sup> – according to the TNM classification (Sobin and Wittekind, 1997)  
<sup>c</sup> – number of unstable / total tested microsatellites  
<sup>d</sup> – data compiled from the references: [17,27,42,50,51,52]
of the 15 MMP+ tumors (27%) (Fig. 1). We have confirmed by sequencing that the abnormal bands are caused by a deletion of one adenine residue in the polyadenine tract (reduction from nine to eight adenines), as previously shown [24]. The mutation frequency in BLM may be a low estimate, because we have excluded cases in which abnormal bands in tumor DNAs were notably weaker than the normal ones, possibly in consequence of a large contamination of tumor with nonmalignant cells or of a low portion of malignant cells displaying the abnormality (Fig. 1). Since no BLM mutation was seen in MMP+ tumors, frameshift mutations in the BLM gene appear to be specifically associated with gastric MMP+ tumors (probability P < 0.01 at Fisher exact test), similar to the previous well-documented alterations in TGFβ-RII, IGFIIR, hMSH3, hMSH6 and BAX genes [14,16,17,32]. Therefore, our data expand the repertoire of alterations associated with MPP+ gastric carcinomas.

Frameshifts in other CDRs microsatellites and relations with BLM frameshifts
The same panel of MMP+ tumors was also analyzed for the presence of frameshifts in microsatellites located in the coding regions of eight other genes (Table 2). We have found variation in sequence length in TGFβ-RII, IGFIIR, hMSH3, hMSH6 and BAX genes, all previously reported to be altered in MMP+ GCs. Data about the TGFβ-RII status for eight (P series in Table 2) of the fifteen tumors were previously reported [27]. The observed mutation frequencies were comparable with those previously reported for all these genes, with the exception of IGFIIR, for which we found a low percentage of frameshifts (Table 2). Interestingly, in comparison with BLM mutations, only TGFβ-RII frameshifts were more frequent (60% vs. 27%, respectively) and BAX frameshifts were as well as frequent (27%). These data suggest that frameshifts in BLM are common alterations in MMP+ GCs. A trend toward an association between mutations in BLM and mutations in BAX and hMSH3 and/or hMSH6 was observed (Table 2) (P = 0.05 at Fisher exact test).

We have also analyzed the RecQL, WRN and CBL genes, which contain short repeats in their coding regions. The first two genes are members of the same helicase gene family as BLM[36,37]. While RecQL is not associated with any human disease, mutations in the WRN gene are cause of the Werner syndrome (WS; OMIM 277700), a rare autosomal recessive disorder clinically characterized by premature aging and increased risk for a variety of neoplasms, and genetically characterized by genomic instability. CBL is a tyrosine kinase receptor [38], whose deregulation was associated with oncogenesis [39]. We have found mutations only in the CBL gene (Fig. 1). Two samples (69R and 14P) were found to carry a 3 bp insertion in the trinucleotide (ATG)6 repeat of the CBL gene (data not shown). As we have previously reported [24], since no frameshift is produced, no functional consequence can be inferred. Both mutations were found in cancers displaying also BLM frameshifts (Table 2). It is noteworthy that this subset of tumors show a statistically significant excess of MSI in coding regions when compared with the MMP+ subset without BLM alterations (P < 0.001, at X² test) (Table 2).

Discussion
Several studies have documented that alternative pathways may exist in gastrointestinal MMP+ and MMP- cancers characterized by different sets of altered genes [40]. A gradual model of mutator mutations ("the mutator that mutates the other mutator") was proposed to define the mutator versus the suppressor pathways in gastrointestinal tumors [41]. By analyzing the timing of mutational events in genetically unstable GCs, it was proposed that the first targets of mismatch repair deficiency are the mononucleotide tracts of TGFβ-RII and BAX. The frameshift mutations of hMSH3 and hMSH6 appear to be secondary mismatch repair lesions, which generate mutations in IGFIIR[42]. The finding that frameshift mutations at TGFβ-RII are the most frequent and the IGFIIR are the less frequent in gastric MMP+ tumors supports to the model in which early
events should be present in the majority of tumors and late mutations only in a minor fraction [43]. This finding is also confirmed in our study (Table 2). BLM frameshift mutations are associated more frequently with first and second step alterations proposed in this model, suggesting that they are secondary to mismatch repair defects (in hMSH3 and/or hMSH6) present in cells which do not undergo apoptosis (possibly in consequence of BAX inactivation). Noteworthy, in case 3P, in addition to BLM, the only frameshifts were found in BAX (see figure 1) and p53 (data not shown) genes, suggesting that, in some instances, BLM alteration may occur without hMSH3 and/or hMSH6 mutations, in cells with abnormal p53-mediated pathway of apoptotic response to DNA damage.

The involvement of BLM in tumorigenesis outside BS patients seems conceivable, since this gene appears to act as a caretaker. BLM mutations are known to cause an increased genomic instability characterized by an elevated number of chromosomal breaks, gaps and rearrangements and by an excessive number of mutations in both coding and noncoding regions, likely originated by unequal sister-chromatid exchange, characteristic feature of the Bloom syndrome [44,45]. Accordingly, BS exhibits a combination of genomic instability and elevated cancer risk, an association found also in other diseases caused by defects in caretaker genes (such as HNPCC, WS, ataxia-telangiectasia and xeroderma pigmentosum). Similar to the mutations found in BS, the frameshifts described in GCs abolish the helicase function of the BLM protein [24]. BLM protein has a proven DNA unwinding activity [46] and could be involved in processes that are disturbed in malignant cells, such as DNA replication, recombination, chromosome segregation, DNA repair and transcription. Indeed, the fission yeast BLM gene homologue (radl2+/rho1+) was shown to regulate the S-phase checkpoint and was proposed to couple chromosomal integrity with cell cycle progression [47,48]. However, the finding of BLM mutations in MMP+ tumors generates a paradox: BLM mutations are predicted to generate chromosomal instability while most of the MMP+ tumors are diploid. Nevertheless, some MMP+ tumors are aneuploid and BLM loss-of-function mutations may have pleiotropic consequences, possibly affecting also the microsatellite instability pathway, as suggested by the description of an increased intra-gene mutations in Bloom syndrome [44,45]. In support to our suggestion is the finding that we have found that BLM is mutated in LoVo cell line, a colon cancer cell line with both microsatellite and chromosomal inatability [43]. Much more, it was recently reported that SGS1 gene, the Saccharomyces cerevisiae homologue of BLM and WRN, suppresses genome instability and homeologous recombination and is redundant with DNA mismatch repair (MMR) for suppressing gross chromosomal rearrangements and for suppressing recombination between divergent DNA sequences [49].

Conclusions
Our results indicate that BLM frameshifts are frequent alterations in GCs and are specifically associated with MMP+ tumors displaying at least 2 unstable microsatellites. Mutations in BLM appear to be frequently associated with frameshifts in BAX and in hMSH6 and/or hMSH3, and tumors with BLM alterations present a number of unstable mono- and trinucleotide repeats located in coding regions significantly higher than that observed in MMP+ tumors without BLM frameshifts. We suggest that BLM loss of function by MSI in gastric tumors is an intermediate mutational event, which may increase the instability of a pre-existent unstable genotype.

Methods

Tumor Samples
Fifty primary gastric tumors and their normal paired tissues (blood or normal gastric mucosa) were selected on the basis of available DNA from a panel of 80 gastric tumors (27, Calin G and Negrini M unpublished data). When possible, areas of tumor tissue with minimal inflammatory cells or minimal stroma, or both, were selected to obtain a neoplastic cell load greater than 50%. All cases were identified histopathologically as adenocarcinomas. Histotype (according to Lauren’s classification) [28] and staging (according to the TNM classification) [29] was known for 47 out of 50 cases (94%) (Table 1). Thirty-two cases were of intestinal histotype and 15 cases of diffuse or mixed type. Twenty tumors were staged I or II, while 27 were in stage III or IV. Regarding the lymph node status, eighteen cases were NO and 29 were N-positive. None of the patients had developed a cancer at an early age, and none had a family history suggestive of genetic predisposition to cancer. High molecular weight DNAs was isolated using established procedures [30].

Assessment of MSI
MSI was revealed with two sets of anonymous markers at loci: (i) D11S1778, D11S1328, D11S922 and D11S1318 [24] or (ii) D2S177, D3S1076, D5S433, D11S904, D17S96 and D18S50 [27]. The primers for these dinucleotide microsatellite loci were obtained from information available through the Genome Database. PCR amplifications were carried out as previously described [24,27]. For all samples the BAT25 and BAT26 microsatellites were analyzed by PCR amplification as described [31]. MSI was assessed if a mobility shift of PCR products from tumor DNA compared with the normal counterpart was identified. Only tumors with at least 2 MSI (>30% of tested microsatellites) were considered MMP+.
Amplification of microsatellites in BLM and in other coding regions (CDRs)
The (A)9 microsatellite located at position 1610–1618 of BLM cDNA sequence was analyzed for all paired samples. Briefly, the microsatellite was amplified by PCR using 50 ng of template DNA, 1 μM of each primer, 1.5 mM of MgCl₂, 50 μM each of dATP, dGTP, and dTTP and 5.0 μM of dCCT, 0.1 unit of Taq DNA polymerase, and 1 μCi [α³²P]dCTP in 10 μl of reaction volume. PCR reactions were carried out for one cycle of 14°C for 5 min followed by 35 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 30 s. PCR products from tumor and corresponding control DNAs were loaded on parallel 6% acrylamide sequencing gels and exposed for visualization by autoradiography on Kodak X-AR films. Detection of mutations in microsatellites located in the CDRs from eight other genes (the (A)10 in TGFβRII, the (G)8 in IGFIIR, the (G)8 in BAX, the (A)8 in hMSH3, the (C)8 in hMSH6, the (A)9 in RecQL, the (A)8 in WRN, and the (ATG)6 in CBL) was done as previously described [14,16,17,24,32]. For verification, each PCR was repeated at least twice. A mutated allele was represented by a band shift of 1 to 3 bp from the normal band with intensity comparable or greater than that of the wild-type band (Fig. 1).

Sequencing of abnormal products
Genomic DNA fragments exhibiting band shifts were amplified under the same conditions except for the omission of labeled dCTP. Before sequencing, the PCR products were purified either directly or after separation in a 2% agarose gel, using Qiagen purification kits. Both sequencing gels and exposed for visualization by autoradiography on Kodak X-AR films. Detection of mutations in microsatellites located in the CDRs from eight other genes (the (A)10 in TGFβRII, the (G)8 in IGFIIR, the (G)8 in BAX, the (A)8 in hMSH3, the (C)8 in hMSH6, the (A)9 in RecQl, the (A)8 in WRN, and the (ATG)6 in CBL) was done as previously described [14,16,17,24,32]. For verification, each PCR was repeated at least twice. A mutated allele was represented by a band shift of 1 to 3 bp from the normal band with intensity comparable or greater than that of the wild-type band (Fig. 1).

Statistical analysis
The statistical analysis of results was performed using the X² test or Fisher’s exact test. A P value of <0.05 was considered statistically significant.

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References
1. Bishop JM: Molecular themes in oncogenesis. Cell 1991, 64:235-238
2. Arzimanoglou IL, Gilbert F, Barber HRK: Microsatellite instability in human solid tumors. Cancer 1990, 82:1808-1820
3. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN, Srivastava S: A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 1998, 58:5248-57
4. Terdiman JP, Gum JR Jr, Conrad PG, Miller GA, Weinberg V, Crawley SC, Levin TR, Reeves C, Schmitt A, Heupburn M, Steisenger MH, Kim YS: Efficient detection of hereditary nonpolyposis colorectal cancer gene carriers by screening for tumor microsatellite instability before germline genetic testing. Gastroenterology 2001, 120:21-30
5. Fishel R, Lescoe MK, Rao MR, Copeland NG, Jenkins NA, Garber J, Kanza M, Kolodner R: The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. Cell 1993, 75:1027-1038
6. Miyaki M, Konishi M, Tanaka K, Kikuchi-Yanoshita R, Muraoka M, Yasuno M, Igray T, Koike M, Chiba M, Mori T: Germline mutation of MSH6 as the cause of hereditary nonpolyposis colorectal cancer. Nature Genet 1997, 17:271-272
7. Nicolaides NC, Papadopoulos N, Liu B, Wei YF, Carter KC, Ruben SM, Rosen CA, Haselton WA, Fleischmann RD, Fraser CM, et al: Mutations of two PMS homologues in hereditary nonpolyposis colorectal cancer. Nature Genet 1996, 22:275-279
8. Papadopoulos N, Nicolaides NC, Wei YF, Ruben SM, Carter KC, Rosen CA, Haselton WA, Fleischmann RD, Fraser CM, Adams MD, et al: Mutation of a mutL homolog in hereditary colon cancer. Science 1994, 263:1625-1659
9. Kinzler KW, Vogelstein: Lessons from hereditary colorectal cancer. Cell 1996, 87:159-170
10. Liu B, Nicolaides NC, Markowitz S, Willson J, Parsons R, Jen J, Padolpho N, Peltomaki P, de la Chapelle A, Hamilton SR, et al: Mismatch repair gene defects in sporadic colorectal cancers with microsatellite instability. Nature Genet 1995, 9:48-55
11. Cunningham JM, Christensen ER, Tester DJ, Kim CY, Roche PC, Burtar L, Thibodeau SN: Hypermutability of the hMLH1 promoter in colon cancer with microsatellite instability. Cancer Res 1998, 58:3455-3460
12. Herman JG, Umar A, Polyak K, Graff JR, Ahuja N, Issa JP, Markowitz S, Willson J, Hamilton SR, Kinzler KW, et al: Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. Proc Natl Acad Sci USA 1998, 95:6870-6875
13. Veigl ML, Kasturi L, Olechnowicz J, Ma AH, Lutterbaugh JD, Periyasamy S, Li GM, Drummond J, Modrich PL, Sedwick WD, Markowitz SD: Biallelic inactivation of hMLH1 by epigenetic gene silencing, a novel mechanism causing human MSI cancers. Proc Natl Acad Sci USA 1998, 95:8698-8703
14. Malkhosyan S, Rampino N, Yamamoto H, Perucchini M: Frameshift mutator mutations. Nature 1996, 382:499-500
15. Parsons R, Myeroff LL, Liu B, Willson J, Markowitz SD, Kinzler KW, Vogelstein B: Microsatellite instability and mutations of the transforming growth factor beta type II receptor gene in colorectal cancer. Cancer Res 1995, 55:5548-5550
16. Rampino N, Yamamoto H, Ionov Y, Li Y, Sawai H, Reed JC, Perucchini M: Somatic frameshift mutations in the BAX gene in colon cancers of the microsatellite mutator phenotype. Science 1996, 275:967-969
17. Souza RF, Appel R, Yin J, Wang S, Smolinski KN, Abraham JM, Zou TT, YQ Shi, Lei J, Cottrell J, et al: Microsatellite instability in the insulin-like growth factor II receptor gene in gastrointestinal tumours. Nature Genet 1996, 4:255-257
18. Souza RF, Yin J, Smolinski KN, Zou TT, Wang S, Shi YQ, Rhyu MG, J Cottrell, Abraham JM, Beken D, et al: Frequent mutation of the E2F-4 cell cycle gene in primary human gastrointestinal tumours. Nature Genet 1996, 14:255-257
19. Dennis PA, Rifkin DB: Cellular activation of latent transforming growth factor beta requires binding to the cation-independent m6phosphate/insulin-like growth factor type II receptor. Proc Natl Acad Sci USA 1991, 88:580-584
20. Lau KM, Stewart CE, Liu B, Bhatt H, Roskindin P, Stewart CL: Loss of the imprinted IGFI2/cation-independent m6phosphate receptor results in fetal overgrowth and perinatal lethality. Genes Dev 1994, 8:2953-2963
21. Yin C, Knudson CA, Korsmeyer SJ, Van Dyke T: Bax suppresses tumorigenesis and stimulates apoptosis in vivo. Nature 1997, 385:637-640
22. Modrich P, Lahue M: Mismatch repair in replication fidelity, genic recombination, and cancer biology. Annu Rev Biochem 1996, 65:101-133
24. Calin G, Herlea V, Barbanti-Brodano G, Negrii M: The coding region of the Bloom syndrome BLM gene and of the CBL proto-oncogene is mutated in genetically unstable sporadic colorectal cancers. Cancer Res 1998, 58:3777-3781
25. German J, Ellis NA: Bloom syndrome. In: The genetic basis of human cancer (Edited by Vogelstein B, Kinzler KW), McGraw-Hill, New York 1998;301-315
26. Sepulveda AR, Santos AC, Yamaoka Y, Wu L, Gutierrez O, Kim JG, Graham DY: Marked differences in the frequency of microsatellite instability in gastric cancer from different countries. Am J Gastroenterol 1999, 94:3034-3038
27. Renault B, Calistri D, Buonsanti G, Nanni O, Amadori D, Ranzani GN: Microsatellite instability and mutations of p53 and TGF-βRII genes in gastric cancer. Hum Genet 1996, 98:601-607
28. Lauren P: The two histological main types of gastric adenocarcinomas: diffuse and so-called intestinal-type carcinoma. Ada Pathol Microbiol Scand 1965, 64:31-49
29. Puranam KL, Blackshear PJ: Molecular cloning: a laboratory manual. Cold Spring Harbor, Cold Spring Harbor Press 1989
30. Sambrook J, Fritsch EF, Maniatis T: Molecular cloning: a laboratory manual. 3rd ed. Cold Spring Harbor, Cold Spring Harbor Press 1989
31. Hoang JM, Cottu PH, Thuille B, Salmon RJ, Thomas G, Hamelin R: Sequence variation at the DTH1 locus: new polymorphic markers. Hum Genet 1989, 80:500-502
32. Markowitz S, Wang J, Myeroff L, Parsons R, Sun L, Lutterbaugh J, Fan RS, Zborowska E, Kinzler K, Vogelstein B et al: Defects in the replication fidelity of simple repeated sequences reveal a new mutator mechanism for oncogenesis. J Biol Chem 1994, 269:16836-16843
33. Oliveira C, Seruca R, Seixas M, Sobrinho-Simoes M: The clinicopathological features of gastric carcinomas with microsatellite instability may be mediated by mutations of different target genes. Am J Pathol 1998, 153:1211-1219
34. dos Santos NR, Seruca R, Constancia M, Seixas M, Sobrinho-Simões: Microsatellite instability at multiple loci in gastric carcinoma: clinicopathologic implications and prognosis. Gastroenterology 1996, 110:38-44
35. MS Wu, Lee GW, Shun CT, Wang HP, Lee WJ, Sheu JC, Lin JT: Clinical significance of microsatellite instability in gastric cancer. Cancer Res 1998, 58:1494-1497
36. Puranam KL, Blackshear PJ: Cloning and characterization of P53 (cDNA cloning of a potential human homologue of the Escherichia coli DNA helicase RecQ). J Biol Chem 1994, 269:29838-29845
37. Yu CE, Oshima J, Fu YH, Wijsman EM, Hisama F, Alisch R, Matthews S, Nakura J, Miki T, Ouais S, et al: The Bloom's syndrome gene. Hum Genet 1997, 100:360-367
38. Ota Y, Samelson LE: The product of the proto-oncogene c-cbl: a negative regulator of the Syk tyrosine kinase. Science 1997, 276:418-20
39. Blake LTJ, Langdon WY: A rearrangement of the c-cbl proto-oncogene in HUT78 T-lymphoma cells results in a truncated protein. Oncogene 1997, 15:757-762
40. Perucchini M, Peinado MA, Ionov Y, Casares S, Malkhosyan S, Stanbridge E: Defects in replication fidelity of simple repeated sequences reveal a new mutator mechanism for oncogenesis. old Spring Harb Symp Quant Biol 1994, 59:339-348
41. Perucchini M: Microsatellite instability: the mutator that mutates the other mutator. Nature Med 1996, 2:630-631
42. Chung YJ, Park SW, Sorg JM, Lee KY, Seo EJ, Choi SW, Rhyu MG: Evidence of genetic progression in human gastric carcinomas with microsatellite instability. Oncogene 1995, 15:1719-1726
43. Calin GA, Gala R, Tibletti GM, Herlea V, Becheanu G, Cavazini L, Barbanti-Brodano G, NencI, Negriii M, Lanza G: Genetic progression in microsatellite instability high (MSI-H) colon cancers correlates with clinico-pathological parameters: a study of the TGF-βRII, BAX, HMSH3, HMSH6, IGFIIIR and BLM genes. Int J Cancer 2000, 89:230-235
44. Groden J, German J: Bloom’s syndrome XVIII. Hypermutability at a tandem-repeat locus. Hum Genet 1992, 90:360-367
45. Langlois RG, Bigbee WL, Jensen RH, German J: Evidence for increased in vivo mutation and somatic recombination in Bloom’s syndrome. Proc Natl Acad Sci USA 1989, 86:6670-6674
46. Karow JK, Chakraverty RK, Hickson ID: The Bloom’s syndrome gene product is a 3’-5’ DNA helicase. J Biol Chem 1997, 272:30611-30614
47. Davey S, Han GS, Ramer SA, Klassen JC, Jacobson A, Eisenberger A, Hopkins KM, Lieberman HB, Freyer GA: Fission yeast rad12+ regulates cell cycle checkpoint control and is homologous to the Bloom’s syndrome disease gene. Mol Cell Biol 1998, 18:2721-2728
48. Stewart E, Chapman CR, Al-Khodairy F, Carr AM, Enoch T: rqh1+, a fission yeast gene related to the Bloom’s and Werner’s syndrome genes, is required for reversible S phase arrest. EMBO J 1997, 16:2682-2692
49. Myung K, Datta A, Chen C, Kolodner RD: SGS1, the Saccharomyces cerevisiae homologue of BLM and WRN, suppresses genome instability and homeologous recombination. Nature 2001, 271:113-116
50. Yin J, Kong D, Wang S, Zou TT, Souza RF, Smolinski KN, Lynch PM, Hamilton SR, Sugimura H, Powell SM, et al: Mutation of HMSH3 and HMSH6 mismatch repair genes in genetically unstable human colorectal and gastric carcinomas. Hum Mutat 1997, 10:474-478
51. Yamamoto H, Sawai H, Perucchini M: Frameshift somatic mutations in gastrointestinal cancer of the microsatellite mutator phenotype. Cancer Res 1997, 57:4220-4226
52. Oztan L, Falchetto M, D’Amico A, Amorosi A, Saiava C, Masala G, Frati L, Cama A, Dalli P, Mariani-Costantini R: Mutations at coding mononucleotide repeats in gastric cancer with the microsatellite mutator phenotype. Oncogene 1998, 16:2767-2772

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