Deletion of the Alu-VpA/MycL1 (1p34.3) Locus is a Negative Prognostic Sign in Human Colorectal Cancer


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Abstract—We examined deletions of the short arm of chromosome 1 and aberrations of the microsatellite locus Alu-VpA/MycL1 (1p34.3) in human primary colorectal adenocarcinomas. Cytogenetically discernible deletions in 1p were found in 45% (14/31) of informative tumors. The 1p- tumors commonly exhibited a polyploid karyotype (Fisher P1 = 0.023) and a larger number of rearranged chromosomes (P2 = 0.045) versus those without 1p deletions. The 1p deletions often combined with chromosome 5 monosomy (X^2 = 6.24; p = 0.013), chromosome 15 monosomy (X^2 = 4.20; p = 0.040), and 11q deletions (P2 = 0.035). Among the 50 carcinomas, 11 (22%) showed Alu-VpA/MycL1 instability, and 14% (6/43 informative) had lost the Alu-VpA/MycL1 allele. The genetic alterations thus revealed were collated with the clinical and morphological features of the tumors. The loss of the 1p material was shown to be correlated with marked karyotype aberrations in colorectal tumors, and Alu-VpA/MycL1 allele deletions were tightly associated with relapses or metastasis within 30 months after surgery.

Key words: colorectal cancer, karyotype, 1p, locus, microsatellite repeat, loss of heterozygosity, Alu-VpA/MycL1, PCR, prognostic sign

INTRODUCTION

Colorectal cancer (CC), as other tumors, is attended with pronounced alterations in the cell genome. The most common of these are changes in the chromosome number and structure, rearrangements and deletions of separate loci, and an elevated mutation background, known as the rer^ phenotype [1-3]. Cytogenetic studies have outlined the spectrum of characteristic anomalies of the karyotype in CC [4, 5]. Deletions of the chromosome 1 short arm (1p) are found in 30-50% of colorectal tumors, including both adenomas and carcinomas [6, 7]. Studies of particular microsatellite markers in colorectal tumors have revealed three spots of most frequent deletions in the 1p34.2-1pter region, and independent interstitial deletions have been shown to occur in these spots [8-10]. There are indications that 1p losses in colorectal tumors correlate with negative prognosis [1, 5].

In this work, our general goal was to find out whether the malignant colorectal tumors harboring clones with 1p deletions exhibit any peculiarities as compared with tumors having no such clones. More specifically, we assessed the Alu-VpA/MycL1 (1p34.3) locus that is close to the MycL1 gene [11, 12] and to a most frequently deleted 1p region SRO C [10].

Alu-VpA/MycL1 is one of the most informative markers of microsatellite instability (MSI) in colorectal adenoma with islets of carcinoma [13]. Though MSI is more typical of hereditary nonpolyposis colorectal cancer, it is also found in 15-40% of sporadic colorectal tumors and, as a rule, is associated with mutations in the DNA repair genes [14-16]. PCR analysis of Alu-VpA/MycL1 can reveal both the loss of heterozygosity and the microsatellite instability (A-MSI). The results of studying the impairment of chromosome 1 and locus Alu-VpA/MycL1 are collated with the clinical and morphological features of the tumors and the post-surgery progress.

EXPERIMENTAL

We used the surgery material of 74 primary colorectal adenocarcinomas from patients aging over 30; cytogenetic examination was performed in 39 cases, PCR assays in 50, and both in 15 cases. The Alu-VpA/MycL1 locus was probed both in the tumor and in the normal colonic mucosa of every patient. The cytogenetic procedure was routine in our lab [17]. For PCR, the DNA was isolated from fresh cryosections [18] and amplification run with primers [5'-TGGCGAGACTCCATCAAG-3'] and [5'-CCTTATGGCTGCAACAATTTT-3'] [11]; the products were resolved in a sequencing gel and transferred semi-dry onto Hybond N or N+ membranes (Amerham), hybridized with [γ-32P](AAAG)_5 (kindly provided by P.M. Chumakov, Engelhardt Institute of Molecular Biology, Moscow), and autoradiographed.
Serial sections of tumor specimens were processed histologically to evaluate the extent of differentiation. Morphological analysis of the material was performed in accordance with the international histological typing of intestinal tumors [19] and the generally recognized Dukes classification [20]. The results of laboratory studies were collated with the clinical and morphological traits of the tumors listed in Table 1. The data were evaluated using the Statistica 5.1 program (StatSoft, Inc.).

RESULTS

Tumor Karyotype Peculiarities

Karyotype analysis was carried out for 39 tumors. One of these had a normal karyotype (a male of 30 with primary multiple cancer). One hyperdiploid tumor exhibited only alterations in the chromosome number, whereas others had cell clones with pronounced aberrations in chromosome number and structure. The ploidy data are given in Table 1. In four tumors, the changes in chromosome number could not be reliably established but the marker chromosomes were identified. In 32 tumors, clones were found with more than three marker chromosomes, in 13 there were five and more markers, and 10 had more than 10 markers.

The most frequent karyotypic alterations in the series examined (36 tumors or 92%) were chr17 short arm changes; 32 tumors had chr18 monosomy; in decreasing frequency, there came 8p deletions (8p- and i8q marker, total 17 tumors), chr5 monosomy (15), deletions in the chr1 short arm (14), and deletions in the chr6 long arm (11).

Karyotype Alterations in Tumors with 1p Deletions

Cytogenetically discernible 1p deletions were found in 14 tumors (36%). Terminal deletions arising from breaks in 1p11 were revealed in three cases, p12 in two, p13 in three, p22 in five, and p31 in one (Fig. 1). Another two tumors supposedly had 1p deletions: loss of 1p34-pter combined with chr1 monosomy, and 1p36 deletion. Some tumors had chr1 monosomy and explicit translocations involving 1p, as well as some combined chr1 aberrations. These cases were excluded from comparative analysis. No 1p deletions were found in 17 tumors.

To evaluate the connection between 1p losses and the extent of karyotype rearrangement, we compared the ploidy and the overall number of chromosome structural alterations in two groups of tumors: with and without 1p deletions. The results in Table 2 show that 1p deletions are significantly correlated with tumor polyploidy and with the presence of numerous chromosomal markers.

We compared the frequency of particular chromosomal aberrations characteristic of CC in the two groups (Table 3), and observed significant correlations of 1p deletions with chr5 monosomy, 11q deletions, and chr15 monosomy, but no correlation with chr6 or chr14 monosomy, deletions 6q-, 9q-, 11q-, rearrangements i8q and i13q, or of chr16 and chr20 trisomy.