High polymorphism in the trisomic portion of a gastric cancer cell line

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Background. Genetic instability is a hallmark of malignancy, and microsatellite instability is a widely appreciated mechanism of generating genetic changes. We have recently observed four markers clustered on chromosome 20 that showed the effects of microsatellite instability in the gastric adenocarcinoma cell line SNU-1. Each affected marker had alleles of three different sizes. The aim of this study was to investigate the origin for this high-density polymorphism on a single chromosome.

Methods. The high polymorphism located on chromosome 20 was confirmed using 37 additional markers. To further evaluate this finding, 15 clones of the cell line were generated and then assayed with the triallelic markers.

Results. All told, almost a third of the markers on chromosome 20 had triallelic patterns, but only 0.3% of the markers not on chromosome 20 showed this result. The number of clones showing allelic variation was an average of 50% greater for chromosome 20 markers than for markers elsewhere. A karyotype analysis showed that the progenitor cell line of SNU-1 was trisomic for chromosome 20, and the high polymorphism on that chromosome is almost certainly due to the trisomy.

Conclusions. Not only are there more chromosome copies and therefore more gene copies subject to mutation in cells containing trisomy, but also more mutations may be passed on to the progeny. This elevated polymorphism increases the repertoire of genetic changes that could affect cellular growth, and may independently increase genomic instability.

Key words: polymorphism, trisomy, gastric cancer

Introduction

Microsatellite instability (MSI) is a phenomenon where there is a change in the size of a microsatellite marker within tumor DNA when compared with normal tissue DNA from the same individual. In gastric cancer, the degree of MSI is correlated with both the biological behavior and the spectrum of specific genetic alterations.¹ ²

In a recent genetic survey of 14 gastric adenocarcinoma cell lines using 351 markers throughout the human genome,³ we detected an unusual cluster of MSI. In the cell line SNU-1, four out of nine markers on chromosome 20 showed MSI, and the rest of the genome showed only one other marker with MSI. A possible cluster was also seen on chromosome 3 in the cell line SNU-638.

Both SNU-1 and SNU-638 have been shown to exhibit MSI⁴ and to be deficient in the proteins of the MutL DNA mismatch-repair pathway.⁵ SNU-1 has been shown to have a mutation in the hMLH1 gene, and SNU-638 has been shown to have a mutation in the hMSH2 gene.⁶ In both of these cell lines, genetic mutations caused by MSI likely play an important role in their growth.⁴

Because normal tissues from the sources of these cell lines were unavailable, in our previous survey the full criteria for documentation of MSI could not be established. The designation of MSI was given to markers where there were three clear alleles with different sizes, because any normal tissue would show at most two alleles. Markers with two alleles may have been subject to MSI, but could not be given that designation without knowing the allele size in matched normal DNA.

The present study was undertaken to confirm the presence of a cluster by using a larger panel of markers, to estimate the chromosome-specific rate of MSI, and to determine the genetic cause of the clustering in the cell line SNU-1.
Materials and methods

Cell lines, culture conditions, and DNA extraction

The cell line SNU-1 was the focus of this study. SNU-1 is also known as CRL-5971 and was obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The sample provided by the American Type Culture Collection had a passage number of 96. The cell line SNU-638 was the gift of Seong-Jin Kim of the National Cancer Institute (Bethesda, MD, USA) and is available from the Korean Cell Line Bank in Seoul, Korea. SNU-1 was derived from a poorly differentiated gastric adenocarcinoma in 1984, and SNU-638 from malignant ascites in 1991. This group has recently published data using original tumor tissue that was the source of SNU-638. Because MSI is best evaluated using matched normal tissue, the group was contacted as to the availability of normal tissue from the SNU-1 patient, but none was archived. A control lymphoblastoid cell line, GM06990, was obtained from the Coriell Institute for Medical Research (Camden, NJ, USA). All cell lines were cultured in RPMI-1640 medium containing 10% fetal calf serum and maintained in a humidified atmosphere of 5% CO₂.

Fifteen clones of SNU-1 were created by diluting a growing culture of cells to a density of 0.3 cells per 150 µl and dispensing that amount into each well of four 96-well microtiter plates. After an hour to allow the cells to settle, wells that contained a single cell were identified visually using an inverted microscope. Those wells were observed daily, and in 15 of them, there was clear progressive cell division with formation of a single colony of cells. Each colony was grown in culture and harvested when the number of cells reached $2 \times 10^8$.

DNA samples from all of the cell lines and SNU-1 clones were obtained using the Genomic-tip 100/G kit (Qiagen, Valencia, CA, USA). Purified DNA from a control lymphoblastoid line, RH01controlRM, was purchased from Research Genetics (Huntsville, AL, USA). DNA concentrations were determined spectrophotometrically.

Microsatellite markers, PCR conditions, and product analysis

Table 1 contains the name, type of nucleotide repeat, and chromosomal position of each marker used in this study. The primer pairs used to amplify the markers were purchased from Research Genetics. The chromosomal locations were compiled from a variety of sources. For chromosomes 3 and 20, the loci are listed in order from the telomeric region of the short arm to...