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BRIEF METHOD

Conventional and Molecular Cytogenetic Characterization of a New Human Cell Line, GIST-T1, Established from Gastrointestinal Stromal Tumor

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A new human cell line, GIST-T1, was established from a metastatic plural tumor from a gastrointestinal stromal tumor (GIST) of the stomach in a Japanese woman, and was characterized by immunohistochemistry, conventional banding methods, comparative genomic hybridization (CGH), and fluorescence in situ hybridization (FISH). Immunohistochemically, GIST-T1 cells were strongly positive for CD34 and c-KIT, but not for desmin, S-100 protein, or a-smooth muscle actin. These findings indicated that the GIST-T1 cell line fully expressed the characteristics of GIST. Conventional banding analysis revealed that GIST-T1 displayed a hypodiploid karyotype with a ring chromosome, several unbalanced translocations, and marker chromosomes. CGH studies showed chromosomal gains and losses on several regions. FISH using painting probes revealed that the ring chromosome was derived from chromosome 1 and some marker chromosomes consisted of more than two different chromosomes. Our study showed that GIST-T1 has a complex karyotype and high-level amplifications involving chromosome bands 3q26.1–27, 5p12–15.1, and 7q21.3–36, where amplification of oncogenes may arise. Combined analysis of classical and molecular cytogenetic techniques provides an accurate cytogenetic assessment, including change in the DNA copy number of GIST-T1. This newly established cell line, GIaST-T1, will be a particularly useful model for studying molecular pathogenesis of GIST.

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CGH studies of GIST have shown some genetic alterations (El-Rifai et al, 2000; Knuutila et al, 2000), and molecular studies of the c-kit gene of GIST revealed the mutation of the gene in GIST (Hirota et al, 1998; Taniguchi et al, 1999). However, the precise molecular and genetic basis of carcinogenesis in GIST still remains unknown. One approach to studying this tumor is to establish well-characterized human tumor cell lines, which are important research resources for studying tumor cell biology, as well as for developing new strategies against tumor cell growth and progression. To our knowledge, no GIST cell line has yet been reported.

The GIST-T1 cell line was established from a metastatic pleural tumor, from a primary gastric GIST in a 47-year-old Japanese woman. The tumor tissue obtained at surgery was minced with knives into small pieces, which were placed in culture flasks and cultured in Dulbecco's minimum essential medium (Sigma-Aldorich, Tokyo, Japan) supplemented with 10% FBS (Gibco BRL, Grand Island, New York) in a humidified incubator containing 5% CO_2 at 37° C.

For the pathologic study, GIST-T1 cells growing on the coverslips were fixed with 95% ethanol, and tissue from a xenotransplanted nude mouse tumor was fixed with 20% buffered formalin solution. The coverslips and dewaxed tumor tissue sections were stained with hematoxylin-eosin (Fig. 1A) and examined with antibodies against vimentin (V9, ×100, Dako, Dakopatts; Kyoto, Japan), desmin (D33, ×30; Dakopatts), α-smooth muscle actin (α-SMA; 1A4, ×200; Dakopatts), S-100 protein (S-100; polyclonal, ×1000; Dakopatts), CD34 (HPCA-1, ×10, Becton Dickinson, Franklin Lakes, New Jersey) (Fig. 1B), c-KIT (polyclonal, ×50, Dakopatts) (Fig. 1C), using the streptavidin-biotin immunoperoxidase method (Histofine SAB-PO kit, Nichirei, Tokyo, Japan). Population doubling time was determined by seeding the cells at the initial density of 10^5 in 24-well plates, and counting was performed for up to 10 days, in triplicate. For the cytogenetic study, subconfluent

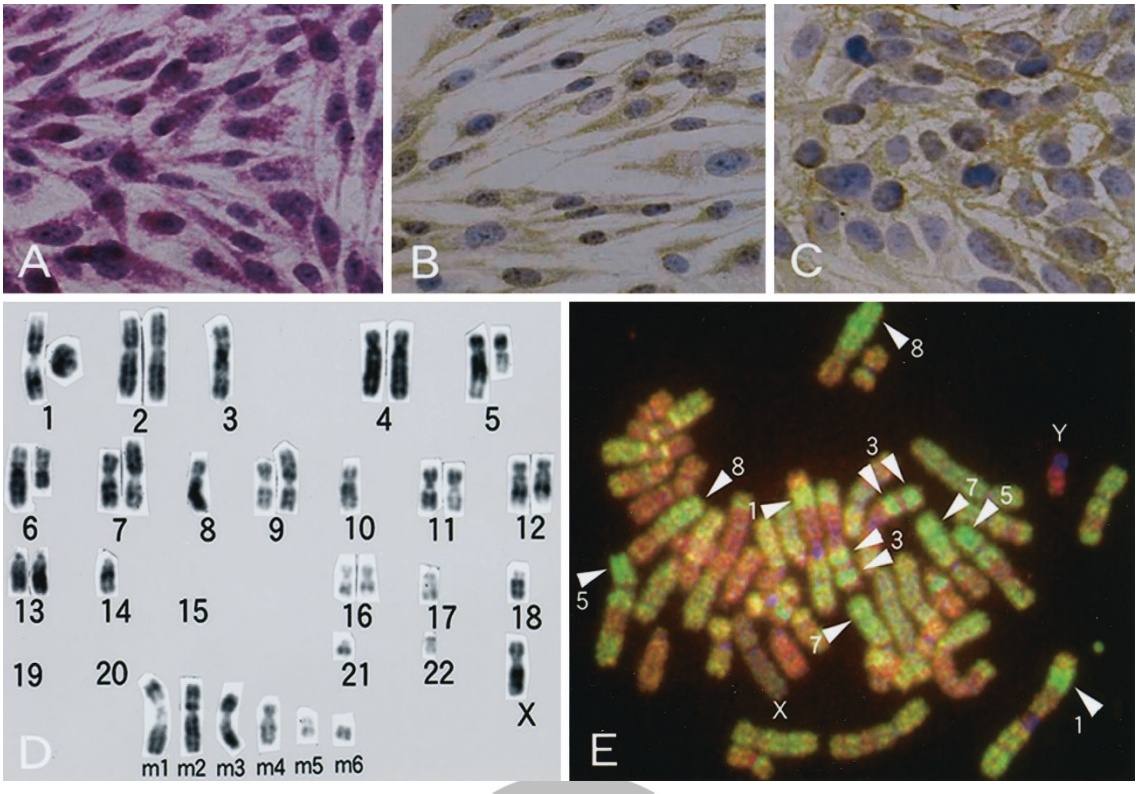

Figure 1.

Light microscopy of GIST-T1 cells. Hematoxylin-eosin staining (A), immunopositive reactions of the cytoplasm for CD34 (B), and c-kit (C). A representative reversed Q-banded karyogram of a GIST-T1 cell (D). Representative CGH image of GIST-T1 (E). Tumor DNA was detected in green (FITC), and normal DNA in red (rhodamine). *Arrowheads* indicate the identified regions of higher levels of amplification on chromosomes 1, 3, 5, 7, and 8.

GIST-T1 cells were treated with Colcemid (Gibco BRL) at the final concentration of 0.1 mg/ml for 4 hours at 37° C. The cytogenetic analysis was conducted as previously described (Taguchi et al, 1997). Ten consecutive metaphases were fully analyzed. The karyotypes of each metaphase were described according to the international system for human cytogenetic nomenclature (ISCN, 1995). For CGH study, DNA was extracted using a kit purchased from Amersham-Pharmacia (Tokyo, Japan). Nick translation of the normal DNA from a healthy donor male (46,XY) was performed with rhodamine-4-dUTP, according to the kit protocol (Amersham-Pharmacia). The tumor DNA from the GIST-T1 cell line was labeled with fluorescein-11-dUTP using a procedure similar to that used for normal DNA. To analyze CGH, 7 FITC and rhodamine fluorescence, specific for the reference genome, respectively, were quantified by a cooled, charged-coupled device camera, which was connected to an M-FISH system (Photometrics, Sekitechnotron, Tokyo, Japan) and the fluorescence banding pattern obtained after DAPI staining was used for chromosome identification. More than five metaphases were combined to obtain profiles of the mean ratio and standard deviation. Chromosomal regions where the green to red ratio exceeded 1.15 were considered overrepresented (gain), whereas regions where the ratio was less than 0.85 were regarded as underrepresented (lost). This value was therefore selected as a cut-off level for the smallest ratio. If the mean green to red ratio exceeded 1.5 in a small segment of the chromosome arm, these regions were considered to represent high-level amplification. Telomeric and heterochromatic regions were excluded from the analysis. For FISH analysis, painting probes of chromosomes 1, 3, 4, 5, 7, 8, and 10 were used (Cambio, Cambridge, England).

The newly established GIST cell line, GIST-T1, derived from a Japanese woman, has maintained cells with 48-hour doubling time for more than 24 months, undergoing more than 30 passages since the initial culture. GIST-T1 cells proved tumorigenic when injected into nude mice, and the xenotransplanted tumor had a similar microscopic morphology to the original tumor. GIST-T1 cells, both in vitro and in vivo, were strongly immunopositive for both c-kit receptor and CD34 antibody (Fig. 1, B and C), which is consistent with the characteristics of GIST (Hirota et al, 1998).

Conventional banding analysis revealed that GIST-T1 displayed a hypodiploid karyotype with a ring chromosome, several unbalanced translocations, and marker chromosomes. The composite karyotype was as follows; 35–39, −X, −1, −3, der(5)add(5)(q11), del(6)(q16), +der(7)t(7;8)(p13;q13), der(9)t(9;10)(p12;q11), −14, −15, −17, −18, −19 × 2, −20 × 2, −22, +r, +3–6 mar [cp10] (Fig. 1D). As identification of unbalanced chromosomal rearrangements involving ring and marker chromosomes was extremely difficult with conventional banding methods, we analyzed them using both CGH and whole chromosome painting methods. Based on the result of CGH, which detected chromosomal imbalances, further characterization of structural chromosomal aberrations was conducted by specific painting

probes. FISH revealed that the ring chromosome was derived from chromosome 1 and some of the marker chromosomes consisted of more than two different chromosomes.

By CGH, high-level amplification (more than 1.5 of the ratio) with a sharp peak was confined to 3q26.1–27, 5p12–15.1, and 7q21.3–36, respectively (Fig. 1 E). It is of interest that three regions of high-level amplifications were detected in this cell line. Because high-level amplification detected by CGH is generally due to the presence of one or more amplified oncogenes in tumors, there may be more than three oncogenes amplified and overexpressed in this cell line. Interestingly, there have been no known oncogenes that map to 5p. In previous investigations of GIST, the most frequent chromosomal abnormalities were observed in losses of chromosomes 14 and 22 by a conventional banding method (Breiner et al, 2000), as also observed in GIST-T1, which may contribute to early GIST pathogenesis. As reported previously on the mutation of *c-kit* gene (Hirota et al, 1998; Taniguchi et al, 1999), our preliminary sequence analysis of exon 11 of the *c-kit* gene on GIST-T1 cells by direct sequence determination revealed a heterozygous deletion of 57 bases. This is predicted to change the wild-type protein sequence beginning at amino acid 5650 from KPMYEVQWKVVEEINGNNYVYIDPTQLPYDHKWEF . . . to KPMYEVQWKVDHKWEF. . .

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