

## Cytogenetical Analysis of the Human Gastric Carcinoma Cell Line TMK-1

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### ABSTRACT

We examined a human gastric carcinoma cell line TMK-1 cytogenetically by G-banding technique. TMK-1 cells were characterized not only by numerical aberrations but also by structural rearrangements affecting various chromosomes. The modal chromosome number was found to be in the hypotriploid range with most ranging between 64 and 66. Flow cytometry revealed near triploid DNA histogram which was correlated well with chromosome counts. Gain of chromosome 7, 9, 11, and X was found in all of the 25 cells examined and trisomy of chromosome 3, 5, 10, 19, 21 and 22 was also frequently detected. Loss of Y chromosome was shown in all of the cells and monosomy of chromosome 6 and 13 was frequently detected. The most conspicuous rearrangements involved chromosome 6, 12, and 16 which showed complex nonreciprocal translocation. Twenty-two cells (88%) had isochromosome of 12q. A brief review is made of reported karyotype of human gastric cancers.

**Key words:** *Human stomach cancer, TMK-1 cell, Cytogenetics, Flow cytometry*

A human gastric carcinoma cell line TMK-1 was established in vitro by the soft agar method from SC-6-JCK, a poorly differentiated adenocarcinoma of a 21-year-old male<sup>9</sup>. Biological properties and morphological appearance have been well examined in our laboratory<sup>9</sup>. Briefly, TMK-1 cells showed parietal cell differentiation which was electron microscopically confirmed by the presence of numerous mitochondria, tubulovesicles and intracytoplasmic canaliculi filled with abundant microvilli. Both human and synthesized gastrin promoted the growth of TMK-1 cells and temporarily increased the content of cyclic adenosine 3', 5'-monophosphate (cAMP) in TMK-1 cells. c-AMP-dependent protein kinase was also activated by gastrin administration.

In the present study, TMK-1 cells were cytogenetically examined to detect a nonrandom chromosome aberration. The results obtained were compared well with the reported karyotypes of human gastric carcinoma.

### MATERIALS AND METHODS

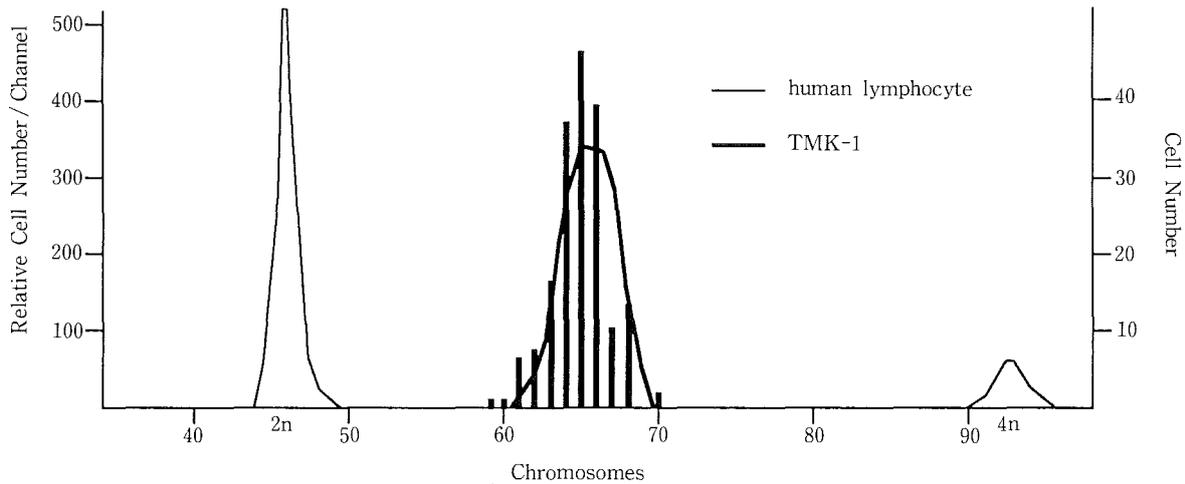
#### Chromosome Analysis:

Cytogenetic analysis was carried out on the third day after subculture of TMK-1 cells in conventional medium containing RPMI 1640 supplemented with 10% fetal calf serum. Colchicine was added at a concentration of 0.1 µg/ml one hour before har-

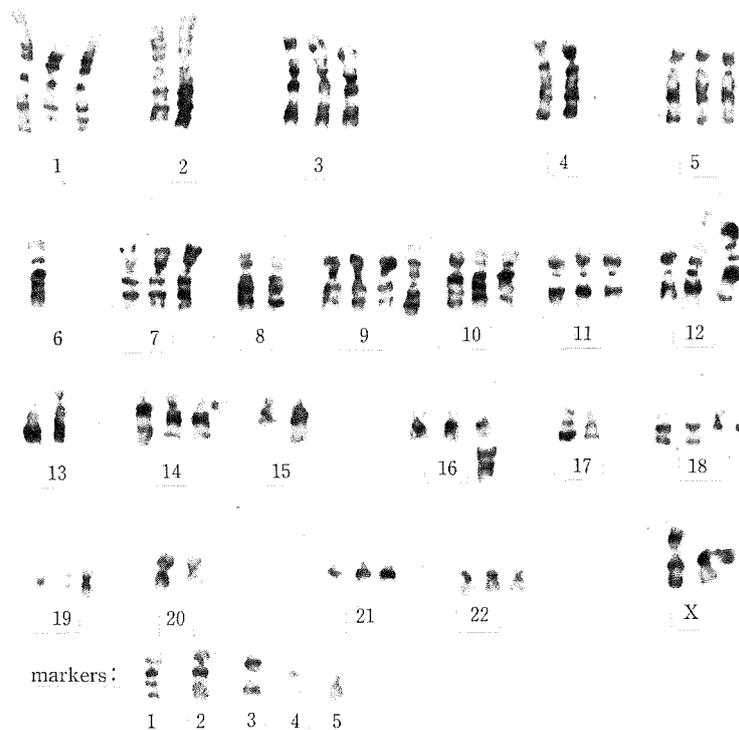
vest. The cells were detached by treatment with 0.025% trypsin-EDTA solution and then treated with 0.075 molar potassium chloride solution for 20 minutes. After hypotonic treatment the cells were fixed three times with cold methanol-acetic acid (3:1). Chromosome preparations were made using the air-drying technique and the slides were kept at room temperature for 7 days before banding. Karyotype analysis was performed using GTG (G-banding by trypsin-Giemsa method) technique<sup>15</sup>. G-banded slides were examined and adequately spread metaphases were photographed using ×1000 or ×800 oil immersion lens with a Nikon photomicroscope. Karyotypes were determined by arranging all photographed metaphases according to the International System for Human Cytogenetic nomenclature scheme<sup>4</sup>.

#### Flow Cytometry:

TMK-1 cells and normal lymphocytes from a healthy volunteer were fixed in 70% ethanol for more than 30 min. Samples were then centrifuged and digested by RNase (Sigma) at 37°C for 30 min. The cells were again centrifuged and resuspended in staining solution (propidium iodide, Sigma, 50µg/ml in PBS). Analysis was performed on an FACScan (Becton-Dickinson, Mountain view, CA). Histogram was analyzed on a Hewlett-Packard computer with the program Consort 30 (BD).



**Fig. 1.** DNA histogram and distribution of chromosome counts. TMK-1 cells show near-triploid histogram, while normal lymphocytes of a healthy volunteer present exclusively diploid pattern. Most of the TMK-1 cells have 64 to 66 chromosomes which coincide well with DNA histogram.



**Fig. 2.** G-banded karyotype of a TMK-1 cell: 66,XX,-Y,+1p+,+3,+5,-6,+7,+9,+9p+,-10,+11,-12,+der(12)t(6;12)(p11,p11).+i(12q),der(13)t(13;?)(p11;?),+der(14)t(14;?)(p12;?),+der(16)t(6;16)(q12;q32),+18q-,+18q-,+19,+21,+22,+5mar(*in vitro*)

## RESULTS

The chromosome counts of TMK-1 cells varied from 59 to 157, but 118 (66.3%) of the 178 cells had 64 to 66 chromosomes. Thus, TMK-1 cells were cytogenetically characterized by hypotriploidy nature. Figure 1 illustrated the DNA histogram and the chromosome counts of TMK-1 cells. TMK-1 cells showed near-triploid histogram, while normal lymphocytes of a healthy volunteer presented an exclusively diploid pattern. A good correlation was observed between DNA histogram and chromosome

counts of TMK-1 cells.

Twenty-five karyotypes were successfully banded and analyzed. Figure 2 shows a representative G-banded karyotype of TMK-1 cells. They were characterized not only by numerical deviation from the diploid karyotype but also showed various structural rearrangements.

We analyzed the gain and loss of whole chromosome with or without structural aberrations (Fig. 3). A gain of chromosome 7, 9, 11, and X without showing structural aberrations was found in all of

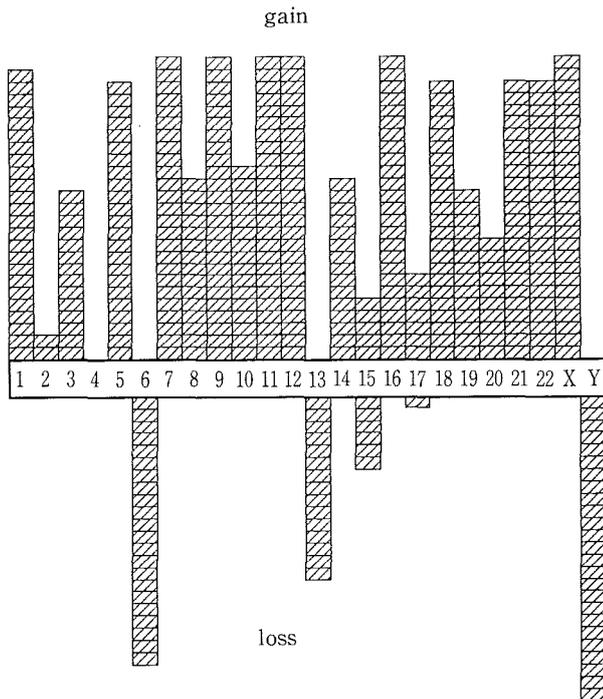


Fig. 3. Distribution of gains and losses of chromosome in 25 TMK-1 cells.

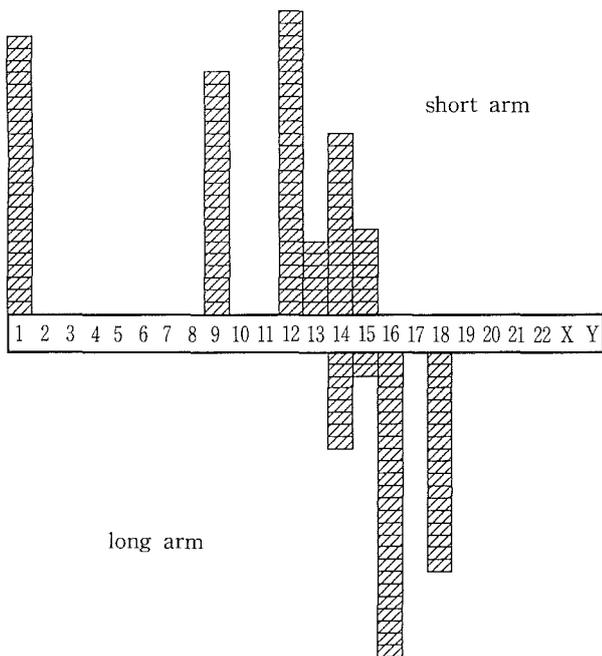


Fig. 4. Distribution of chromosome involvement of structural rearrangements in 25 TMK-1 cells.

the 25 cells examined. Trisomy of chromosome 5, 21 and 22 was seen in 24 cells (96%), while trisomy of chromosome 10 was noted 16 cells (64%), chromosome 8 in 15 (60%), chromosome 3 and 19 in 14 cells (56%). Loss of Y chromosome was found in the all cells. Monosomy 6 and 13 was detected in 22 (88%) and 15 (60%) cells, respectively.

Figure 4 shows the frequency of structural aberrations of TMK-1 cells. 1p+ and 9p+ from unknown origin were detected in 23 (92%) and 20

(80%) cells, respectively. 13p+, 14p+ and 15p+ were found in 6 (24%), 15 (60%) and 7 cells (28%), respectively, while 14q+ and 15q+ were noted in 8 cells (32%) and in 2 (8%), respectively. 18q- was also frequently detected. The most conspicuous rearrangements occurred in chromosome 6, 12 and 16 which showed complex nonreciprocal translocation. Short and long arm of chromosome 6 translocated to chromosome 12 and 16, respectively (Fig. 2). Derivative chromosome 12 and 16 due to translocation were detected in all the cells examined. Breakpoints were assumed to be 6p11, 6q12, 12p11 and 16q32. Moreover, 22 cells had isochromosome of 12q [i(12q)].

Marker chromosomes existed in all the cells examined with the number ranging between 4 and 8 in each karyogram. double minutes (DMs) and homogeneously staining regions (HSR) were not observed.

### DISCUSSION

We have examined the karyotype of TMK-1 cells, human gastric poorly differentiated adenocarcinoma. TMK-1 cells were characterized not only by numerical aberrations but also by various structural rearrangements. Gain of X and trisomy of chromosome 7, 9, and 11 were found in all the cells examined and trisomy of chromosome 3, 8, 10, and 19 was also frequently detected. Of the various structural rearrangements, chromosome 12 and 16 showed the most conspicuous changes due to non-reciprocal translocation from chromosome 6. Twenty-two cells had isochromosome of 12q [i(12q)].

Chromosome counts of TMK-1 cells were found to be in the hypotriploid range which coincided well with DNA histogram estimated by FACScan analysis. Recently, Remvikos et al<sup>13</sup> examined 35 human colorectal adenocarcinomas and found excellent correlation between modal peak values of histograms and chromosome counts. Thus, DNA histogram appears to indicate chromosome numerical abnormalities of tumor cells.

There has been few reports on karyotypic changes of human gastric cancers examined by banding technique. Ochi et al<sup>7</sup> found trisomy X in both the primary and metastatic mucin-producing adenocarcinomas of a white female. Later, they analyzed primary gastric cancers from four male patients<sup>8</sup>. No less than 67 numerical and 83 structural changes were identified as clonal. The most frequent numerical aberrations comprised a gain of chromosome 12 and loss of Y chromosome (in three cases each). They detected no recurrent structural abnormalities, but breakpoints at bands 1p22, 3p21, and 19p13 appeared to be frequently involved. Ferti-Passantonopoulou et al<sup>2</sup> examined five cases of intestinal type gastric cancer. Three were near-diploid and two were near-triploid. Aberrations of chromosome 9 were present in four cases; as trisomy, as i(9), and twice as 9p+. Chromosome 8 in-

involvement was found in three cases.

The chromosome aberrations of the human gastric cancer reported by Ochi et al<sup>7,8)</sup> and Ferti-Passantonopoulou et al<sup>2)</sup> were also found in TMK-1 cells; gain of chromosome 8, 9, 12 and X, and missing Y. The complexity of chromosome changes might be due to the clonal evolution with progress of gastric cancers as do most solid malignant tumors which are characterized by complex chromosomal rearrangements at the time of cytogenetic analysis. Clonal evolution also occurs in the tumor cells of hematologic malignancy<sup>3)</sup>. All of the cases reported by Ochi et al<sup>7,8)</sup> and Ferti-Passantonopoulou et al<sup>2)</sup> were advanced gastric cancers mostly classified as stage IV and the specimens were obtained from primary tumor in seven cases and from ascitic effusion in three cases. It does not yet appear possible to determine a non-random aberration specific for human gastric cancer. Missing Y chromosome, for instance, has been frequently described in tumor cells of hematologic malignancies, and of a variety of solid tumors, epithelial and nonepithelial, including meningiomas, renal cell carcinoma and malignant fibrous histiocytoma<sup>3,6)</sup>, as well as in bone marrow cells of healthy elderly men<sup>11,12,14)</sup>.

Examination of small, early gastric cancer and adenoma may provide more useful information in determining initial "tumor-specific" chromosomal changes and a nonrandom chromosome aberration. Gastric adenomas are well known to show variable histological atypicality and sometimes coexist with tubular adenocarcinoma<sup>5)</sup>. Of interest is a recent cytogenetic analysis which demonstrated the gene for familial adenomatous polyposis (FAP) on chromosome 5, most probably near band 5q21-q22<sup>1)</sup>. Moreover, interstitial deletion of 5q was found in at least 20% of sporadic colorectal adenocarcinomas<sup>10,16)</sup>. Both early gastric cancer and adenoma, however, have never been successfully examined cytogenetically.

In conclusion, it would be invaluable for further research to elucidate the link between karyotype showing clonal evolution, to both the progression of gastric cancers as well as to the expression of various oncogenes or tumor growth factors.

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