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Cytogenetic studies of human esophageal squamous cell carcinoma and gastric adenocarcinoma cell lines

Kimio Tanaka[1] and Naoki Hirabayashi[2]

[1]*Dept. of Radiobiology, Research Institute for Environmental Sciences, Hachazawa 2-121, Takahoko, Rokkasho, Kamikita, Aomori 039-3213, Japan;* [2]*Department of Surgery, Research Institute for Radiation Biology and Medicine, Hiroshima University, Kasumi 1-1-1, Minami-ku, Hiroshima 734-8552, Japan*
kmtanaka@ies.or.jp

Abstract

Esophagus and gastric cancer are one of the most common in Asian countries, but molecular event in the carcinogenesis remains as largely unidentified associated oncogenes. Detailed karyotypic analyses were performed on 4 cell lines derived from malignant cells of a patient with esophagus carcinoma of both diagnosis and metastasis stages and 2 cell lines from 2 independent patients with gastric cancer of metastasis stages. Most of these cells examined were near triploid with modal chromosome number of 59-73. These cells had a complex karyotype with more than 10 rearrangements. The karyotypic pattern was relatively stable; consistent alterations involved long arm of chromosomes 2, 9 and 12 and short arm of chromosomes 3 in both diagnosis and metastasis stages in esophagus cell lines. Those alterations of gastric cancer involved chromosomes 7 and 11. Higher rearrangements associated with translocations and deletions were observed at 2q23 of chromosome2, 3p14 of chromosome 3, 4q11 of chromosome 4 and 11q25 of chromosome 11 in esophagus cancer; while 7p13 of chromosome 7 and 11p15 of chromosome 11 in gastric cancer. The overall chromosome pattern was not similar between these cancers, although these tumors arise from anatomically or biologically related site. Unknown oncogenes localizing on these chromosome breakpoints for translocation or deletion region might be associated with the pathogenesis of esophagus or gastric cancer. These newly established cell lines will be useful tools in the study of the molecular pathogenesis and for testing new therapeutic reagents for these cancers in the future.

Keywords: Esophagus cancer, gastric adenocarcinoma, karyotypic analyses, metastasis, human cell line.

Introduction

Esophagus and gastric cancers are common especially in Asian countries. The prognosis of the patients with this malignancy is poor, especially when metastasis exists. They have been ranked as 4th and 3rd in the cancer-caused deaths in Japan, respectively. Also, gastric cancer is the 4th most frequent type of cancer and second most frequent cause of cancer mortality worldwide. Chromosome abnormalities are common in these tumor cells, which will be an ideal clinical and biological indicator to reveal new and recurrent oncogenic changes relevant to this cancer development. Molecular events in the carcinogenesis of these cancers remain, although a few relevant genes such as *Tp53*, *RB* and *cyclin D1* in esophagus cancer and *K-RAS* and *MET* in gastric cancer have been identified (Montesano *et al.,* 1996; Mita *et al*., 2009; Asaoka *et al.,* 2010). Established cancer cell lines will be useful for isolating oncogenes as well as cellular changes relating to tumor pathogenesis and progression. Only a modest number of esophagus and gastric carcinoma cell lines have been isolated so far. Only a few detailed banding analysis has been reported in these tumor cell-lines (Wuu *et al*., 1986; Ito *et al*., 1989; Wang-Peng *et al*., 1990; Park *et al*., 1990; Yang *et al*., 2008). In the present study, we describe the chromosome abnormalities of these esophagus and gastric carcinoma derived cell lines established in our laboratory.

Materials and methods

Cell lines

The esophagus cell lines were established from a tumor biopsy method of a 77-year-old female patient with squamous cell carcinoma. The patient had relapse 16 months after the therapy. The original tumor specimens obtained both stages before the therapy and at metastasis stage were initiated for establishing cell lines. One cell line (CH1) and 3 cell lines (EH-5, EH-7 & MRO) were established from specimens before therapy and at the metastasis stage, respectively. The gastric cancer cell lines (NSHv & MATUv) were established from primary tumor biopsies of 70 and 68 year old female patients with gastric adenocarcinoma respectively. These cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 1.2% L-glutamine and 0.8% streptomycin. An half of the medium was replaced with fresh medium twice each week. Subculture was performed fortnightly and the cells were examined cytogenetically at passage 4 to 8.

Cytogenetic analysis

Cells in exponential growth after set up culture with fresh medium for 48 h were exposed to colcemid (0.02 µg/ml) for 1 h to arrest adequate number of mitoses for karyotypic examinations. The cells were removed from the surface of the flask by trypsinization and then exposed to hypotonic solution consisting of a 1:4 mixture of 1% sodium citrate and 0.075 M KCl for 30 min. The

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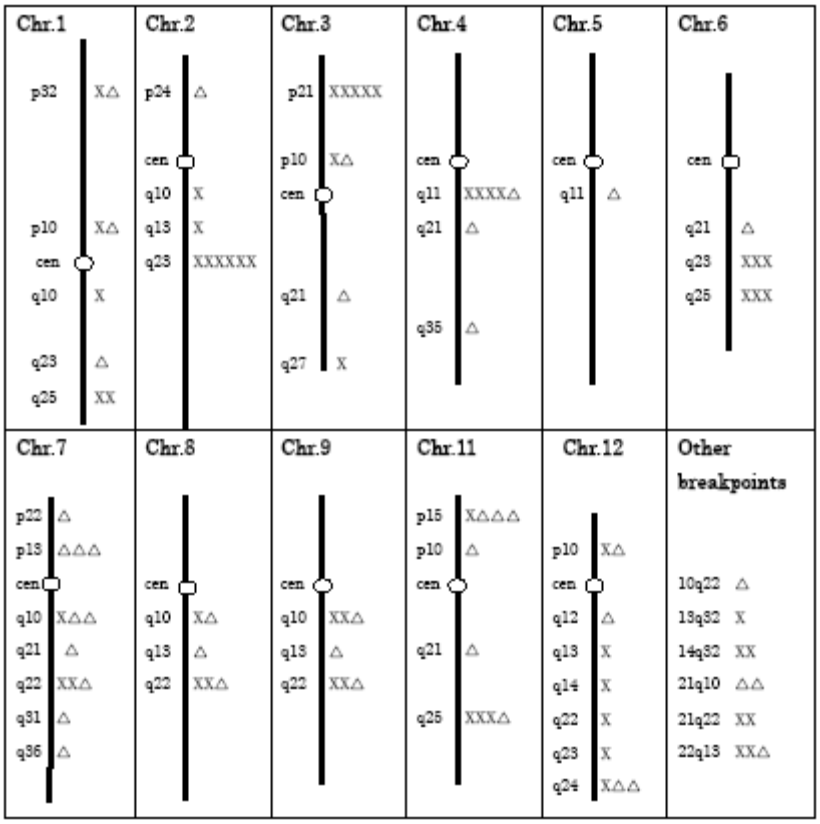
cells were fixed with a 3:1 mixture of methanol/acetic acid. Metaphases analyzed were examined using Trypsin G-banding techniques. Chromosome counts were performed on 11-43 metaphase plates. Karyotypes were analyzed from 12-15 well spread, banded metaphase chromosomes. Chromosomes were identified according to the International System for Human Cytogenetic Nomenclature (ISCN2005).

Fig. 1. Chromosome breakpoints related with structural chromosome aberrations such as translocations & deletions in 4 esophagus cell lines and 2 gastric cancer cell lines.



Esophagus cancers & gastric cancers are shown as X and △, respectively

Results

Esophagus cell lines

We reported the establishment and cytogenetic characterization of four continuous cell lines derived from human primary and metastasis esophagus tumors and compared their properties of chromosome aberrations. These all cell lines were grown as a single, non-organized layers, similar to fibroblasts with former formation, heterogeneous cell division and cell cycle approximately 40 h. All cell lines were characterized not only by numerical aberrations but also by structural rearrangements affecting various chromosomes. The modal chromosome numbers found to be in the hypotriploid to hypertriploid ranging between 59 and 73. Most cells at this passage at four levels was near triaploid with a modal chromosome number of 72 in CH1 cell line. Fig. 2 and 3 show G-banded representative karyotypes of these cell lines (CH1 & EH5). In CH1 cell line, the chromosome pattern showed slight variation from cell to cell and 11 structurally rearranged chromosomes were constantly identified in most cells. Another 2 markers were found in 41% and 20% of the metaphases respectively. The karyotype of this cell-line contained rearrangements of add(1), del(2), der(3)ins(3;?)del(3)(q27), del(6), add(7), der(8;9), del(8), i(9), del(9), der(12)ins(12;?)t(12;?12;?) and add(12), accompanying loss of chromosomes 14, 17 and 21 lost from neartriploid range. Iso chromosome 9 was also observed in cell line established from primary sample.

The cell line had also one each of ring chromosome and double minute (dmin) chromosome. Whole karyotypes of these cell lines are listed in Table 1. Three cell lines were established from the tumor specimens at relapse stage of the same esophagus patient. More complex aberrations were added such as der(2)t(2;3)ins(3), add(7), add(11) and der(?)t(?;?;2) with homogenous staining region (HSR) and 3-7 dmin chromosomes in 3 cell lines established from tumors at metastasis stage, in which metastasis was found. In important, several rearrangements such as del(2), der(3)ins(3)del(3), del(4), del(6), der(8;9), del(8), der(12)ins(12;?)t(12;?;?) add(22) were commonly found to all 4 cell lines established from same patient, which is shown in underlined in Table 1. Our interpretation the origin of the associating derivative chromosomes is presented in Table 1. Chromosome losses were found slightly higher in chromosomes 14, 16 and 17 (Table 2). Structural chromosome abnormalities were frequently observed in long arms of chromosomes 2, 7, 9 and 12 (designated as 2q,7q, 9q & 12q respectively) in 6, 3, 4 aberrations and in short arms of chromosomes 3 and 11 (designated as 3p & 11p, respectively) in 4 and 3 rearrangements among the 4 cell lines (Table 3). Thus deletions of 2q13-ter, 3q27-ter, 4q11-ter, 6q23-q25, 8q22-ter, 9q22-ter were seen commonly in all cell lines 8 (Table 1). The most affected chromosome bands were 2q23 of chromosome2, 3p21 of chromosome 3, 4q11 of chromosome 4 and 11q25 of chromosome 11 (Fig. 1).

Gastric cell lines

These 2 cell lines were grown as single non-organized layers similar to fibroblasts with former formation, heterogeneous cell division and cell cycle approximately 40 h, same as those in esophagus cell lines. Two cell lines had modal chromosome numbers of 61-171 and 63-96 accompanying many numerical aberrations and structural rearrangements affecting various chromosomes

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Table 1. Karyotypes of 4 cell lines established from an esophagus cancer patient.

Name of cell lines	Composite Karyotypes (chromosome aberrations found in several side clones)
CH1	72, XX,-X, -X, add(1)(q25)x2, -2, del(2)(q13), -3, der(3)ins(3;?)(p21;??)del(3)(q27), -4, -4,del(4)(q11)x4, -5, del(6)(q23q25)x3, -7, add(7)(q22), der(8;9)(q10;q10), del(8)(q22)x2, -9, i(9)(q10) , del(9)(q22),-10, -11, add(12)(q23), der(12)ins(12;?)(q13;?)t(12;?;12;?)(q14;?;q22;?), add(12)(q24), -13, -14, -14, -15, -16, -16, -17, -17, -18, -20, -21, -21, -22, add(22)(q13)x2, +ring, +1dmin [10] (aberrations with diploid or hyper diploid range[28])
EH-5	59-69, XX, -X, -X, -1, del(2)(q13), der(2)t(2;3)(q23;p21)ins(3)(p14;??), -3, der(3)ins(3;?)(p21;??)del(3)(q27), -4, +del(4)(q11)x3, -5, -6, -6, del(6)(q23q25), -7, add(7)(q22), der(8;9)(q10;q10)x2, del(8)(q22)x2, -10, -10, -11, add(11)(p15), -12, der(12)ins(12;?)(q13;?)t(12;?;12;?)(q14;?;q22;?), -13, -14,-14, -15, -15, -16, -16, -17, -17, -18, -18, -19, -20, -20, -21, -21, -21, add(22)(q13)x2, +der(?)t(?;2)(?;q23), 0-3 dmin [8]/ 71-83, XX, -X, -X, add(1)(q25), del(2)(q13)x2, + der(2)t(2;3)(q23;p21)ins(3)(p14;??)x2, -3, der(3)ins(3;?)(p21;??)del(3)(q27), -4, +del(4)(q11)x3, -6, -6, del(6)(q23q25), -7,-7, add(7)(q22), der(8;9)(q10;q10), +del(8)(q22), -9, -9, -10, add(11)(p15), add(11)(q25), -12, add(12)(q23), + der(12)ins(12;?)(q13;??)t(12;?;12;?)(q14;?;q22;?)x2, -13, -13, -14, -14, -15, -16, -16, -16, -17, -17, -18, -19, -20, -20, -21, -22, +add(22)(q12)x3., +mar6x2, +mar7x2, 3 dmin [2] (Other additional chromosome aberrations were mar 1, add(11)(q25), mar 5 loss of normal chromosome 6 ,ring chromosomes, i(7)(q10) and new markers (mar 6 and mar 7).)
EH-7	70-73, XX, -X, -X, +add(1)(q25), del(2)(q13), der(2)t(2;3)(q23;p14)ins(3)(p21;??), , -3, -3, der(3)ins(3;?)(p21;??)del(3)(q27), +4, del(4)(q11)x2, -5, -5, -6, del(6)(q23q25)x2, -7, add(7)(q22), der(8;9)(q10;q10)x2, +del(8)(q22)x2, -9,-10, -10, -11, add(11)(q25), -12, der(12)ins(12;?)(q13;??)t(12;?;12;?)(q14;?;q22;?), -13, -15, -16, -16, -17, -17,-18, -18, -20, -21, -21, -22, add(22)(q13), +der(?)t(?;?;2)(HSR;?;q23),+mar2, +mar3, 0-3 dmin[2]/ 54-63, XX, -X, -X, der(1;2)(p10;q10), -2, del(2)(q13), der(2)t(2;3)(q23;p21)ins(3)(p14;??), -3,-3, der(3)ins(3;?)(p21;??)del(3)(q27), del(4)(q11)x2, -5, -6, -6, -6, del(6)(q23q25), -7, add(7)(q22), -8, del(8)(q22), der(8;9)(q10;q10), -9, -10, -11, add(12)t(12;?)(p10;?), der(12)ins(12;?)(q13;?)t(12;?;12;?)(q14;?;q22;?), -13, -13, -14, -14, -15, -16, -16, -16, -17, -17, -18, -18, -20, -21, -21, -22, add(22)(q13), +der(?)t(?;?;2)(HSR;?;q23), +mar1, 0-3 dmin[3] (One cell with 59 modal chromosome number had del(9)(q22) for additional chromosome aberrations.)
MRO	65-68, XX, -X, -X, -1, del(2)(q13), der(2)t(2;3)(q23;p21)ins(3)(p21;??), -3,-3, der(3)ins(3;?)(p21;??)del(3)(q27),-4, -4, del(4)(q11)x3, -5,-5, -6, -6,-6, -7, add (7)(q22), der(8;9)(q10;10), del(8)(q22), -9, -10, +11, -12, add(12)(q23), der(12)ins(12;?)(q13;??)t(12;?;12;?)(q14;?;q22;?), -13,-14,-14, -15, -16,-16,-16, -17, -17, -18, -18, -19, add(21)(q22), add(21)(q22),-22, -22,add(22)(q13), +der(?)t(?;?;2)(HSR;?;q23), +mar, 0-7dmin [10] (Extra chromosome aberrations had del(1)(p22), der(1;3)(q10;p10), add(7)(q22) , del(13)(q32) and losses of chr. 14, 16, 17, 18, or add (1)(q25), loss of chr.13)

Dashed structural chromosome aberrations were commonly found in primary established CH1 cell line at diagnosis stage & other 3 cell lines established at relapse stage.

(Table 5). Fig. 4 and 5 show G-banded representative karyotypes of these cell lines (NSHv & MATUv respectively). Precise karyotypes of these gastric cell lines are listed in Table 4. In NSHv cell line, the chromosome pattern showed slight variation from cell to cell and 16 structurally rearranged chromosomes were constantly identified in most cells. The karyotype contained rearrangements of i(3), add(3), add(4), 2 type of add(7), der(7)t(7;?)del(7), del(7), del(8), add(9), add(10), del(11), add(11), add(12), i(21) and 2 marker chromosomes, accompanying loss of chromosome 19 lost from complete tetraploid range. Iso chromosomes 9 and 21 were also observed in cell line established from primary tumors. MSHv cell line had also 1-121 double minute chromosomes and the distribution of number of double minute chromosomes in a cell are listed in Table 6.

MATUv cell line had more simple aberrations such as der(13;14) and tri(1;?;4), accompanying monosomy of chromosomes 10, 13, 14, 17, 18, 19, 21 lost from tetraploid range and 1 ring and 2 double minute chromosomes (Table 4). Higher number of rearrangement were found in short arm of chromosome 11 (11p) and long arm of chromosomes 4,7, 8 and 12 (4q, 7q, 8q & 12q) in the 2 cell lines (Table 3). Thus deletions

of 7q31-ter, 7q22-ter, 8q13-ter, 11p15-ter, loss of whole short arm of chromosomes 21 and 7 in MSHv cell line were seen but those were not found in MATUv cell line (Table 4). The slightly more affected chromosome bands were 7p13 of chromosome 7, 11p15 of chromosome 11 (Fig. 1), although analyzed cell lines were quite a few. Breakpoint regions associated with chromosome rearrangements were different between esophagus and gastric adenocarcinomas (Fig.1).

Discussion

The establishment of esophagus and gastric cancer cell lines can facilitate the search for mechanism underlying its pathogenesis. These newly established cell lines will be useful tools in the study of the molecular pathogenesis and biological behaviour of these cancer cells and for testing new therapeutic reagents for these cancers in the future. Though esophagus cancer is common in some parts of the world especially in the Orient, but only a few chromosomal banding studies have been reported. In a study an epidermoid carcinoma of the middle 3rd of the esophagus of a 58 year old man was cultured and after many passages showed hypotetraploidy with a modal

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Fig.2. A representative G-banded karyotype of CH1 cell line established at prognostic stage from a esophagus cancer patient. Marker chromosomes are shown as mar. Arrow indicates abnormal chromosomes.

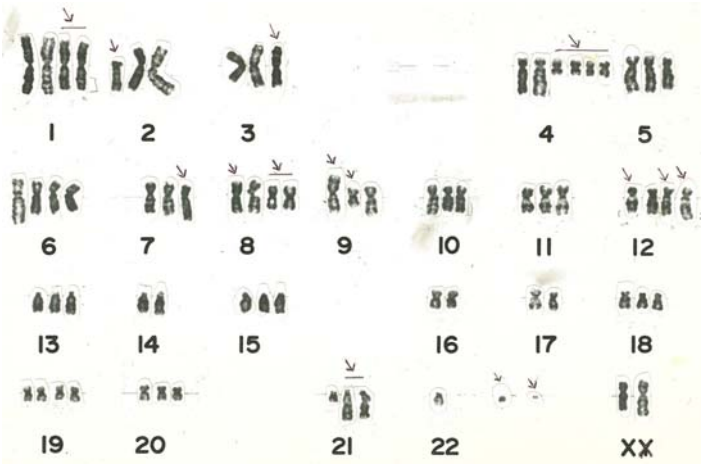


Fig.3. A representative G-banded karyotype of EH5cell line established at metastasis from a same esophagus cancer patient as shown in Fig.1. Marker chromosomes are shown as mar. Arrow indicates abnormal chromosomes.

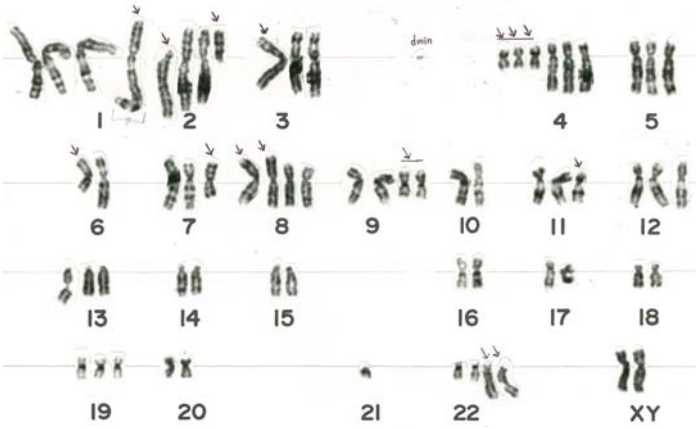


Fig. 4. A representative G-banded karyotype of NSHv cell line established at metastasis from gastric cancer patient no.1. Marker chromosomes are shown as mar. Arrow indicates abnormal chromosomes. This metaphase had 25 dmin chromosomes, which is shown in bottom of the karyotype.

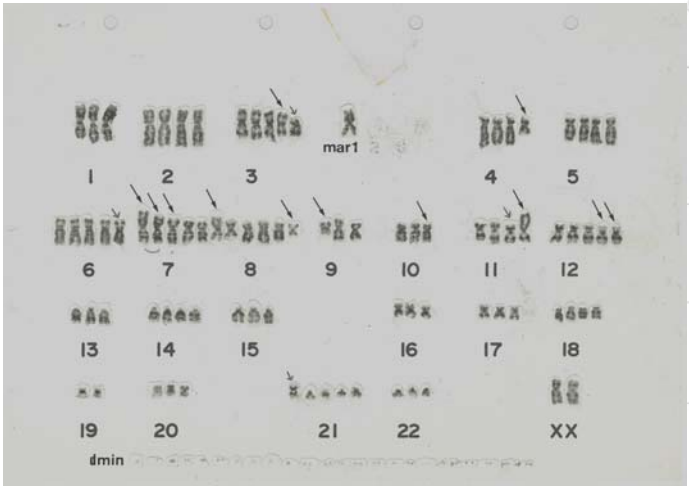
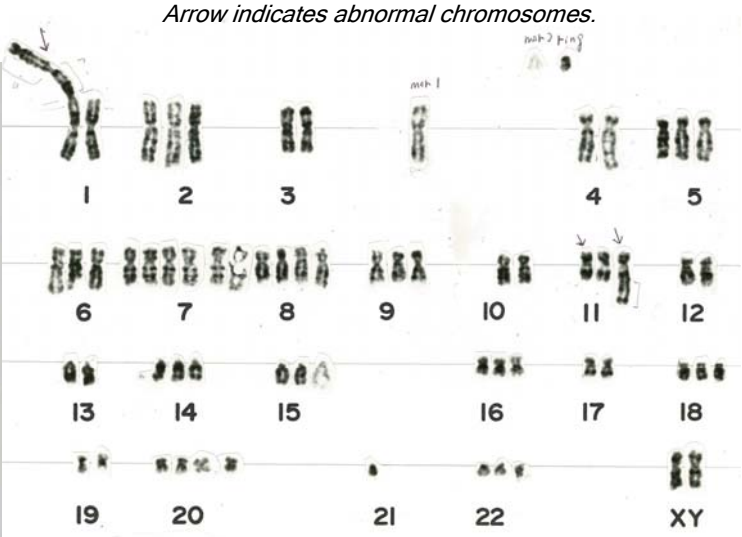


Fig. 5. A representative G-banded karyotype of MATUv cell line established at metastasis from gastric cancer patient no. 2. Marker chromosomes are shown as mar. Arrow indicates abnormal chromosomes.



number of 73, no normal chromosomes of 1 and Y and structural rearrangements of chromosomes 1, 9, 14, X and Y, as well as complex alteration (Wuu *et al*., 1986). In addition, the cell line had structural chromosome aberrations such as iso chromosome[i(7p)], del(6)(q21) and der(12)t(12;?)(q24;?), which was also found in our present analysis (Fig.1 & Table 2 & 3). This suggests that the breakpoint regions of 12q24 and also deletion regions of 6q21-6qter and 7cen-7qter might have important roles in pathogenesis of esophagus cancer. As these deletion regions contain identified oncogenes of *MYB*, *ROS* on chromosome 6, *MET* on chromosome 7, so the study on whether these oncogenes are associated with esophagus cancer will be needed. The affected regions of chromosomes 7 and 12 have also been observed in other solid tumors such as lung cancer and ovarian cancer, stomach cancer and breast cancer and seminoma respectively. Several chromosomal studies on primary tumors using G-banding method reveled more abnormalities of chromosomes 1, 3, 6, 7, 9, 11 and relatively high incidence of chromosomes of 2, 4, 5, 12, 12, 17 and 21 (Xiao *et al*., 1991; Rosenblum-Vos *et al*., 1993; Rao *et al*., 1995; Menke-Pluymers *et al*., 1996; Manoel-Caetano *et al*., 2004). Chromosome breakpoint regions formed with a translocation is considered to be containing recurrent oncogene associated with pathogenesis or progression of esophagus cancer. In our present G-banding analysis revealed that several chromosomal regions such as 2q23, 3p14, 4q11, 6q23, 6q25 and 11q25 had more translocation than others. This breakpoint other than 3p14, which locates *FHIT*, *PCAT* and *ACA1* genes, has not been reported. These

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breakpoints might be recurrent oncogenes relating to esophagus cancer development. Four cell lines were established from a esophagus cancer patient. All of three cell lines had the same abnormalities, which indicates that most of these abnormalities, mentioned above were not from secondary change but persisted from the original clone.

al., 2005) in esophagus cancers. Therefore, there is another possibility that cytogenetic features in esophagus cancer might be different between western and oriental countries.

The results of array study correlates well with the present cytogenetic approach. Although an array CGH and SNP array are currently using powerful technique for

Table 2. Number of numerical chromosome aberrations in each chromosome in 4 esophagus cancer cell lines & 2 gastric cell lines.

Esophagus cancer cell lines																							
Chrom. Gain																							
0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***	***12***	***13***	***14***	***15***	***16***	***17***	***18***	***19***	***20***	***21***	***22***	***X***	***Y***
0	0	1	1	2	**3**	1	0	1	1	0	0	2	**4**	1	**4**	**4**	**3**	0	1	2	1	1	0
Chrom.Loss																							
Gastric cancer cell lines																							
Chrom. Gain																							
0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***	***12***	***13***	***14***	***15***	***16***	***17***	***18***	***19***	***20***	***21***	***22***	***X***	***Y***
0	0	0	1	0	1	0	0	0	1	0	0	1	1	0	0	1	1	2	0	1	2	0	0
Chrom.Loss																							

Table 3. Number of breakpoints related to structural chromosome aberrations in each chromosome in 4 esophagus cancer cell lines & 2 gastric cell lines.

Esophagus cancer cell lines																							
Short arm of chromosome(p)																							
1	1	**4**	0	0	0	0	0	0	0	**3**	1	0	0	0	0	0	0	1	0	0	0	0	0
1	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***	***12***	***13***	***14***	***15***	***16***	***17***	***18***	***19***	***20***	***21***	***22***	***X***	***Y***
2	**6**	1	1	0	1	**3**	2	**4**	0	2	**4**	1	0	0	0	0	0	0	0	2	1	0	0
Long arm of chromosome(q)																							
Gastric cancer cell lines																							
Short arm of chromosome(p)																							
2	1	1	0	0	0	2	0	1	0	**3**	1	0	0	0	0	0	0	0	0	0	0	0	0
1	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***	***12***	***13***	***14***	***15***	***16***	***17***	***18***	***19***	***20***	***21***	***22***	***X***	***Y***
1	0	1	**3**	1	1	**4**	**3**	1	1	2	**3**	0	0	0	0	0	0	0	0	2	1	0	0
Long arm of chromosome (q)																							

Present cell lines had abnormalities of short arm of chromosome 1 at 1p10, which are found in variety of cancers. Moreover, centromere portions of chromosomes 2q10, 4q11, 7q10, 8q10 and 9q10 had breakages in addition to homogenous staining region (HSR), dmin chromosomes and ring chromosome, although their incidences were not predominant. One paper report using multiple fluorescence *in situ* hybridization (M-FISH) method mentioned a Chinese esophagus patients having many rearrangements near or at many centromeric portions at 8q10, 21q10, 3p10, 3p11, 1p11, 5p11 , 5q11, 14p11 and 14q11 with HSR and ring chromosomes (Jin *et al*., 2004). Unknown oncogenes locating near these centrometic regions might also be associated with the pathogenesis of esophagus cancer. Thus our cell line also had chromosomal deletions of 2q13-ter, 3q27-ter, 4q11-ter, 6q23-q25, 8q22-ter, 9q22-ter were seen commonly in all cell lines. Although the deletions at 4q21-22 have been reported (Rumpel *et al*., 1999), other deletions have not been reported using the loss of heterozygodity (LOH) studies. LOH analysis suggests that deletions of chromosome 4q, 5q, 9p, 9q, 13q 17q 17p and 18q are present (Blount *et al*., 1993; Wagata *et al*., 1991; Tarmin *et al*., 1994; Maesawa *et al*., 1997; Petty *et al*., 1998; Galipeau *et al*., 1999; Hu *et al*., 2004; Yang *et*

detecting very tiny chromosomal regions having deletion and amplification, chromosomal translocations are detected only by conventional G-banding analysis. Recent studies using comparative genomic hybridization (CGH) represents the first whole-genome analysis in esophageal adenocarcinoma and squamous carcinoma for associated chromosomal aberrations that may be involved in either the genesis and progression of this malignancy, in which chromosome gains of 6p, 7q, 7p, 8q, 10q, 15q, 17q and 20q and chromosome losses of 4q, 5q, 9p, 14q, 16q, 17p, 18q and Y (Moskaluk *et al*.,1998; Van Dekken *et al*.,1999; Pack *et al.,* 1999; Kamitani *et al*., 2002). Further, small size regions associated with gene deletion and amplification could be detected by array CGH, not G-banding analysis. Then currently developed array CGH such as single nucleotide polymorphism (SNP) microarray also detected small deleted region of chromosome 9p at 9p22.3-22.2, which involves *CDKN2A*(*p16*) gene and chromosomal amplification of 17q12-21.1, involving *ErbB*, *RARα* and *TOP2A* genes (Akagi *et al.,* 2009), and deletion of chromosome 3, involves p300/CBP associated *PCAT* gene and amplifications of chromosome 8 at 8q24, involving *MYC*, chromosome 11 at 11q13, involving *CCND1* gene (Jin *et al*., 2004) and chromosome 20 at 20q12-13, involving

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Table 4. Karyotype of 2 cell lines established from 2 independent gastric cancer patients

Name of cell lines	Composite Karyotypes (chromosome aberrations found in several side clones)
NSHv	82-89,XXXX, -X, -X, -1, i(3)(p10), add(3)(q21), add(4)(q21), + 6, add(7)(p13)x2, +add(7)(p22), +der(7)t(7;?)(p22;?)del(7)(q31), +del(7)(q22), del(8)(q13), -9, add(9)(q10), add(10)(q22), del(11)(p15), add(11)(p10), -12, ; add(12)(p10)x2, -13, -15, -16, -17, -19, -19, -20,+i(21)(q10), -22,+mar1, 1-121 dmin [15] (Other additional chromosome aberrations were mar 1, add(11)(q25), mar 5, loss of normal chromosome 6 ,ring chromosomes, i(7)(q10), new markers (mar 6 and mar 7) and add(11)(p15). (Other additional chromosome aberrations were i(1)(p10), del(1)(q23), del(2)(p24), del(6)(q21),i(8)(q10), ins(12)(12;?)(q12;??), 3-5 copies of i(21)(q10),mar 2 and mar 3.)
MATUv	63-70, XXXX, -X, -X, -2, -3,-4,-4, -5, +9, +9, -10, -10, der(11)t(4;11)(q11;q21), -13, -13, -13, der(13;14)(q10;q10),-14,-14, -15, -16, 17-,-17, -18, -18, -19,-19, -21, -21, -22, +ring, 0-2 dmin [8] / 63-66, XXXX, -X, -X, -1,-1, tri(1;?;4)(p36;?;q35), -2, -3,-3, -4,-4,-5, -6, +8, +8, -9, -10, -10, -11, der(11) t(4;11)(q11;q21), -12, -12, -13, -13, -14, -15, -16, -17, -17, -18, -19, -19, -21, -21, -21, -22, +mar1, +ring [2] (Other additional aberrations were del(1)(p32), dic(4;?)(q35;?), der(5)t(5,?)(q11;?), del(7)(p13), de(7)(q21), der(7;9)(q10;p10), add(7)(q36), del(8)(q22), add(11)(p15), add(12)(?q24), del(12)(q24),add(22)(q13), mar2 and mar3.)

AIBI, *BTAa* and *T2A*. It has been hypothesized that cancer progression develops as a consequence of an acquired genetic instability and the subsequent cytogenetic evolutions with accumulated genetic errors. In esophagus cancers, Barrett's esophagus cancer is considered to be premalignant stage of the esophagus adenocarcinoma. Barrett's esophagus cancer is associated with *TP53* (also known as *p53*) mutations and *CDKN2A* (also known as *p16*) and nonrandom LOH (Barrett *et al*., 1999). Esophagus carcinoma had deletion of 3p and 17p in premalignant stage (Shimada *et al*., 1996). Aneuploidy or increased tetraploidy populations occur in more than 90% of esophagus adenocarcinoma. The clonal evolution of the cancer might develop from preneoplastic cells with mutations of *TP53* and *CDKN2A* is acquired LOH at 5q, 13q, and 18q, which is subsequently occurred aneuploidy and tetraploidy (Barrett *et al*., 1999). Translocations found recurrently might be developed at primary change of the cancer. Amplifications such as *MYC* and other genes at 20q, or HSR or dmin are considered to be associated with late changes.

Gastric cancer is also one of the most common in Asian countries, but molecular event in the carcinogenesis remains and largely unidentified associated oncogenes. Gastric cancer is a significant mortality; therefore further understanding of its molecular basis is required. A few cell lines have been established and several chromosome changes such as trisomies 8 and 7, monosomy 1, and rearrangements of chromosome 12q and 17p have been revealed, which will be helpful to discover new oncogenes associating with gastric cancer. Furthermore, using modern cytogenetic technique, CGH, high incidences of gains of 7q, 8q and 20q, and losses of 13q, 17p, 17q and 18q (Kokkola *et al*., 1997,1998; Sakakura *et al*., 1999; Noguchi *et al*., 2001; Kimura *et al*., 2004; Buffart *et al*., 2009; Tomioka *et al*., 2010). The abnormalities of gain of 1q32.3 and losses of 13q (*FOXO1A*, *ATP7β*) and 18q22.1 (*SMAD2, SMAD4*) and amplification of 7p12(*EGFR*), 12p13-ter and 20q13.21(*ZNF217*, *CYP24*) are closely relating to poor prognosis and lymph node metastasis and histological cancer type, respectively. LOH analyses revealed the abnormalities at 1p21.1 (*AMY2A*), 7q31 (*MET*, *FGR2*, *ErbB2*) and 1p13.1 ($TOP\ II\alpha$).

Very few studies on chromosome aberrations in gastric cancer has been performed by G-banding analysis (Van der Riet-Fox *et al*., 1979; Ochi *et al*., 1986; Ferti-Passantonopoulou *et al*., 1987; Lima *et al*., 2004). In present report, we reported on cytogenetic analyses of 2 gastric cell lines, in which both were established from metastatic carcinomas. Rearrangements were observed highly at 11p, 4q, 7q, 8q and 12q. Chromosome breakpoints were found on chromosome 7 at 7p13 and chromosome 11 at 11p15 for 3 sites and 7q10, 12q24 and 21q10 for 2 sites, which were rerating to rearrangements of add(7), del(11) and add(11), der(7;9)(q10;q10) add (12) and del(12) and i(21q). Chromosome rearrangement of specific region of 11p13-15 has been previously reported in both stages of primary and metastasis of gastric or esophagus cancer (Rodriguez *et al*., 1990). However, most of other abnormalities were not coincide with those of previously reported. Also, cytogenetic evidence of many dmin chromosomes in one cell line showed amplification of oncogene, like *MYC* and other unknown oncogene. Further studies, using large number of patients' samples are necessary to identify cancer specific regions associating with new oncogenes.

Gains and amplification of *MYC*, *EGFR*, 18q21.1 and 20q13 region, and loss of 17q were found commonly in both esophagus and gastric cancers (Maesawa *et al*.,

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Table 5. Modal chromosome numbers of 30 cells in NSHv cell line from gastric cancer.

NSHv cell line																			
61	66	68	72	73	74	79	82	83	84	85	86	87	88	89	101	102	110	135	171
1	1	2	1	2	1	1	1	1	1	2	1	4	3	2	1	1	1	1	2

Modal chromosome numbers of 11 cells in MATUv cell line from gastric cancer						
MATUv cell line						
63	64	65	66	67	70	96
3	1	1	2	2	1	1

Table 6. Number of double minute (dmin) chromosomes per cell in observed 30 cells in NSHv cell line from gastric cancer.

1	14	16	25	26	27	30	33	35	36	40	41	44	56	59	60	61	74	103	105	121
1	1	3	1	1	1	3	1	1	2	1	3	2	1	1	1	1	1	2	1	1

Mean number of dmin per cell was 45.5.

1997; Petty *et al*., 1998; Rygiel *et al*., 2008). However, present overall patterns of chromosome rearrangements found in gastric cancer were not similar to those of esophagus cancer (Fig.1 & Table 2 & 3), although the analyzed cell lines were small, which was not consisted with the previous result (Rodriguez *et al*., 1990). The result may be suggesting tumors arise from these abnormal sites are biologically or anatomically different. Current techniques of M-FISH analyses of cancer cell lines will reveal more important genetic changes such as disease specific translocations for esophagus and gastric cancers affecting chromosomes sites that harbor genes known to pathogenesis in tumorogenesis and progression of human neoplasias (Wu *et al*., 2006; Yang *et al*., 2008).

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