Geographical difference of chromosome aberrations between Japanese and American small cell lung cancer cell lines

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Abstract
Lung cancer is the leading cause of cancer-related death worldwide. Cytogenetic analysis have been performed last two decades, but comparative analysis of karyotypes of small cell lung cancer (SCLC) from between Japan and America has not been precisely studied. Six Japanese and one American SCLC cell lines examined were hyperdiploid to near-tetraploid with modal number of 52-91. These cells had a complex karyotype with more than 10 rearrangements. The karyotypic patterns were relatively consistent alterations involved long arm of chromosomes 1, 3, 7 and 11, and short arm of chromosomes 1 and 3. Higher rearrangements specially associated with translocations and deletions were observed in short and long arms of chromosomes 3 (3p21 & 3q25), and recurrently long arms of chromosome 7 (7q36 and 7q23). Although the chromosome of SCLC is too complex and G-banding analysis could not resolve all of many of the karyotypic abnormalities seen, several potentially site-specific abnormalities such as deletions of chromosome 3p, 7q and 3q, and amplifications of 3p, 7q, 1q, 11p, 2p and 12p in 6 SCLC cell lines established from chemotherapy resistant patients tumor cells. Losses of short arm chromosome 11 and 12 (11p, 12p) and long arm of chromosome 13 (13q), and amplifications of chromosomes 2, 11, 12, 13 and 19 (2p, 11p, 12p, 13q, 19p) were recurrently identified in the several cell lines, being different from published chromosomal abnormalities in American SCLC, which suggests geographical difference of SCLC karyotype. Also, these abnormal patterns were largely different from non-small cell lung carcinoma (NSCLC). Unknown oncogenes localizing on these chromosome breakpoints for translocation or deletion region might be associated with the pathogenesis of SCLC. Present analysis can provide information on significant genes involved pathogenesis of SCLC.

Keywords: Chromosome aberration, lung cancer.

Introduction
Lung cancer is the leading cause of cancer death in Japan and worldwide, accounting for over 4931 deaths in 1975-1977 in Japan alone (Yoshimura & Yamashita 1982). Clinical statistical analysis according to histological type was performed. Lung cancer can be histologically sub-classified into 4 major categories: squamous cell carcinoma (epidermoid carcinoma), lung adenocarcinoma, and undifferentiated large cell carcinoma, comprising non-small cell lung cancer (NSCLC), and undifferentiated small cell carcinoma (SCLC). Epidermoid carcinoma accounted for 46.6% of male cases and 18.8% in female cases, while adenocarcinoma was 61.2% of female cases and 30.3% in male cases in Japan. SCLC accounted for 11.5% in male cases and 8.3% in female cases. Squamous carcinoma was predominant in male, and male and female sex ratio was 8:1, but lung adenocarcinoma was higher in female. 5-year survival rates were 14.4%, 14.4%, 11.9%, 5.4% and 1.3% for epidermoid carcinoma, adenocarcinoma, large cell carcinoma, small cell carcinoma intermediately classified type and oat cell type, respectively (Yoshimura & Yamashita, 1982). SCLC has poor prognosis and remains from target therapy.

Although the squamous cell carcinomas are most common, the majority of the lung tumors so far cytogenetically investigated have been the small-cell type. Whang-Peng have reported a very specific chromosome abnormality, del(3)(p14p23) in SCLC (Wang-Peng et al., 1982). The segment 3p14-3p23 always seems to be part of the lost region for common deleted regional segments, when the breakpoints were different among authors (Miura et al. 1992). RFLP analysis of 3p markers confirmed almost universal loss of the region (Bauch et al., 1987). Deletions and other rearrangements involving short arm of chromosome 3 (3p) have been also reported previously in several NSCLC tumors and cell lines (Zech et al., 1985; Jin et al., 1988; Bello et al., 1989; Miura et al., 1990a, b; Testa et al., 1994). Therefore, the del(3)(p14p23) is well established as a specific marker for SCLC and NSCLC of lung cancers. It is found predominantly in SCLC, but it has been present in only some cases or nearly all NSCLC cases examined. More data are needed on other chromosome abnormalities before the prevalence of deletion of short arm of chromosome 3(3p- on) in SCLC tumors and other histological type tumors.
Fig. 1. Chromosome breakpoints related to structural chromosome aberrations in 6 Japanese SCCL cell lines and an American SCLC cell line and reference cases*

Chromosome abnormalities are a common in these tumor cells, which will be a good clinical and biological indicator as well as suitable indicator to reveal new, recurrent oncogene changes relevant to development of these cancers. Molecular events in the carcinogenesis of these cancers remain, although a few relevant genes such as Tp53, RB, STK11, EGFR and CDKN2A in lung cancer have been identified (Greulich 2011). Established cancer cell lines will be useful for isolating oncogenes as well as for cellular changes relating to tumor pathogenesis and progression. Geological difference of tumor karyotype has been observed in leukemias and lymphomas, but comparative karyotype analysis between Japanese and American SCLC cases has not been performed. In this report, we describe the establishment of these cell lines and detailed karyotypes of seven SCLC cell lines. Although the karyotype in SCLC appeared to be very complex, several specific and recurrent abnormalities and breakpoint cluster sites have been identified that should be helpful to focus further molecular investigation. Present analysis revealed a possibility that karyotype of SCLC is geographically different.

Materials and methods

Cell lines

Six Japanese lung cancer cell lines (K#1-1-1, NKM, HT, SWD, OHf, FJt) were established from plural fluid of 54 to 71-year-old Japanese male or female patients with SCLC. For comparison, one female cell line (M417) was established from 66 year-old American SCLC patient. These seven patients whose samples were used for establishing cell line had been received chemotherapy using cyclophosphamide, vincristine, adriamycin, etoposide and vincdesine. The original tumor specimens obtained at stage after the therapy or at metastasis stage were initiated for establishing cell lines. These cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 1.2% L-glutamine, and 0.8% streptomycin. One half of the medium was replaced with fresh medium twice each week. Sub-cultivation was performed once every two weeks. Cells were examined cytogenetically at passage 20 to 80 of these cell lines.

Cytogenetic analysis

Cells in exponential growth after set up culture with fresh medium for 20-48h were exposed to colcemid (0.02 ug/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 minutes. The cells were fixed with a 3:1 mixture of methanol/acetic acid. Metaphases analyzed were examined using trypsine G-banding techniques. One American cell line (M417) was stained by Q-bandning.

Chromosome counts were performed on 15-35 metaphase plates. Karyotypes were analyzed from 12-15 well spread, banded metaphase chromosomes. Chromosomes were identified according to the
Results

SCLC cell lines

We reported the establishment and cytogenetic characterization of 6 Japanese SCLC cell lines and one American SCLC cell line derived from human metastasis lung tumors, and compared their properties of chromosome aberrations. These all cell lines were grown as single, non-organized layers, similar to fibroblasts with former formation, heterogeneous cell division, and cell cycle approximately 20-38 h.

All cell lines were characterized not only by numerical aberrations but also by structural rearrangements affecting various chromosomes. In Japanese 6 cell lines, the modal chromosome numbers found to be in the hypotriploid to hypertriploid ranging between 51 and 91. Most cells at this passage were hyper-diploid with modal number of 52, 51 and 62 in 3 cell lines (K#1-1-1, NKM, SWD), and near triploid with a modal chromosome number of 69 and 74 in 2 cell lines (HT, OHf). Distribution of chromosome breakpoints in 6 Japanese cell lines is shown in Fig.1. Fig. 2, 3 and 4 show G-banded representative karyotypes of these cell lines (K#1-1-1, HT and OHf, respectively). In K#1-1-1 cell line (Fig.2), six structurally rearranged chromosomes were constantly identified in most cells. The karyotype had add(2), add(6), der(12)t(2;12), add(13)(q14) and 2-3 marker chromosomes.

In HT cell line (Fig.3), the chromosome pattern showed slight variation from cell to cell, and twelve structurally rearranged chromosomes were constantly identified in most cells. Another six markers were found in 20-64% of the metaphases. The karyotype of this cell line contained rearrangements of add(2), two kinds of der(3), add(3), two kind of add(6), add(7), add(8), add (9), add(11), add(12) and 2 double minute (dim) accompanying loss of chromosomes 2, 7, 15 and 22 lost from neartriploid range.

In OHf cell line (Fig.4), eighteen structurally rearranged chromosomes were constantly identified in most cells. Figure 4 shows G-banded representative karyotypes of the cell line. Another 15 markers were found in about 30-80 % of metaphases. The karyotype of this cell line contained der(1), add(1), add(3), add(4), del(5), der(7), add(8), add(11), del(11), another add(11), add(12), del(12), add(14) and add(?17), accompanying loss of 4, 13, 14, 15, 17, 19, 21 and 22 lost from near-tetraploid range. The cell line had also one homogenous staining region (HSR) at 7q36-7q25 region of the der(7) chromosome.

In an American SCLC cell line (M417), (Fig.5), ten rearranged chromosomes such as add(1), t(3;7;?), der(4)t(?3;4), add(?9), add(11) and der(21)t(?7;22) were identified constantly, which karyotype is shown in Fig.5.
This cell line had an abnormality on chromosome 3p similar to Japanese SCLC, but not on chromosome 7q.

Whole karyotypes of these 7 cell lines are listed in Table 1. In important, several types of short arm or long arm of chromosome 3 rearrangements such as der(3)q(3;7), der(3)t(3;19), der(3)t(3;7), add(3)(q25), add(3)(p13), add(3)(p11) and t(3;7;) were predominantly found to all 7 cell lines established, although the breakpoints were variable from 3p21, 3p13, 3p11, 3q12, 3q21 and 3q25 (Fig.1), which is shown in bold letter in Table 1. Common deleted regions of 3p15-pter, 7q36-qter, 3q25-qter, 8q24-qter and 11p15-pter were seen in 7, 7, 4, 3 and 3 cell lines. Common amplified regions were found at 3p13-3q21 in 15 copies, 7q11-7q22 in 12 copies, 1q11-qter and 11p15-q21 in 8 copies each, 12p11.2-qter and 6p23-qter in 6 copies each, and 4pter-4q35, 4p13-pter, 13pter-q14, 4q21-qter in 4 copies each in 8 cell lines, in which number of copy was counted including normal chromosomes. Thus the most affected chromosome bands were 7q36 of chromosome 7, 3q25 of chromosome 3, 3p13 of chromosome 3, 7q32, 4q31, 5q13 and 8q24.3 (Fig. 1).

**Discussion**

The establishment of SCLC 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 behavior of these cancer cells and for testing new therapeutic reagents for these cancers in the future.

Though lung cancer is common in the world, but almost no comparative study on karyotype using chromosomal banding studies between Japanese and American cases have been reported. A karyotype study on American 6
fresh patients and 7 cell lines with SCLC showed hypodiploidy to near-tetraploidy with modal numbers of 38-78, no normal chromosomes of 17, 21 and 22 in each one case, and structural rearrangements of chromosomes 3, 17 and 13 as well as complex alteration (Miura et al., 1992). In addition, the tumors and cell lines had structural chromosome aberrations such as deletion of chromosome 3p in 13 cases, add(17)(p13) in 12 cases, rearrangement at 13q14 of chromosome 13 in 10 cases and alteration of chromosome 5q in 12 cases, which were also found in our present analysis except 17p13 and 13q14 abnormalities (Fig.1 and Table 1). For comparison, an American cell line (M417) and published samples from reference no 24 (Mimura et al., 1992) are shown in Fig. 1.

More accurate analysis using array comparative genomic hybridization (CGH) revealed that decrease DNA copy number on 3p, 5q, 10, 16q and 17p, and frequent gain of DNA copy number on 3q, 1p and 14q (Ashman et al., 2002), and loss of regions implicated regions being 3p13-14, 4q32-35, 5q22-35, 8p21-22, 10q25, 13q13-14, and 17p12-13, and common gains include regions being 3q26-29, 5p12-13, 8q23-24 and 19p13.1 (Heiway & Betticher, 2004).

Loss of 5q region by CGH was coincident with highly observed isochromosome 5p or deletion of chromosome 5q in SCLC (Miura et al., 1992; Hartel et al., 2008). In comparison with American cases, present Japanese cell lines showed different results, which

<p>| Table 1. Karyotypes of 7 SCLC cell lines established |
|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Composite Karyotypes (chromosome aberrations found in several side clones)</th>
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<tbody>
<tr>
<td>K #1-1</td>
<td>50-57, X, +X, add(Y)(q12), add(2)(q31), der(3)(3;7)(p21;q11), -4, del(4)(q21q31), +add(1)q13, der(12)(12q12)13q13, add(13)(q14), +21, +4, +17, +mar1, +mar2, +mar3 [4]</td>
</tr>
<tr>
<td>NK1</td>
<td>58-73, XY, add(1)(q25), add(1)(q12), add(1)(p13), +add(1)(p22), +add(1)(p11), del(2)(p21), der(3)(3;19)(q5;p3)x2, add(3)(q25), +add(3)(q21), +6, add(8)(q22), +der(8)(7;8)q13;q13, +add(9)(p22), +add(11)(q23), -13, +del(13)(?q27;14q)x2, +15, -19, -20, -21, +mar1, +mar2, +mar3, +mar4, +mar5, +mar6, +mar7 [6]</td>
</tr>
<tr>
<td>SWD</td>
<td>47-54, XY, add(1)(q12), -2, +add(1)(q11), +add(1)(q11), add(3)(q25), +add(3)(p13), +add(4)(q31), der(4)(?:7;4)q31(3), add(4)(q31), -5, +del(7)(p15;cen-q23), +add(6)(q15), +add(7)(q36), +der(7)(?:7;7q36;7), add(8)(q24), -9, +add(9)(q22), der(11)(11;12)(p11.2;11.1), +11(q10), -12, +add(12)(p11.2), -13, -17, +18, -19, -21, +22, -22, +mar1, +mar2, +mar3, +mar4, +mar5, +mar6, +mar7, +8 [10]</td>
</tr>
<tr>
<td>OHF</td>
<td>75-82, XX, der(1)(?:1;?):(?:p11.2-cen-q44::?), add(1)(p36.1), +2, +add(3)(p11), -4, +add(4)(q35)x2, +del(5)(q31)x2, +6, +6, +7, der(7)(?:7;7q36;7r5;q25), +add(8)(q24.3), +10, +add(11)(q32)x2, +del(11)(q23), +add(11)(q21)x2, +add(12)(p11.2), +del(12)(p13), -13, -14, +add(14)(q32), -15, -17, +add(17)(?):q21, +18, -19, +20, -21, -22, -22, +mar1, +mar2, +mar3, +mar4, +mar5, +mar6, +mar7, +mar8, +mar9, +mar10, +mar11, +mar12, +mar13, +mar14, +mar15 [0]</td>
</tr>
<tr>
<td>FJt</td>
<td>88-95, XY, +X, +add(1)(q21), +add(1)(q23), +2, +2, +add(3)(p11), +add(3)(p13)x2, +4, +5, +5, +6, +del(6)(q21), +7, +add(7)(q32), +add(8)(q24.3), +11, +add(11)(p15), +12, +13, +14, +add(14)(q32), +15, +15, +17, +18, -19, -21, +mar1, +mar2, +mar3, +mar4, +mar5, +mar6, +mar7, +mar8, +mar9, +mar10, +mar11, +mar12, +mar13, +mar14, +mar15, +mar16 [7]</td>
</tr>
<tr>
<td>M417</td>
<td>53, X, X, +add(1)(q42), +3(3;7)(?:pter??:q36;p13;?):, der(4)(?:7;4)(pter-p21::pter-p14), +7, +add(9)(pter-q11), +add(11)(p14), +add(15)(q22), +der(17)(?:7;17)(pter-p11, +der(19)(?:7;19)(pter-p21;pter-p33), +21, +der(21)(?:7;11q1-pter-q13))</td>
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*American SCLC cell line; Bold: abnormalities on chromosome 3p; underlined: abnormalities on chromosome 7q

Table 2. Number of numerical chromosome aberrations in each chromosome in J ap enese 6 SCLC cell lines (Italic letters show chromosome number. Bold letters show higher number of aberrations.)

| Chromosome Gain |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 1               | 1               | 1               | 2               | 2               | 1               | 3               | 1               | 2               | 0               |
| 1               | 2               | 3               | 4               | 5               | 6               | 7               | 8               | 9               | 10              |
| 1               | 11              | 12              | 13              | 14              | 15              | 16              | 17              | 18              | 19              |
| 2               | 20              | 21              | 22              | X               | Y               |

Table 3. Number of breakpoints related to structural chromosome aberrations in each chromosome in J ap enese 6 SCLC cell lines (Italic letters show chromosome number. Bold letters show higher number of aberrations.)

| Short arm of chromosome |
|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|------------------------|
| 1                      | 1                      | 2                      | 3                      | 4                      | 0                      | 0                      | 0                      | 0                      | 0                      |
| 1                      | 2                      | 3                      | 4                      | 5                      | 6                      | 7                      | 8                      | 9                      | 10                     |
| 1                      | 11                     | 12                     | 13                     | 14                     | 15                     | 16                     | 17                     | 18                     | 19                     |
| 2                      | 20                     | 21                     | 22                     | X                      | Y                      |

Long arm of chromosome
had hyper-diploid to near-tetraploidy modal number and higher abnormalities on chromosome 3p, 3q and 7q, slightly higher in 5q, but no abnormalities on 13q14 and 17p13. In addition, out of 6 Japanese cell line, only one cell line had HSR and dmin chromosomes independently, while in the American SCLC cases, abnormal banded region (ABR), HSR and dmin were more observed in 4 of 13 cases observed (31%) (Miura et al., 1992). Therefore, there is a possibility that cytogenetic features in SCLC might be different between western and oriental countries.

Chromosome breakpoint regions formed with a translocation, deletion and amplification are considered to be containing recurrent oncogene associated with pathogenesis or progression of SCLC. Present study suggests that the breakpoint regions of 3p13, 3q26 and 7q36, and also deletion regions of 1q44-qter, 6q21-qter, 8q24.3-qter, 11p15-qter, 12p13-qter and 13q14-q22? might have important genes for pathogenesis of SCLC. These chromosomal breakpoints associated with deleted or amplified regions might contain identified genes of FHIT, RASSF1 and Fus1 at 3p13 (Zabarvsky et al., 2002), MDR/ABCB1 and MET on chromosome 7 (Yabuki et al., 2007; Engelman et al., 2007), MYC at 8q24.3 and RB1 at 13q14 (Rygaard et al., 1990; Yuan et al., 1999), so the study on whether these oncogenes are associated with SCLC will be needed. No oncogene has been identified on 12p13, but amplification and over expression of DYRK2 gene at 12q14 in lung adenocarcinoma has been reported (Miller et al., 2003). Then currently developed array CGH such as single nucleotide polymorphism (SNP) microarray on SCLC also detected small deleted region involving RB1(RB) and CDKN2A(p16), (Nagayama et al., 2007) and chromosomal amplifications of 1p36.1 containing WNT4 (Garnis et al., 2005), of 5p13 of chromosome 5 involving SKP2 (Coe et al., 2005; Yokoi et al. 2009), of chromosome 2 involving ABCB1 (Kitada et al., 2009) and TERT of chromosome 18q (Salido et al., 2009) and of 2q12.21 of chromosome 22 containing CRKL (Kim et al., 2010).

Further, small size regions associated with gene deletion and amplification could be detected by array CGH, not G-banding analysis. The affected regions of 3q26, 7q36 of chromosome 7 and of 12p11.2 of chromosome 12 have also been recurrently observed in NSCLC, which had also been observed in NSCLC (Lee et al., 1987; Miura et al., 1990a, 1990b; Testa et al., 1998), esophagus cancer and ovarian cancer, stomach cancer, and breast cancer and seminoma. Array CGH analysis revealed that copy number alteration at 3q27.1 highlighted the connection of THPO, SOX2 and PIK3CA novel oncogene activation at 3q27 and tumor cell growth in NSCLC (Baik et al., 2009; McCaughan et al., 2010). Less frequent gain of copies of chromosomal region such as 7q22.3-31.31 and 12p11.23-13.2 in NSCLC identified by CGH analysis (Dehan et al., 2007), being different from present karyotype results on SCLC, which are implying that chromosome patterns of NSCLC and SCLC are different. Genome-wide high resolution analysis also suggested that SCLC and NSCLC had different specific genetic alterations and expressions (Girard et al., 2000; Wistuba et al., 2001).

The etiology of SCLC is strongly tied to cigarette smoking. And then, more than 80% of lung cancers are attributed to tobacco exposure. However, since only a fraction of long-term smokers of about 15% will develop lung cancer in their lifetime, it is proposed that genetic factors play a role in individual susceptibility (Sheilds 2002). All SCLC patients, whose tumor specimens were used for establishing present cell lines, had severe resistance to chemotherapy. Levels of glutathione S-transferase (GST-P) correlate with the resistance to cisplatin and carboplatin in human cancer cell line (Wakagawa et al., 1988). Similar finding was observed in our 6 Japanese cell lines examined. GSTs are family of enzymes that detoxify hydrophobic electrophiles that have been implicated in the pathogenesis of lung cancer. GST related genes mapped on chromosomes 1p36.1, 1p31, 6p12.2, 8p21.1 and 12q13-q14. No correlation between resistance to chemotherapy in the patients and chromosome deletions and amplifications on these region involving GST-related genes were observed in these 6 Japanese cell lines. These drug-resistant lung cancer cell lines has an increased copy number in the MDR/ABCB1 locus region on 7q32 of chromosome 7 and significantly higher number or chromosome 7 alterations (Slovak et al., 1987; Ueda et al., 1986; Yabuki et al., 2007). The presence of recurring chromosome 7 alterations did not always contain oncogenes EGFR at 7q11.2 and MET at 7q31.2 in NSCLC having amplified chromosome 7. Other genes such as FTSJ2, NUDT1, TAF6 and POLR2 were identified as candidate gene on chromosome 7 associated with drug-resistant (Campbell et al., 2008).

Since variant SCLC cells have a less differentiated neuroendocrine phenotype than their classical counterparts, it was of interest to examine the effects of a differentiation inducer such as retinoic acid and vitamin A on variant and classical SCLC. Expression of retinoic acid α (RARα), retinoic acid β(RARβ) and retinoic acid γ(RARγ) was examined SCLC cell line (Martin et al., 1990). Variant SCLC is distinguished from classical histology by changing growth rate, morphology, MYC amplification, a loss of some biochemical markers and some chemoch and radio resistance. The variant SCLC grows and leads a morphological change after exposure to retinoic acid, similar to classical SCLC (Doyle et al., 1989). No correlation between differentiation to RARs in the patients and chromosome deletions and amplifications on these regions involving RARα, RARβ and RARγ genes were observed in these 6 Japanese cell lines.
Current techniques of M-FISH analyses, gene expression array, methylation-specific PCR, whole DNA sequencing of cancer cell lines and tumors will reveal more important genetic changes such as disease specific translocations for lung cancers affecting chromosomes sites that harbor genes known to pathogenesis in tumorigenesis and progression of human neoplasias (Inamura & Ishikawa 2007; Meiju et al., 2011), which will be therapeutic targets for cancer chemoprevention.

Acknowledgments

We thank Prof. Miura I. of St. Marianna University of School of Medicine for important comments and Prof. Resau J. of Maryland Cancer Center for providing tumor samples. The study was supported in part by Grants-in-Aid for Scientific Research from Ministry of Education, Culture, Sports, Science and Technology of Japan (K.T.).

References


