Original Article

Chromosomal and Genetic Analysis of a Human Lung Adenocarcinoma Cell Line OM

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Abstract

Background: Lung cancer has become the leading cause of death in many regions. Carcinogenesis is caused by the stepwise accumulation of genetic and chromosomal changes. The aim of this study was to investigate the chromosome and gene alterations in the human lung adenocarcinoma cell line OM.

Methods: We used Giemsa banding and multiplex fluorescence *in situ* hybridization focusing on the human lung adenocarcinoma cell line OM to analyze its chromosome alterations. In addition, the gains and losses in the specific chromosome regions were identified by comparative genomic hybridization (CGH) and the amplifications of cancer-related genes were also detected by polymerase chain reaction (PCR).

Results: We identified a large number of chromosomal numerical alterations on all chromosomes except chromosome X and 19. Chromosome 10 is the most frequently involved in translocations with six different interchromosomal translocations. CGH revealed the gains on chromosome regions of 3q25.3-28, 5p13, 12q22-23.24, and the losses on 3p25-26, 6p25, 6q26-27, 7q34-36, 8p22-23, 9p21-24, 10q25-26.3, 12p13.31-13.33 and 17p13.1-13.3. And PCR showed the amplification of genes: Membrane metalloendopeptidase (*MME*), sucrase-isomaltase (*SI*), butyrylcholinesterase (*BCHE*), and kininogen (*KNG*).

Conclusions: The lung adenocarcinoma cell line OM exhibited multiple complex karyotypes, and chromosome 10 was frequently involved in chromosomal translocation, which may play key roles in tumorigenesis. We speculated that the oncogenes may be located at 3q25.3-28, 5p13, 12q22-23.24, while tumor suppressor genes may exist in 3p25-26, 6p25, 6q26-27, 7q34-36, 8p22-23, 9p21-24, 10q25-26.3, 12p13.31-13.33, and 17p13.1-13.3. Moreover, at least four genes (*MME*, *SI*, *BCHE*, and *KNG*) may be involved in the human lung adenocarcinoma cell line OM.

Key words: Cell Line; Chromosomes; Genes; Lung Cancer

Introduction

Approximately, 1.6 million new cases of lung cancer are diagnosed each year throughout the world.[1] Both genetic and epigenetic changes contribute to the development of human cancer.[2] Since carcinogenesis is caused by the stepwise accumulation of genetic changes,[3] it is very important to understand the alterations of chromosome and gene in cancer cells. The traditional Giemsa banding (G-banding) technique is applied to detect cell sample with a higher mitotic index, but lung cancer cells have a lower mitotic index,[4] which makes karyotypic analysis difficult. In our study, combining with the latest molecular biology techniques, we investigated the chromosomal and genetic alterations in the human lung adenocarcinoma cell line OM. Here, we summarized the chromosomal and genetic abnormalities detected in lung cancer cells and discussed the possible implication of those alterations in the processes of tumorigenesis.

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Methods

Cell line

The lung adenocarcinoma cell line OM was established in the second Department of Biology Kochi Medical School, Kochi

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University, Japan. The cells in cell line OM were from human lung adenocarcinoma tissues. After cultured and purified, these cells developed into a special cell line for cancer research.

Chromosome preparation and Giemsa banding analysis

The cultures were harvested and G-banding of the chromosomes was performed by hypo-osmolality, fixation, trypsinization, and Giemsa (Invitrogen Corp., Carlsbad, CA, USA) staining. The subsequent karyotype description followed the recommendations of the ISCN (1995).

Multiplex fluorescence *in situ* hybridization

The slides after denaturation were dehydrated in graded series of ethanol and air dried. The 24 XCyte mFISH kit (Vysis Corp., USA) with five fluorophores were used for hybridization. Samples were evaluated, and images were captured using an Olympus BX60 fluorescence microscope (Olympus, Tokyo, Japan) and analyzed with the aid of image analysis software (CytoVision, Applied Imaging Corp., Santa Clara, CA, USA).

Comparative genomic hybridization

DNA extraction was performed with 2-ProPanol (Sigma-Aldrich, Japan). After washing with ethanol, dried, concentrated, and purified, the sample and reference genomic DNAs were digested and random primed labeled with Cyanine-5/Cyanine 3-dUTP (Vysis Corp., USA). Cohybridization of these DNAs to a comparative genomic hybridization (CGH) arrays was performed for 72 h at 37°C. After washing, the chromosomes were counterstained with DAPI. Array CGH was scanned on Olympus BX60 (OLYMPUS Corp., Japan) and data were extracted and analyzed using Multiscan medical image analysis system (SONY Corp.,Tokyo, Japan).

Genomic DNA polymerase chain reaction

Extracted DNA from a normal human was used as the internal control. All the primers were provided by the Invitrogen Corp., (Invitrogen Corp., Carlsbad, CA, USA). The amplified samples were analyzed by electrophoresis at 100 V on 1.5% agarose gels stained with ethidium bromide. The visual of the bands of amplified DNA were captured by a camera in the device.

Statistical analysis

SAS 9.3 (SAS Institute Inc., Cary, NC, USA) is useful statistical software to analyze this kind of data. An exact 95% confidence interval (*CI*) using the Clopper–Pearson method was given for the positive rate of abnormal chromosomes and genes. Qualitative data were expressed as a frequency. The *CI* value of reference standard was 0.3%.

Results

Karyotype analysis

Characterizations of complex chromosomal abnormalities in the human lung adenocarcinoma cell line OM were showed by G-banding analysis, and the wrong number of chromosomes was present in almost all cells. Either aneuploid or polyploid karyotype was observed which displayed chromosome instability in the human lung adenocarcinoma cell line OM. G-banding showed chromosomal aberrations on all chromosomes except chromosome X (95% *CI*: 0–64%) and 19 (95% *CI*: 0–64%) [Figure 1].

Multiplex fluorescence *in situ* hybridization analysis

The structurally abnormal chromosomes were analyzed by multiplex fluorescence *in situ* hybridization (M-FISH). As was illustrated in Figure 2, chromosome 10 is the most frequently (95% *CI*: 29–100%) involved in translocations with six different interchromosomal translocations, and its frequency was 4–6 times in each sample. The other chromosomes involved in translocation were chromosomes 1, 2, 3, 5, 6, 7, 8, 9, 11, 16, and 18, and the 95% *CI* minimum value of each statistical datum was more than 9%.

Comparative genomic hybridization analysis

CGH revealed that the gains in the region of chromosome bands 3q25.3-28, 5p13, 12q22-23.24, and the losses in that of 3p25-26, 6p25, 6q26-27, 7q34-36, 8p22-23, 9p21-24, 10q25-26.3, 12p13.31-13.33, and 17p13.1-13.3 [Figure 3]. The 95% *CI* minimum value of each statistical datum was more than 15%.

Polymerase chain reaction analysis of genomic DNA

According to the results obtained by CGH, gene analysis was performed in the 3q24-28 region. The results of the polymerase chain reaction (PCR) experiments showed that the genes membrane metalloendopeptidase (*MME*) (95% *CI*: 1–88%), sucrase-isomaltase (*SI*) (95% *CI*: 54–100%),

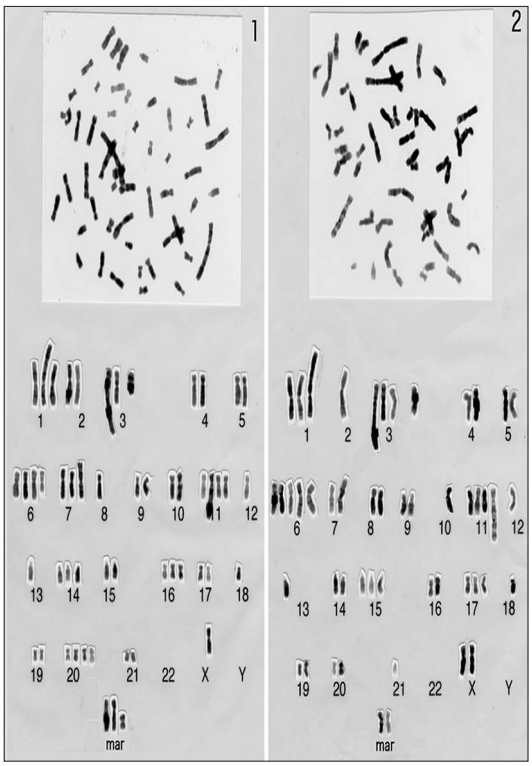

Figure 1: Giemsa-banding analysis revealed chromosomal aberrations on all chromosomes except, 4, 8, 14, 15, 17, and 19. The wrong number of chromosomes was present in almost all cells. Either aneuploid or polyploid karyotype was observed.

butyrylcholinesterase (*BCHE*) (95% *CI*: 16–100%), and kininogen (*KNG*) (95% *CI*: 1–72%) were amplified in this region. However, the amplification of genes Histidine-rich glycoprotein (*HRG*, 95% *CI*: 0–60%) and Mucin (95% *CI*: 0–52%) were not observed [Figure 4].

Discussion

Lung cancer remains the leading cause of cancer-related morbidity and mortality worldwide.[5] Unlike in leukemia research, lung cancer cell has a lower cell mitotic index,[6] which makes karyotypic analysis difficult. Using modern genetic technologies, we investigated the chromosome and gene alterations in human lung adenocarcinoma of cell line OM. The goal of our study is to identify the alterations of chromosome and gene in human lung adenocarcinoma cell line OM, which may provide new insights into the mechanisms of tumorigenesis.

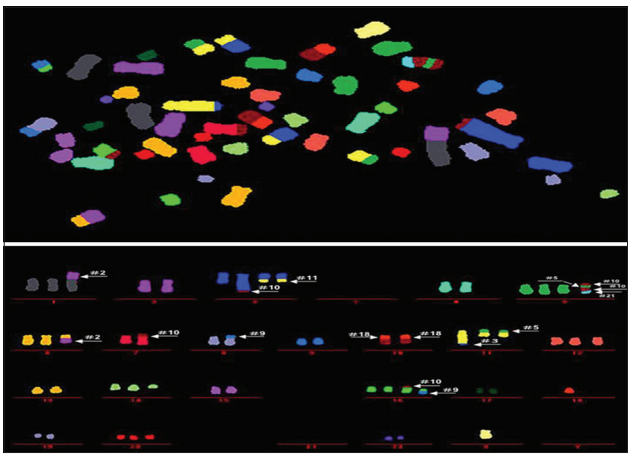

Figure 2: Multiple numerical and structural aberrations were observed in the majority of cells from multiplex fluorescence *in situ* hybridization image. Chromosome 10 is the most frequently involved in translocations with four different interchromosomal translocations. The other chromosomes involved in translocation were chromosomes 2, 5, 9, 11, 18 and 3, 21.

With high-resolution G-banding techniques, both the numerical and structural chromosomal abnormalities were observed. G-banding analysis revealed that the changes in chromosome number were present in most cells, and either aneuploidy or polyploidy was identified within them. In OM cell line, certain chromosomes frequently participated the structural rearrangement, prominent among them being chromosome 3, 6, 7, 9, 10, 11, 18, and Y.

Although G-banding is a fundamental technique for cytogenetic study, it has limited utility in instances of cryptic or very complex rearrangements.[7] M-FISH is a powerful technique that can be used for identifying uniquely all 24 chromosome types of the human genome.[8] The M-FISH image showed that the highest frequency of translocation was in chromosome 10. In genetics, a chromosome translocation is caused by rearrangement of parts between nonhomologous chromosomes, and the distinct classes of chromosomal rearrangements create proto-oncogenes.[9] Loci on chromosome 10 include microseminoprotein beta, which encodes beta-microseminoprotein, a primary constituent of semen and a proposed prostate cancer biomarker.[10] Moreover, multiple tumor-suppressive genes are located in chromosome 10,[11] and the translocations of chromosome

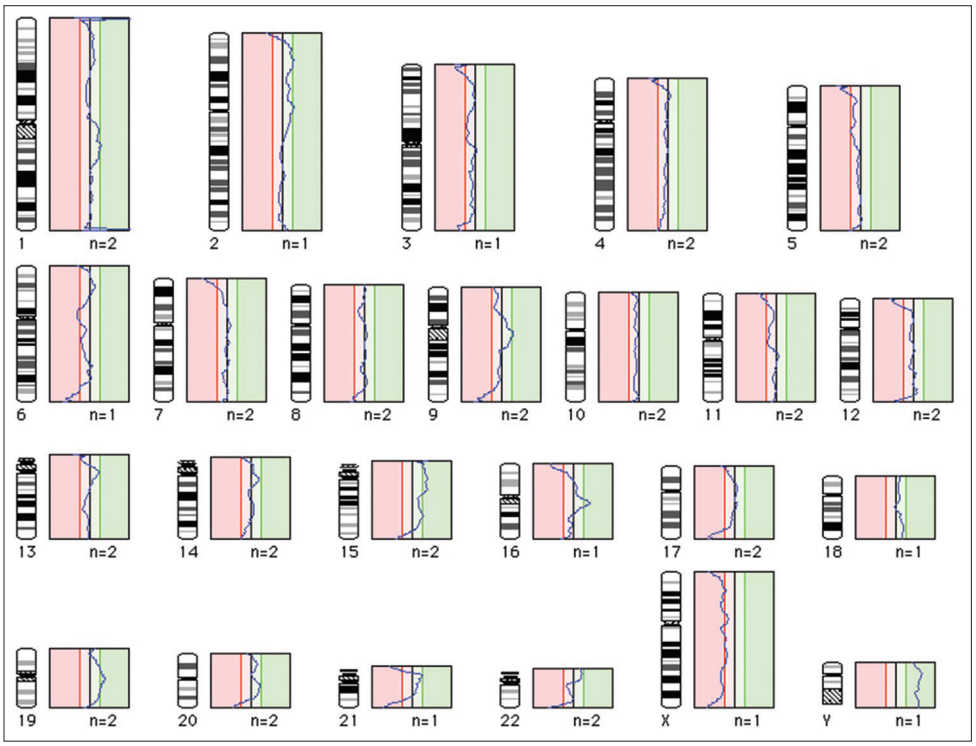

Figure 3: Array comparative genomic hybridization analysis showed partial deletion of in 3p24.1-26, 6p25, 6q25.2-27, 7q32-36, 8p22-23, 9p21-24, 10q25-26.3, 12p13.31-13.33, and 17p131-13.3 together with duplication at 3q24-28, 5p13, and 12q22-23.24.

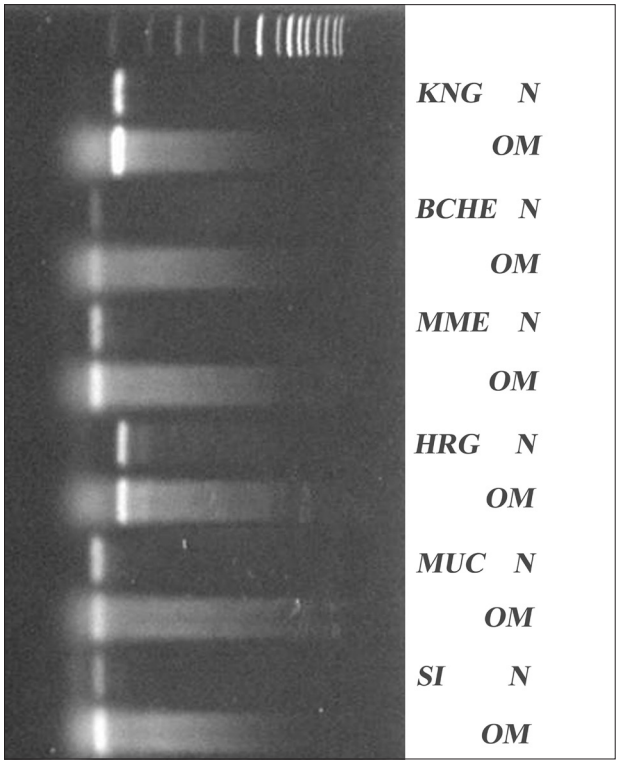

Figure 4: Agarose gel electrophoresis of polymerase chain reaction amplification products from the human lung adenocarcinoma cell line OM. N: Negative control (normal human lung cell line); OM: Human lung adenocarcinoma cell line OM. *MME*: Membrane metalloendopeptidase; *SI*: Sucrase-isomaltase; *BCHE*: Butyrylcholinesterase; *KNG*: Kininogen; *HRG*: Histidine-rich glycoprotein; *MUC*: Mucin.

10 may lead to damage of these tumor-suppressive genes. Therefore, frequent translocations of chromosome 10 in cell line OM may be catastrophic genomic events and play key roles in tumorigenesis.

In general, regional gains on chromosome indicate that oncogenes may exist in this region, while the chromosome losses have been interpreted as an evidence that the region has affected a certain tumor suppressor genes.[12] CGH analysis revealed that gain regions were observed on chromosomes 3q25.3-28, 5p13, and 12q22-23.24, which indicated that there may exist oncogenes that were activated in the gain regions. While loss regions were detected in chromosome 3p25-26, 6p25, 6q26-27, 7q34-36, 8p22-23, 9p21-24, 10q25-26.3, 12p13.31-13.33, and 17p13.1-13.3, which indicated that some tumor suppressor genes may be located in these regions. It is well-known that tumor suppressor genes p16 and p53 were located in chromosome 9p21 and 17p13, respectively.[13]

Based on the above results, we detected amplification of genes at chromosome band 3q24-28 by PCR in the cell lines. The genes *BCHE*, *MME*, *KNG*, and *SI* were amplified in the chromosomal region. The *BCHE* gene provides instructions for making the pseudocholinesterase enzyme,[14] which has a variety of physiological effects such as the metabolism of the cocaine and heroin[15] and the breakdown of organophosphorus esters.[14] Recent studies indicated that *BCHE* gene was amplified in the cells of lung carcinomas,[16] which were consistent with our results. Brass *et al*.[17] observed the amplification of genes *BCHE* and *SLC2A2* at 3q26 in squamous cell lung carcinomas, whereas no amplification has been found in genes *MME* and *KNG*. *MME* gene is expressed in both normal and neoplastic cells as a common acute leukemia antigen.[18] In squamous cell lung carcinoma, the high *MME* gene expression was significantly associated with poor overall survival.[19]

There were many studies on the expression of *MME* gene in lung cancer cells; however, these results were quite conflicting. Many factors may be responsible for the difference among the results, such as experimental conditions, experimental methods,[19] and pathological types of lung cancer. The *KNG* gene, which is one of the members of a cystatin-like superfamily, has potential roles in angiogenesis and (or) tumor development.[20] Kinins that derived from *KNG* involved in cell proliferation, leukocyte activation, cell migration, endothelial cell activation, and nociception.[21] Kashuba *et al*.[22] thought that *KNG* may be a novel therapeutic target in chronic lymphocytic leukemia and the possible association with prognosis. There are few reports on the expression of *KNG* gene in lung cancer cell. We observed that *KNG* gene was amplified in lung adenocarcinoma cell line OM, which suggested that *KNG* gene was likely to be involved in tumorigenesis of lung cancer cells. *SI* gene encodes an SI enzyme that is expressed in the intestinal brush border.[23] Although there are numerous studies elucidating the role of *SI* gene in the digestion of dietary carbohydrates,[24] there are limited data indicating a specific involvement of *SI* gene in the genesis of human cancers. Our experiments demonstrated that *SI* gene was amplified on chromosome band 3q24-28. Since cancer is attributed to multi-genetic alterations accumulated in the cells, we speculated *SI* gene may be involved in tumorigenesis.In summary, our findings suggested that most of those examined cells exhibited multiple complex karyotypes in human lung adenocarcinoma cell line OM. Chromosome 10 was frequently involved in chromosomal translocation, which may be catastrophic genomic events and play key roles in tumorigenesis. According to the results of CGH, we speculated that the oncogenes may be located at 3q25.3-28, 5p13, and 12q22-23.24, while tumor suppressor genes may exist in 3p25-26, 6p25, 6q26-27, 7q34-36, 8p22-23, 9p21-24, 10q25-26.3, 12p13.31-13.33, and 17p13.1-13.3. In human lung adenocarcinoma cell line OM, at least four genes (*MME*, *SI*, *BCHE*, and *KNG*) were involved in carcinogenesis. It may be helpful to deeply understand the tumorigenesis of lung cancer.

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Conflicts of interest

There are no conflicts of interest.

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