Journal of Cellular Biochemistry Supplements 28/29:74–80 (1997)

Molecular Cytogenetic Alterations in the Early Stage at Human Bronchial Epithelial Cell Carcinogenesis

Xiang-Yang Dong, Yong-Jie Lu, Tong Tong, Yong-Jun Wang, Su-Ping Guo, Jin-Feng Bai, Nai-Jun Han, and Shu-Jun Cheng*

Department of Etiology and Chemical Carcinogenesis, Cancer Institute (Hospital), CAMS & PUMC, Beijing, The People's Republic of China

Abstract Lung carcinogenesis is a multi-step process involving activation of oncogenes and inactivation of tumor suppress genes. Many molecular and cytogenetic alterations occur in the early stages of carcinogenesis. We have developed an effective culture system for human bronchial epithelial cells and lung cancer cells. Four immortalized human bronchial epithelial cell lines were established by transfecting the epithelial cells with plasmid DNA containing the early region of SV40. Some molecular and cytogenetic alterations, such as 3p-, 2q-, 9p-, c-*myc* translocation t(8;14) (q23; q32), were found in one immortalized bronchial epithelial cell line M when approaching malignant transformation. An increase in cell proliferation and decrease of apoptosis were noted in the late passages of the immortalized cell line M. Some molecular cytogenetic alterations were also observed in human primary non-small cell lung cancers. Molecular cytogenetic alterations during the early stage of carcinogenesis of human bronchial epithelial cells may be useful as biomarkers for both diagnosis and intermediate endpoint of chemoprevention of lung cancer. J. Cell. Biochem. Suppls. 28/29:74–80.

Key words: bronchial epithelium; carcinogenesis; lung cancer; molecular cytogenetic alterations; chemoprevention

Lung cancer can be classified into four major types: small cell carcinoma (representing 25%), squamous cell carcinoma (30%), adenocarcinoma (25%,) and large cell carcinoma (15%), with uncommon or mixed types making up the remaining 5% [1].

Lung cancer is now the number one cause of cancer death for both men and women. An estimated 170,000 new cancer cases were reported in the United States in 1993; 80% of these were non-small cell lung cancer (NSCLC) [2]. The mortality rate for NSCLC has remained constant since 1985, despite advances in cytotoxic drug development, radiotherapy, and patient management. Surgical resection remains the preferred cure, but patients who undergo surgical resection have a long-term survival rate of only 50% [3]. Characterizing the molecular events that transform bronchial epithelial cells into invasive lung cancer cells may provide effective tools for early detection and diagnosis of lung cancer.

As initially suggested by the results of model systems [4], and more recently by studies of the molecular pathogenesis of colon cancer [5], a multi-step model of epithelial carcinogenesis has evolved. Multiple genetic changes, including mutations, deletions, gene amplification, or translocations, occur during the transformation of a normal cell into a malignant one.

Presently, there is minimal informative data about the sequence of events leading to lung cancers. Current theories suggest that as many as 10 to 20 events, including alterations of oncogenes and tumor suppressor genes, must occur by the time lung cancer becomes clinically evident [6].

To decrease the incidence rate and improve the cure rate of lung cancer, comprehensive studies of the genetic mechanisms of lung cancer development must be carried out to obtain some specific biomarkers which can be used to diagnose lung cancer as early as possible. Another approach is to use chemoprevention measures in high-risk populations, which requires intermediate biomarkers to evaluate the effect

*Correspondence to: Shu-Jun Cheng, Department of Etiology and Chemical Carcinogenesis, Cancer Institute (Hospital), CAMS & PUMC, PO Box 2258, Beijing 100021, The People's Republic of China.

Received 26 February 1997; Accepted 2 December 1997

of chemoprevention and/or treatment. Recently research groups are focusing on looking for biomarkers that accompany the development and carcinogenesis of lung cancer.

BRONCHIAL EPITHELIAL CELL LINE M

The majority of human NSCLC originates from carcinogenesis of the bronchial epithelium [1]. Many model systems have been designed to study the carcinogenesis of human bronchial epithelium [7–10]. One common and practical system is to generate immortalized cell lines with simian virus 40 (SV40) early region genes. Using a plasmid containing the early region of SV40, we transfected human bronchial epithelial cells and established four immortalized SV40T-transformed cell lines; one was termed cell line M. Our discussion in this paper is mainly limited to cell line M. Derived from one noncancerous individual, bronchial epithelial cells were cultured from an explant of biopsy specimen as described previously [10]. The cells were cultured in a modified serum-free medium in collagen-coated dishes. After being transfected with SV40T and selected by G418, the surviving cells grow vigorously and can be passed continuously. Interestingly, an obvious crisis was not observed in the cell line M, which was rarely reported before. The cells' epithelial origin has been confirmed by the presence of keratin (KARO 7650 CAM5.2 CLONE). The cells show a constant expression of SV40T. When cells of the early passage of cell line M were compared with those of the later passage, many alterations in both phenotype and genotype were observed. Following are the primary results of our study.

Alterations of Growth Factor Requirement

We tested the colony forming efficiency (CFE) of cells of M at passage 16, M(p16) and 71, M(p71). Two thousand cells were planted in 60-mm dishes and cultured in MCDB-151 Serum Free Medium (MSFM) with the addition of different growth factors. After 6 days of incubation, colonies were scored under phase-contrast microscopy, and only colonies of more than 10 cells were counted. Cells per colony were enumerated in 25 randomly-chosen colonies from each group. CFE was calculated as CFE = colonies/cells planted $\times$ 100%. Cell doubling time (PD/D) was calculated using the following formula:

$$\text{PD/D} = \frac{\log \text{cells/colony}}{\log 2\text{Xt}} \quad \text{t} = \text{days cell cultured.}$$

The results were summarized in Table I, which shows that cells of the later passage M(p71) grew faster than those of the early passage M(p16) ($P < 0.01$). Meanwhile, later passage cells become more independent of EGF.

Resistance to Serum Differentiation

Cells of the later passage M(p71) were more resistant to serum-induced differentiation than those of the early passage M(p16) (see Table II).

Anchorage Independent Growth Ability in Soft Agar

Small colonies were observed in dishes planted with M(p71) cells. After 1 week of culture, colonies larger than 100 μm were scored; CFE% of M(p71) was 6.45 $\pm$ 0.223. No colony was found for M(p16). Also, cells of early passage grow as a monolayer, but cells picked from a colony growing in soft agar can grow in multilayer.

Cytogenetic Analysis

Cytogenetic analysis was carried out in cells of passage 14, 30, 71, and 97 by standard methods [11]. Cells in passage 14 were diploid with a chromosome mode of 43; chromosome modes of M(p30), M(p71), and M(p97) were 85, 77, and 67, respectively. All of these cells possessed complex karyotypes with many structural and

TABLE I. Growth Factor Requirement of M(p16) and M(p71)*

	CFE%		PD/D	
Medium	M(p16)	M(p71)	M(p16)	M(p71)
MSFM	10.40 $\pm$ 0.707	17.125 $\pm$ 0.742	0.79 $\pm$ 0.08	0.98 $\pm$ 0.08
MSFM-EGF	1.875 $\pm$ 0.177	16.900 $\pm$ 0.283	0.69 $\pm$ 0.08	0.85 $\pm$ 0.08

*CFE: colony forming efficiency; PD/D: per doubling/day; EGF: epidermal growth factor; MSFM-EGF: deprivation of EGF from MSFM. CFE% and PD/D between M(p16) and M(p71) $P < 0.01$; CFE% and PD/D of M(p16) between MSFM and MSFM-EGF, $P < 0.01$.

TABLE II. Resistance to Serum Differentiation Between M(p16) and M(p71) Cells*

	CFE%		PD/D	
Medium	M(p16)	M(p71)	M(p16)	M(p71)
MSFM	10.40 ± 0.707	17.125 ± 0.742	0.79 ± 0.08	0.98 ± 0.08
MSFM + 2%NBS	7.325 ± 0.177	41.575 ± 2.298	0.77 ± 0.12	0.97 ± 0.08
MSFM + 10%NBS	1.175 ± 0.106	32.775 ± 2.580	0.77 ± 0.13	0.94 ± 0.10

*NBS: newborn bovine serum. CFE% and PD/D between M(p16) and M(p71) $P < 0.01$; CFE% between MSFM and MSFM + NBS $P < 0.01$; PD/D between MSFM and MSFM + NBS $P > 0.05$.

numerical chromosome aberrations. Cell line M shows the alterations of chromosome mode, from diploid to tetraploid, then aneuploid. This chromosome mode change sequence reflects a common pathway from a normal diploid cell to a malignant aneuploid cell. This procession is probably related to the alteration of cell cycles that control cell growth and death. When normal diploid cells in cell line M were damaged by some external factors such as SV40T, most cells died, but the survivors gained a growth advantage and divided continuously. However, the genome of the cells became unstable, resulting in random chromosome loss and aberration along with cell proliferation, and complexed karyotypes appeared.

Tumorigenicity Assay

Cells from passage 22 and 67 were injected s.c. into 3–4-week-old athymic nude mice. No tumor was found after inoculation for 6 months.

Immortalized cell line M grew more and more malignant. The cells of later passages were growth-independent of EGF, had increased resistance to serum differentiation, grew anchorage-independently in soft agar, lost contact inhibition, and possessed abnormal karyotypes. However, malignant transformation was not complete since no tumors formed in nude mice. Cell line M was still at the premalignant stage. Based on the above phenotype alterations, we screened the genotype alterations of this cell line, and found various molecular and cytogenetic changes. We hope to identify specific biomarkers accompanying the carcinogenesis process in human bronchial epithelial cells. Previous results [12–14] are discussed below.

C-*myc* Gene Translocation

The proto-oncogene c-*myc*, whose products bind DNA and regulate gene transcription, is frequently activated by amplification in many cancers [15]. Although the L-*myc* gene has been associated with complex intrachromosomal rearrangements and the creation of a fusion gene [16], no interchromosomal recombinations involving *myc* genes have been observed in lung cancers. In our M cell line (p13), FISH analysis demonstrated that the c-*myc* gene is translocated to 14q32 [14]. This translocation is also found in Burkitt's lymphoma, in which c-myc is translocated to one of the immunoglobin loci and results in an aberrant expression of this gene [17,18]. The mRNA level of the c-*myc* in M(p15) and M(p72) was also tested by FISH. Both passages showed a strong positive result. Immunohistochemical assay indicated a high level expression of c-*myc* protein. Although the normal physiologic roles of the *myc* genes have yet to be defined, c-*myc* is an early response gene which is critical with respect to stimulating quiescent cells into and through the Go phase of the cell cycle. Activation of c-*myc* appears to be sufficient to initiate DNA synthesis [19]. The activation of c-*myc* by translocation, which occurs in the early stage in cell line M, may cause vigorous growth of the cells without an obvious crisis.

Chromosome Deletion Involved in 3p and 2q

Using a chromosome 2- and chromosome 3-specific DNA probe (from American Type Culture Collection, Rockville, MD) combined with DAPI(4*6-diamidino-2-phenylindole) banding, we found 3p-deletion occurred at passage 14 M(p14), and another chromosome abnormality, 2q-, occurred at passage 30 [13]. Cytogenetic analysis has confirmed that lung cancers are associated with multiple genetic alterations. The commonly detected abnormality involves 1p, 3p, 5q, 7q, 9p, 11p, 13q, and 17p [20–22]. Among these abnormalities, 3p was the first reported nonrandom chromosomal abnormality in lung cancers [23], as well as a variety of other neoplasms, in particular renal cell cancers [24]. The region of deletion in NSCLC appeared to be

3p23. This suggests that tumor suppressor gene(s) exist in the region. The candidate tumor suppressor genes include c-*erb*-A-β(thyroid hormone receptor), RARβ(retinoic acid receptor), as well as the phosphotyrosine phosphatase-γ gene (PTP-γ)[25]. 2q- has rarely been reported in human lung cancer before. However, we found that 2q- exists in cell line M and two other immortalized human bronchial epithelial cell lines we established [13].

It shows that 2q- is also a frequent genetic alteration in the human bronchial epithelial cell lines. Tsuchiya et al., 1992 [26] found that losses of chromosome 2q occurred in 31% of NSCLC by RFLP. And Shiseki et al., 1994 [22] obtained the same result in the study of primary NSCLC. Also, 2q aberration is found in human small cell lung cancer, as well as alveolar rhabdomyosarcoma [27], hairy cell leukemia [28], colorectal carcinoma and neuroblastoma [29]. These results indicate the presence of one or more putative tumor suppressor gene(s) on the long arm of chromosome 2. Loss of the gene(s) may contribute to the carcinogenesis of human bronchial epithelial cells. In cell line M, we found both 3p- and 2q- as nonrandom genetic alterations. What is more, 3p- seems to occur earlier than 2q-. The connection and meaning of these sequential alterations needs further investigations.

9p Deletion

Cytogenetic deletions involving chromosome bands 9p13–9p22 have been reported in at least 50% of NSCLC [30–31]; a critical 9p deletion region has been defined in NSCLC through loss of allelic heterogeneity studies using cancer cell lines. A consensus deletion region was located at chromosome bands 9p21–9p22 [32,33]. These data indicate that tumor suppressor genes at 9p21–9p22 contribute to neoplastic transformation in many NSCLCs. The candidate genes, cyclin-dependent kinase inhibitors p15 and p16, both map to 9p21, and are frequently deleted/mutated in many kinds of tumors, including lung cancer [34,35]. Using biotinylated P1 clone 1063 DNA, which contains both p15 and p16 genes, and a digoxigenin-labeled chromosome 9-specific DNA probe, we applied dual color FISH to cells of M(p18), M(p36) and M(p97). No deletion of p15/p16 was found in M(p18), but in M(p36) and M(p97) the p15/p16 showed a partial deletion. In cells of M(p36), there were four chromosome 9, but 80% of the cells showed only three P15/p16 hybridization signals. In the cells of M(p97), three chromosome 9 with only two p15/p16 signals were observed [36]. Immunohistochemical analysis utilizing monoclonal antibodies reactive with p16 has revealed concordant results with the FISH test in cell line M. The cells of M(p20)showed a strong positive stain of P16 monoclonal antibodies, while only a negative or very weak positive stain was found in later passages. Although p15/p16 were not completely deleted, once one is deleted, all other loci are commonly inactivated by mutation or methylation. Loss of p15/p16 may contribute partly to the neoplastic transformation of cell line M. Both p15 and p16 are negative control factors of the cell cycle, whose product is involved in inhibiting the transition of the cell cycle; especially, p16 inhibits the ability of CDK4 and CDK6 to phosphorylate the Rb protein [37]. Loss of function of these two genes may force the cell cycle out of control, causing further malignancy.

Integration of SV40 at 12q 23

SV40 is commonly used to transform and immortalize cells. Maintaining the transformed state requires the integration of virus DNA into the genome of the cells and constant expression of SV40T [38,39]. Much work has been done to study SV40T integration site of SV40T transformed human cells [38,40]. Previous studies show that this integration site is random in different cell lines, but it is specific in a particular cell line [38,40,41]. We have studied the integration sites of SV40 in SV40T transformed epithelial cells by FISH, in cell Mp13, p30, and p71. The SV40T has a high and stable expression in every passage of the cells. In M(p13) SV40 integrated mainly at 12q 23, but in some cells SV40 was also found on other chromosome regions, such as 2p and 5q. In M(p30) and M(p71), the hybridization signal was almost exclusively in 12q [13]. The specific integration site may correlate with the transformation of cell line M, conferring some kind of growth advantage to these cells. This specific integration may activate oncogenes or growth factors. Interestingly, the insulin-like growth factor I(IGF-I), a gene related to the SV40 transformation of mammalian cells, is located at 12q 23 [42]. In SV40T-transfected cells, SV40 can activate the IGF-I promoter and markedly increase secretion of IGF-I, resulting in high-level IGF-I expression, which could stimulate growth of the cells [43].

This high expression of IGF-I may explain why later passage cells become independent of EGF. However, the subtle relationship between SV40 integration, IGF-I activation, and transformation of cell line M needs further investigation.

SENSITIVITY TO PDD (CIS-PLATIN) INDUCED APOPTOSIS

Using in situ terminal deoxynucleotidyl labeling assay, we tested PDD-induced apoptosis of cells of M(p16) and M(p73). When the PDD concentration was 25 μM, induced apoptosis of M(p16) and M(p73) was 27.4 and 5.8%, respectively ($P < 0.01$); when the concentration of PDD was 50 μM, the induced apoptosis of M(p16) and M(p73) was 49.4 and 8.8%, respectively ($P < 0.01$). Apoptosis was easier to induce in earlier passage cells than in later passage cells.

Cell numbers are regulated by a balance between proliferation and cell death in vivo [44]. Recently, studies on oncogenesis have focused on regulating cell proliferation. The product of *bcl*-2 gene is known to play a role in protecting cell survival and inhibiting apoptosis; a high level of *bcl*-2 expression was observed to block apoptosis. Immunohistochemical tests of cell line M show a higher level of *bcl*-2 expression in later passage cells than in early passage cells. Discussed above are our preliminary studies on the cell line M; further studies are in process. Among the phenotype and genotype alterations accompanying cell line M passage, some may occur only in vitro, while others may indeed reflect the development and carcinogenesis of human bronchial epithelium. Since cell line M is in the early stage of carcinogenesis, these alterations can be considered early events in human bronchial epithelium carcinogenesis. However, early cytogenetic and molecular alterations found in vitro should be further investigated in human lung cancer. If we find the same alterations in lung cancer samples, these early events will be useful as biomarkers for early human lung cancer detection.

HUMAN PRIMARY NSCLC

Twelve human primary NSCLC specimens (each case included tumor tissue and the adjacent bronchial epithelial cells) were collected from the Cancer Hospital, Chinese Academy of Medical Sciences. All tumors were classified according to the standard criteria of the World Health Organization (WHO). Nine men and three women, median age 57 years (29–69) with six squamous cell carcinomas and six adenocarcinomas received no chemotherapy and radiotherapy before surgical resection. Specimens were delivered to the laboratory immediately after surgical resection. Tumor tissues were washed in L15 medium with antibiotics, cut into small pieces and digested in collagenase, then cultured in modified MCDB151 medium for 3–7 days before harvesting. Bronchial mucosa was digested with pronase E and the bronchial epithelial cells cultured for 1 to 2 weeks. Metaphase chromosome slides were made by standard cytogenetic methods. We investigated the molecular and cytogenetic alterations in these primary NSCLCs. In each case, the bronchial epithelial cells had a normal chromosome mode of 46, while 83.3% (10/12) companion neoplastic cells were aneuploid, showing genome instability and imbalance during lung carcinogenesis. FISH tests showed that 2q- and 3p- were frequently occurring events; 2q- was found in three out of seven primary NSCLC specimens (the other five specimens were not done).

2q- was also found in one case of bronchial epithelial cells adjacent to the tumor. 3p- was found in 45.67%(5/12) of the specimens. Also, loss of chromosome 3 was found in two specimens. In the 12 case specimens, two specimens had c-*myc* translocation; one case was t(8;14), the other was t(8;22). Immunohistochemical analysis showed that in addition to these two cases, two more showed high-level c-*myc* protein expression, indicating that c-*myc* amplification is common in lung cancer, in which c-*myc* translocation was a major cause. In another study, 9p- was found in four out of nine clinical NSCLC specimens.

3p- and 9p- were frequently found in human lung cancer samples. 2q- was another nonrandom chromosome abnormality, not only in human bronchial epithelial cell lines but also in primary NSCLCs, indicating that these alterations were nonrandom events during human lung cancer carcinogenesis development. It further confirmed that cell line M was a good in vitro model system for lung cancer research.

Growth factor and receptor abnormalities play an important role in lung cancer development. TGF-α and EGF-R are frequently targeted in human lung cancer cases. An immunohistochemical analysis of 26 NSCLCs (18 squamous cell carcinomas and 8 adenocarcino-

mas) found that TGF-α and EGF-R had a much higher level of expression in tumor cells than in cells adjacent to the tumor tissue. High expression may have a positive correlation with human lung cancer development [45]. Our results, together with previously reported data, suggest that lung cancer is a multiple genetic disease. Although great progress has been made in understanding the molecular genetics of lung cancer, our knowledge of early activation events remains preliminary. It is paramount to identify which, if any, of the genetic lesions associated with lung cancer are critical early steps. By applying the techniques of modern molecular biology, we could conceivably devise approaches to identify early-stage lung cancer in high-risk patients. This may allow us to have an effective impact on this deadly disease at an early and more treatable time point.

ACKNOWLEDGMENTS

We thank Dr. A. Kamb for the generous donation of the P1 1063 clone that recognizes both p15 and p16 genes, and Dr. S.R. Chaganti for the JISE cosmid, which contains the human c-myc gene.

REFERENCES

1. Sobin LH (1982): The World Health Organization's Histological Classification of Lung Tumors: A comparison of the first and second editions. Cancer Detect Pre 5:391–406.
2. Boring C, Squires T, Tong T (1993): Cancer statistics. CA Cancer J Clin 43:7–26.
3. "Cancer Facts and Figures, 1994" (1994): Atlanta, GA: American Cancer Society.
4. Bishop JM (1987): The molecular genetics of cancer. Science 235:305–311.
5. Fearson ER, Vogelstein B (1990): A genetic model for colorectal tumori genesis. Cell 61:759–767.
6. Minna J, Maneckjiee R, D'Amico D, Bader S, Bodner S, Broers J, Buchhagen D, Carbone D, Chiba I, Curiel D, Fedorko J, Jensen S, Knutsen T, Linnoila I, Mitsudomi T, Nan M, Oie H, Pass H, Russeu E, Steinberg S, Takahashi T, Unger T, Viallet J, Whang-Peng J, Gazdar A (1991): Mutations in dominant and recessive oncogenes and the expression of opoid and nicotine receptors in the pathogenesis of lung cancer. In Harlow E (ed): "Origins of Human Cancer: Comprehensive Review." Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, pp 781–789.
7. Lechner JF, LaVeck MA (1985): A serum free method for culturing normal human bronchial epithelial cells at clonal density. J Tissue Culture Methods 9:43–48.
8. Jetten AM, Shirley JE, Stoner GD (1986): Regulation of proliferation and differentiation of respiratory tract epithelial cells. Cell Biol Toxicol 2:53–62.
9. Reddel RR, Ke Y, Gerwin BI, McMenamin MG, Lechner JF, Su RT, Brash DE, Park JB, Rhim JS, Harris CC (1988): Transformation of human bronchial epithelial cells by infection with SV40 or adenovirus-12 SV40 hybrid virus, or transfection via strontium phosphate coprecipitation with a plasmid containing SV40 early region genes. Cancer Res 48:1904–1908.
10. Fang J, Cheng SJ, Mu JW (1992): Effect of growth factors on the growth of human bronchial epithelial cells and lung cancer cells (in Chinese). Carcinogen Teratogen Mutagen 4:7–10.
11. Seabright M (1971): A rapid banding technique for human chromosomes. Lancet 2:971–972.
12. Lu YJ, Dong XY, Guo SP, Ke Y, Cheng SJ (1996): Integration of SV40 at 12q23 in SV40-immortalized human bronchial epithelial cells. Carcinogenesis 17: 2089–2091.
13. Lu YJ, Dong XY and Guo SP, Zhang RG, Ke Y, Zhang LZ, Xu LH, Cheng SJ: 2q-, a nonrandom chromosomal abnormality in human nonsmall cell cancer. Carcinogenesis 17:1589–1593.
14. Lu YJ, Cheng SJ, Guo SP, Xu LH, Dong XY (1998): Detection by FISH of c-*myc* translocation in SV40T-transformed human bronchial epithelial cells and human lung cancer cell lines. Cancer Genet Cytogenet (in press).
15. Marcu KB, Bossne SA, Potel AJ (1992): Myc function and regulation. Annu Rev Biochem 6:809–860.
16. Sekido Y, Takahashi T, Makela TP, Dbata Y, Ueda R, Hida T, Hibi K, Shimokata K, Alitalo K, Takahashi T (1993): Complex intrachromosomal rearrangements in the process of amplification of the L-*myc* gene in small cell lung cancers and adjacent benign lung. Cancer Res 53:2379–2385.
17. Nichols J, Nimer SD (1992): Translocation factors, translocation, and leukemia. Blood 80:2953–2963.
18. Groce CM, Nowell PC (1985): Molecular basis of human B cell neoplasia. Blood 65:1–7.
19. Evan GI, Wyllie AH, Gilbert CS, Littlewood TD, Land H, Brooks M, Waters CM, Penn LZ, Handcock DC (1992): Induction of apoptosis in fribroblasts by c-myc protein. Cell 69:119–128.
20. Minnar JD (1993): The molecular biology of lung cancer pathogenesis. Chest 103:445s–456s.
21. Sato S, Nakamura Y, Tsuchiya E (1994): Differences of allelotype between squamous cell carcinoma and adenocarcinoma of the lung. Cancer Res 54:5652–5655.
22. Shiseki M, Kohono T, Nishikawa R, Sameshima Y, Mizoguchi H, Yokota J (1994): Frequent allelic losses on chromosome 2q, 18q and 22q in advanced non small cell lung carcinoma. Cancer Res 54:5643–5648.
23. Whang-Peng J, Kao SC, Lee EC, Bunn PA, Carney DN, Gazdar AF, Minna JD (1982): Specific chromosome defect associated with human small cell lung cancer; deletion 3p(14–23). Science 215:181–182.
24. Boldog FL, Gemmill RM, Wilke CM, Glover TW, Nillson AS, Chandrasekharappa SC, Brown RS, Li FP, Drabkin HA (1993): Positional cloning of the hereditary renal carcinoma chromosome translocation break point. Proc Natl Acad Sci USA 90:8509–8513.
25. LaForgia S, Morse B, Levy J, Barnea G, Cannizzaro LA, Li F, Nowell PC, Boghosian-Sell L, Flick J, Weston A, Harris CC, Drabkin H, Patterson D, Croce CM, Schlessinger J, Huebner K (1991): Receptor protein-tyrosine phosphatase gamma is a candidate tumor suppressor gene at human chromosome region 3p21. Proc Natl Acad Sci USA 88:5036–5040.

26. Tsuchiya E, Nakamura Y, Weng SY, Nakagawa K, Tsuchiya S, Sugano H, Kitagawa T (1992): Allelotype of non small cell lung carcinoma comparison between loss of heterozygosity in squamous cell carcinoma and adenocarcinoma. Cancer Res 52:2478–2481.
27. Douglass EC, Valentine M, Etcubanas E, Parham D, Webber BL, Houghton PJ, Green AA (1987): A specific chromosome abnormality in rhabdomyosarcoma. Cytogenet Cell Genet 45:148–155.
28. Haglund U, Juliusson G, Stellan B, Gahrton G (1994): Hairy cell leukemia is characterized by clonal chromosome abnormalities clustered to specific regions. Blood 83:2637–2645.
29. Kohno T, Morishita K, Takano H, Shapiro DN, Yokota J (1993): Homozygous deletion at chromosome 2q33 in human small cell lung carcinoma identified by arbitrary primed PCR genomic fingerprinting. Oncogene 9:103–108.
30. Lukeis R, Ball D, Irving L, Garson OM, Hasthorpe S (1993): Chromosome abnormalities in nonsmall cell lung cancer pleural effusions: Cytogenetic indicators of disease subgroups. Genes Chromosomes Cancer 8:262–269.
31. Testa JR, Siegfried JM, Liu Z, Hunt JD, Feder MM, Litwin S, Zhou JY, Tagushi T, Keller S (1994): Cytogenetic analysis of 63 nonsmall cell lung carcinomas: Recurrent chromosome alterations amid frequent and wide spread genomic upheaval. Genes Chromosomes Cancer 11:178–194.
32. Olopade OI, Buchhagen DL, Malik K, Sherman J, Nobori T, Bader S, Nau MM, Gazdar AF, Minna JD, Diaz M (1993): Homozygous loss of the interferon genes defines the critical region on 9p that is deleted in lung cancers. Cancer Res 53:2410–2415.
33. Center R, Lukeis R, Dietzsch E, Gillespie M, Garson OM (1993): Molecular deletion of 9p sequences in nonsmall cell lung cancer and malignant mesothelioma. Genes Chromosomes Cancer 7:47–53.
34. Kamb A, Gruis N, Weaver-Feldhaus J, Liu QY, Harshman K, Tavtigian SV, Stockert E, Day RS III, Johnson B, Skolinick MH (1994): A cell cycle regulator potentially involved in genesis of many tumor types. Science 264:436–440.
35. Washimi O, Nagatake M, Osada H, Veda R, Koshikawa T, Seki T, Takahashi T (1995): In vivo occurrence of P16(MST1) and p15(MST2) alterations preferentially in nonsmall cell lung cancer. Cancer Res 55:514–517.
36. Dong XY, Lu YJ, Guo SP, Gao SG, Cheng SJ (1996): The establishment and application of the dual color fluorescence in situ hybridization. Acta Academiae Medicinae Sinicae 18:319.
37. Serrano M, Hannon GJ, Beach D (1993): A new regulatory motif in cell cycle control causing specific inhibition of cyclinD/CDK4. Nature 366:704–707.
38. Kucherlapati R, Hwang SP, Shimizu N, McDougall JK, Botchan MR (1978): Another chromosomal assignment for a simian virus 40 integration site in human cells. Proc Natl Acad Sci USA 75:4460–4464.
39. Wright WE, Pereira-Smith OM, Shay JW. Reversible cellular senescence (1989): Implications for immortalization of normal human diploid fibroblasts. Mol Cell Biol 9:3088–3092.
40. Croce CM, Girardi AJ, Koprowski H (1973): Assignment of the T-antigen gene of simian virus 40 to human chromosome C-7. Proc Natl Acad Sci USA 70:3617–1620.
41. Kao C, Wu SQ, DeVries S, Reznikoff WS, Waldman FM, Resnikoff CA (1993): Carcinogen induced amplification of SV40 DNA insert at 9p12–21.1 associated with chromosome breakage, deletions, and translocations in human uroepithelial cell transformation in vitro. Genes Chromosomes Cancer 8:155–166.
42. Macaulay VM (1992): Insulin like growth factors and cancer. Br J Cancer 26:311–320.
43. Porcu P, Ferber A, Pietrzkowski Z, Roberts CT, Adamo M, Leroith D, Baserga R (1992): The growth stimulatory effect of simian virus 40T antigen requires the integration of insulin like growth factor I with its receptor. Mol Cell Biol 12:5069–5077.
44. Marx J (1993) Cell death studies yield cancer clue. Science 259:760–761.
45. Wang H, Chen L, Lin LM, Han MJ, Sun HX, Dai JW, Zhao CX, Cheng SJ (1996): Expression of transforming factor α and p53 in non small cell lung cancer by immunohistochemistry study. Chin J Oncol 18:84–87.