

Taylor & Francis
Taylor & Francis Group

Cancer Biology & Therapy

ISSN: 1538-4047 (Print) 1555-8576 (Online) Journal homepage: https://www.tandfonline.com/loi/kcbt20

# Detection of deletions and/or amplifications of genes related with lung cancer by multiplex ligation-dependent probe amplification (MLPA) technique

Emre Tepeli, Muhammed Hamza Muslumanoglu, Ahmet Uludag, Nur Buyukpinarbasili, Muhsin Ozdemir, Murat Oznur, Huseyin Aslan & Sevilhan Artan

**To cite this article:** Emre Tepeli, Muhammed Hamza Muslumanoglu, Ahmet Uludag, Nur Buyukpinarbasili, Muhsin Ozdemir, Murat Oznur, Huseyin Aslan & Sevilhan Artan (2009) Detection of deletions and/or amplifications of genes related with lung cancer by multiplex ligation-dependent probe amplification (MLPA) technique, Cancer Biology & Therapy, 8:22, 2160-2164, DOI: 10.4161/cbt.8.22.9840

**To link to this article:** https://doi.org/10.4161/cbt.8.22.9840

Published online: 15 Nov 2009.

Submit your article to this journal

Article views: 175

View related articles

Citing articles: 4 View citing articles

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=kcbt20

Cancer Biology & Therapy 8:22, 2158-2163; November 15, 2009; © 2009 Landes Bioscience

# Detection of deletions and/or amplifications of genes related with lung cancer by multiplex ligation-dependent probe amplification (MLPA) technique

Emre Tepeli,[1,*] Muhammed H. Muslumanoglu,[1] Ahmet Uludag,[1] Nur Buyukpinarbasili,[2] Muhsin Ozdemir,[1] Murat Oznur,[1] Huseyin Aslan[1] and Sevilhan Artan[1]

[1]Eskisehir Osmangazi University School of Medicine Department of Medical Genetics; Eskisehir, Turkey; [2]T.C Ministery of Health Yedikule Pulmonary Medicine Hospital Department of Pathology; Istanbul, Turkey

**Key words:** lung cancer, molecuar genetics, MLPA, amplification, deletion, tumorsoppressor, oncogene

Background: Lung cancer has the leading mortality rate among all cancers and it is the second most common cause of death following cardiovascular diseases.
The aim of the study was determining deleted and/or amplified regions of 64 different loci previously associated with lung cancer, by using Multiplex Ligation-dependent Probe Amplification (MLPA).
Results: The most frequently seen deletions in lung cancerous tissues were in 2p, 3p, 13q, 17p, 16p and the most frequently seen amplifications were in 17q, 8p and 5q. We observed same deletions in the same regions in normal lung tissues as in cancerous tissues in lower frequencies. Deletions in 5q, 8p, 9q, 10p, 11p. 11q, 12p, 14q, 17q and 21q probe regions were seen especially in cancerous tissues.
Materials/methods: One hundred non small cell lung cancer (NSCLC) tissue samples which had been previously examined histopathologically were included in this investigation. DNA extracts of normal lung tissues from the same patients were used as control group in the study.
Conclusions: As a conclusion, it was determined that MLPA is an alternative technique which can give cheap, fast and reliable results in the screening of lung cancers. The findings obtained in the study are compatible with the literature. MLPA is one of the most important molecular techniques which have been developed recently and it can be used in cancer screening easily and reliably.

This manuscript has been published online, prior to printing. Once the issue is complete and page numbers have been assigned, the citation will change accordingly.

## Introduction

Lung cancer has the leading mortality rate among all cancers and it is the second most common cause of death following deaths from cardiovascular diseases.[1,2] Although it is generally accepted that the tobacco smoke causes lung cancer, not everyone who smokes develops lung cancer. Epidemiologic studies have shown that smokers are 14 times more likely to develop lung cancer than nonsmokers, but only about 11% of heavy smokers develop lung cancer in their lifetime. As a result, some have suggested that genetic factors may predispose people to lung cancer development.[3-5]

Extensive molecular genetic studies of lung cancer have shown that clinically overt lung cancers have multiple genetic and epigenetic alterations (%20 per tumor). In addition, several studies have demonstrated that the preneoplastic cells and the histologically normal bronchial epithelium harbor many of these abnormalities, which has suggested that human lung cancer develops from normal epithelial cells through a multistep process involving successive genetic and epigenetic abnormalities, usually accompanied with cigarette smoking.[6,7] These abnormalities contribute to the initiation, development, and the maintenance of lung cancer.

There were a lot of studies about dominant oncogenes like *MYC* and *RAS* and some tumor suppressor genes like *TP53* and *RB*. Additionally, in the studies of loss of heterozygosity in chromosome 2q, 3p, 5q, 9p, 11p, 13q, 17p, 18q and 22q due to deletions have been demonstrated. Also genomic instability seen in lung cancers may include lots of structural anomalies like deletions, amplifications and translocations.[8,9]

Recently, MLPA has proved to be a reliable tool for the diagnosis of genetic diseases characterized by gene deletions and amplifications.[10] This system allows the simultaneous hybridization and ligation of several probes in a single reaction tube, followed by PCR amplification and analysis by capillary electrophoresis. In MLPA technique only 20 ng DNA is enough for reactions, one reaction provides information on up to 45 targets and can distinguish sequences that differ in only a single nucleotide, and is able to detect small copy number differences.

*Correspondence to: Emre Tepeli; Email: emre_tepeli@yahoo.com
Submitted: 08/04/09; Accepted: 08/18/09
Previously published online: www.landesbioscience.com/journals/cbt/article/9840

**Table 1.** Disrtubition of patients among to grade

| Stage | n | % |
|---|---|---|
| Grade I | 2 | 2 |
| Grade II | 12 | 12 |
| Grade III | 86 | 86 |
| Total | 100 | 100 |

**Table 2.** Disrtubition of patients among to TNM

| TNM clasifications | n | % |
|---|---|---|
| T3N1M0 | 32 | 32 |
| T2N1M0 | 26 | 26 |
| T2N0M0 | 10 | 10 |
| T3N0M0 | 10 | 10 |
| T4N1M0 | 6 | 6 |
| T2N2M0 | 4 | 4 |
| T4N0M0 | 4 | 4 |
| T3N2M0 | 2 | 2 |
| T2N0M1 | 2 | 2 |
| T3N2M1 | 2 | 2 |
| T4N2M0 | 2 | 2 |
| Total | 100 | 100 |

This study was aimed to determine deleted and/or amplified regions of 64 different loci previously associated with lung cancer by using MLPA technique and SALSA MLPA ProbeMix 126/127 kits were used which were designed specifically for lung cancer.

## Results

**Clinical, demographic and pathological characteristics of patients.** Sixty eight males and 32 females in the study as patients. The mean age of the patients was 58, 98. Fifty four of the patients were adenocarcinomas (ADC) cases and 46 of the patients were squamous cell carcinomas (SCCs) cases. Ninety percent of the patients were smokers and %26 had cancer history in their families. In **Tables 1 and 2** the distribution of the patients according to the grade and the TNM classification have shown.

**MLPA findings.** Images of MLPA analysis and chromosomal locations are shown in **Figures 1 and 2**.

DNA samples isolated from healthy lung tissues near cancer lesions and cancer tissues both from the same patients analyzed and results are shown in **Table 3**.

According to these results, most frequent deletions detected in 2p, 3p, 13q, 17p, 16p regions, and amplifications were in 17q, 8p, 5q regions. No deletions and amplifications detected in short arm of the chromosome 9. In healthy lung tissues near cancer lesions similar results obtained. But in only one patient 17q amplification was detected.

In **Figure 3**, the image of the analysis belongs to a patient with 2p, 9p and 13q deletions is shown.

## Discussion

Cancer is one of the most important health problems of today. Its high frequency and mortality have caused an increase in cancer research. In many studies made until now to clarify the genetic etiology of lung cancer, different techniques and tissue types have been used, many genes that have been related with lung cancer have been defined and still continue to be defined. That is why genomic alterations seen in lung cancer rest on a fairly wide scale. These alterations range from single nucleotide alteration to chromosomal gains or losses. That is why it is very difficult to analyze the genetic etiology of lung cancer.

In our study, alterations in chromosome 2p, 3p, 5q, 8p, 9p, 9q, 10p, 11p, 11q, 12p, 13q, 14q, 16p, 17p, 17q and 21q regions were analyzed by MLPA method. As a result of these analyses, especially the deletions of tumoral tissue at 2p, 3p and 13q conspicuous. Even though there were many studies made using the MLPA method, there hasn't been any publication for lung cancer yet. In this sense, our study was the first of its kind. Our datas were compared with results obtained from printed studies which used different techniques.

In a study made by Yan et al. in 2005, they have analyzed the genomic alterations in lung tumor tissues by using Comparative Genomic Hybridization (CGH) and have determined deletions at especially 1p, 2q, 3p, 4p, 4q, 6p, 6q, 8p, 13q, 13p and 16p chromosomal regions. They showed the most frequent alterations among these regions in 3p with a ratio of 56.4%, 5q with 53.8% and 13q with 51.3%.[11] In another study published by Dacic et al. in 2005, they analyzed alterations by using microsatellite markers in 1p, 3p, 5q, 9p, 9q, 10q, 17p and 22q regions in DNA samples obtained from the paraffin tissue of 20 patients with lung adenocarcinoma and determined the heterozygosity loss in especially the 3p region.[12] Yoshino I et al. researched heterozygosity by using microsatellite markers loss in 49 lung cancer patients with adenocarcinoma, 22 lung cancer patients with squamous cell carcinoma and *FHIT*, *TP53*, *CDKN2*, *HMLH1*, *HMSH3* genes. Heterozygosity loss was observed in 67% of patients with adenocarcinoma and 90% of patients with squamous cell carcinoma.[13]

Chromosome 2p and 3p are the most sensitive regions in terms of genomic instability.[14] In many studies performed on patients, more deletions have been determined in these areas compared with other chromosomal regions. In these studies, deletion frequencies varying between 25–75% at 2p and/or 3p have been observed. When these observed deletions compared with the clinical and demographical properties of the patient, it was determined that they are more commonly seen in especially early stage tumors and long-term smokers. These data led us to think that the genetic alterations that trigger carcinogenesis could be the deletions at 2p and 3p. In the epigenetic studies, it has been shown that beside the increase in the CpG region methylation during the start of carcinogenesis, an overall methylation decrease was observed in the whole genome. Increasing DNA hypomethylation causes the chromatin structure more sensitive thereby laying the groundwork for genomic alterations. The high ratio of deletions at the 2p and 3p chromosomal regions, which are the most sensitive to genomic instability, is an indicator that epigenetic alterations play an important role in the carcinogenesis process. Again the tumor suppressor genes in 2p and 3p regions are the

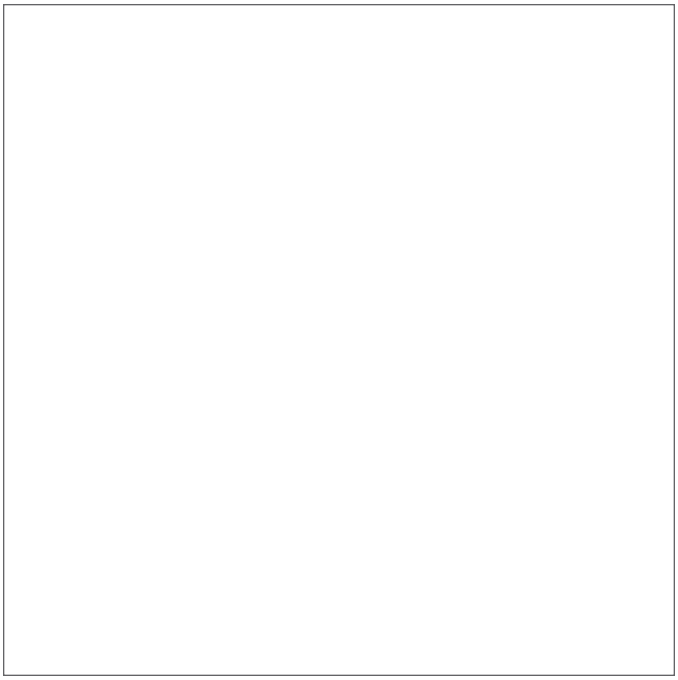


**Figure 1.** AUTHOR: please provide figure legend.

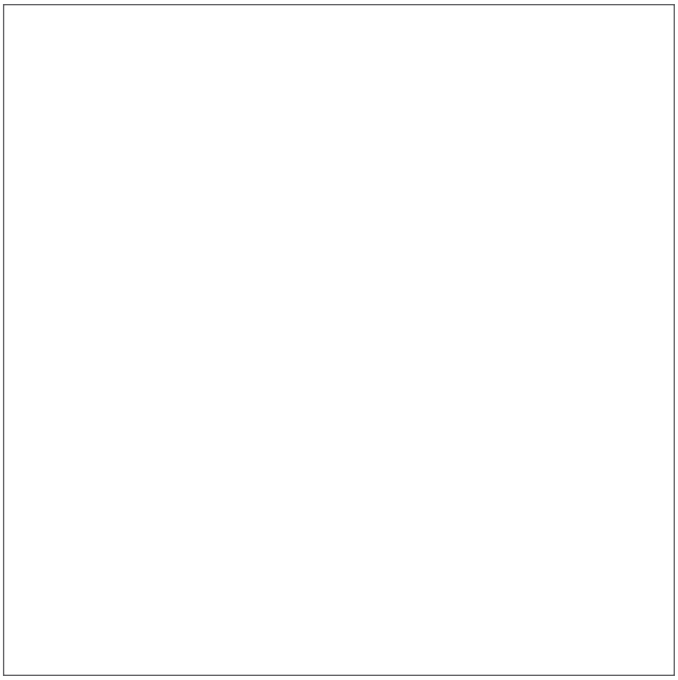


**Figure 2.** AUTHOR: please provide figure legend.

AUTHOR: please provide Figures 1–3.

main reasons of these alterations. Especially *FHIT* gene is one of the important tumor suppressor gene candidates in lung cancer.

The data obtained in our study was found to be in compliance with literature. Among 100 tumor tissues 2p deletion was seen in 72 patients (72%) and 3p deletion was seen in 64 patients (64%). Again, in the neighboring lung tissues that did not contain tumor tissue, 2p deletion was observed in 12 cases (12%) and 3p deletion was observed in again 12 cases (12%). The fact that these deletions were observed in healthy tissues not containing tumor tissue led us to think that there were genomic alterations in these tissues however there hasn't been any neoplastic alteration yet. Because in the tumoral areas of the healthy tissue samples that we studied, these alterations were observed. When the histopathological and demographic properties of patients with 2p and 3p deletions were compared, it was determined that the ratio of 3p deletion in squamous cell carcinomas was statistically significantly higher compared with adenocarcinomas ($p = 0.01$). This data was found to be in compliance with literature as well.

In our study, 13q deletion had the highest rate (76%). In literature there weren't many studies that analyzed 13q alterations in lung cancer cases. In the specific studies, alterations in chromosome 13 are related with prostate and breast cancers.[15] Whereas in studies aimed at lung cancer, loss of heterozygosity has been analyzed and losses of 40–80% ratios have been observed. Losses of 13q12.2, 13q14.1 and 13q22 chromosomal regions were more frequent in NSCLCs. In addition to this some tumor suppressor genes such as *BRCA2* are settled in 13q12.1 region. However the role of these genes in NSCLC is not clear yet. In the study performed by Kwong F, et al. in 2002, the allelic loss in 13q14.3 region, where *RB1* gene is also located at, was found to be 16.7% which was less than the expected ratio. Contrary to this study, Reismann showed that *RB1* gene is inactive in NSCLSs.[16] In addition to this, *DICE1* gene localized at D13S284 microsatellite region, which is similar to *RB1* gene, was also shown among the candidate tumor suppressor genes which are planned for research. In our study these regions were analyzed and a high ratio of deletions of 76% was observed. Because there aren't many studies performed related to these regions in lung cancer, we were not able to compare our data as we would have liked.

In our study, another anomaly which was frequently (36%) observed especially in afflicted tissues was the 17p chromosome deletion. It has been reported that the *TP53* tumor suppressor gene localized at chromosome 17p plays an important role in lung cancer as well as in many other cancer types. *TP53* gene is the highest observed mutant gene in human cancers and is localized in 17p13 locus. Whereas the mutations of this gene are observed in 50% of all cancers, it is observed in 90% of NSCLCs, in 65% of squamous cell cancer, in 60% of large cell cancers and in 33% of adenocancers.[17-22] In our study whereas 17p deletion was observed in 40.7% of the 55 adenocarcinoma cases, this ratio was 34.7% for our squamous cell carcinoma cases. No statistically significant difference was observed among these two groups for the frequency of 17p deletion.

Another chromosomal region associated with lung cancer is 11q region. 11q13 chromosome anomalies are related with the tumor increase in Multiple Endocrine Neoplasia Type 1 (MEN1) patients. An increase in the frequency of loss of heterozygosity in lung adenocarcinomas in 11q22-24 region has been shown.[23] This revealed the existence of 3 possible tumor suppressor genes. It is thought that another tumor suppressor gene related with the development and advancement of lung cancer is localized in 11q23 region. In a study published by Peltzmann S, et al. in 2001, by microsatellite marker aimed at 11q region in lung carcinoid cases and determined loss of heterozygosity in this region in 65%

**Table 3.** All MLPA results including cancer tissue and healty lung tissues near cancer lesions

| Chromosomal region | Tumoral tissue | | Normal lung tissue | |
|---|---|---|---|---|
| | Deletion (%) | Amplification (%) | Deletion (%) | Amplification (%) |
| 2p | 72 | - | 12 | - |
| 3p | 64 | - | 12 | - |
| 5q | 12 | 3 | 2 | - |
| 8p | 8 | 4 | 2 | - |
| 9p | - | - | 2 | - |
| 9q | 16 | - | - | - |
| 10p | 22 | - | 6 | - |
| 11p | 2 | - | 2 | - |
| 11q | 20 | - | 2 | - |
| 12p | 20 | - | 6 | - |
| 13q | 76 | - | 14 | - |
| 14q | 4 | - | - | - |
| 16p | 30 | - | 2 | - |
| 17p | 38 | - | 4 | - |
| 17q | 6 | 9 | 2 | 1 |
| 21q | 16 | - | 4 | - |

©2009 Landes Bioscience
Do not distribute

**Figure 3.** AUTHOR: please provide figure legend.

of the analyzed patients.[23] In our study, the ratio of 11q deletions among the analyzed afflicted tissues was observed to be 20%. This ratio was a lot less when compared with studies in literature. Because in the literature, the 11q alterations were studied on a much more specific group, especially in neuroendocrine tumors of the lung. That was why we thought that slightly higher ratios have been observed.

In the studies made until now, it has been determined that the polymorphisms on the p arm of chromosome 12 are related with the lung cancer development and prognosis.[24] The most important gene for the tumor development localized on 12p is *KRAS*. In the study published by Lei J, et al. in 2003, they have studied the chromosome 12p region in lung adenocarcinoma cases and have determined loss of heterozygosity in 12p region in approximately 50% of the tissues they analyzed. Also, besides finding the *KRAS2* mutation ratio as 40% in this publication, they have determined that all the patients having this mutation also have loss of heterozygosity on 12p. Again in this publication it has been claimed that *KRAS2* is a strong tumor suppressor gene for lung cancer.[24] In our study we analyzed different probe regions in 12p region and observed deletion in 20% of the tumor tissues. Even though there was no probe designed specifically for *KRAS* gene among the probemixes that we used, we thought that there might be a deletion in this gene as well due to the fact that we observed losses in different regions.

In the cytogenetic studies and loss of heterozygosity analyses, 21q deletion was frequently observed in NSCLC.[25,26] This situation indicated that there might be tumor suppressor genes in 21q region. However, since information about this region is not clear, the tumor suppressor genes that were thought to be in this region were not clearly defined. In the study made by Lee EB et al. on the heterozygosity loss in chromosome 21 in NSCLC patients, they have observed the loss of heterozygosity in at least one of the regions of 21q21.2, 21q21.3 and 21q22.1 in 56.4% of the patients. In a similar study made by Takashi K, et al. they observed a heterozygosity loss in 21q region with a 51.1% ratio.[27] The 16% ratio of 21q deletion was found to be smaller compared with some studies in literature. The basis for the difference between results obtained from our study and other studies in literature could be the difference of the method used, the diversity of the number

of patients, the different clinical, histopathological, demographic properties of the patients studied and the monoclonality observed in the cancer cells.

The P126 and P127 kits designed by MLPA for lung cancer and used in our study were designed to determine larger genomic alterations in lung cancer. The chromosomal region alterations that stand out in literature were considered while preparing the probes. Even though the MLPA method is sensitive enough to determine even single base alterations, the design of these kits are not ordinarily target the gene but the chromosomal alterations. The most important reason for this is more desirable to scan more regions in one study. The technique had kits aimed at different tumor suppressor genes that can analyze the gene alterations more broadly. That is why the results obtained are more superficial. Also even though there are many more chromosomal regions associated with lung cancer, these regions are not present in the kit. Among these regions 1p, 1q, 4p, 4q, 6p, 6q, 8q, 16p and 18p can be given as examples. But still, important alterations related with lung cancer can be analyzed with the present kit.

Some disadvantages of the method can be listed as the fact that the results are directly related with the sample quality, that it can not scan triploidies and balanced translocations and that it can not distinguish mosaic situations.

As a result, most of the genomic alterations in lung cancer could be analyzed by MLPA method. And this might be done with low cost and in a short amount of time. By using the data obtained, gene specific alterations could be analyzed later by using different kits and more detailed results might be obtained. Also additional probes colud be prepared for the regions already in the kit thereby increasing the analyzed regions. MLPA method is one of the most important molecular techniques developed in recent years and it is a method that might be used very easily in the genomic scan of cancer as well. The data we obtained would be a source for many researchers who would perform similar studies.

## Materials and Methods

**Study population and specimens.** This study was performed by analyzing 100 archival samples which had been previously examined histopathologically and diagnosed as "lung cancer" in Istanbul Yedikule Hospital of Chest Medicine. The sample DNA's have extracted from 5 micron paraffin-embedded tissue sections which have contained cancerous tissues were used as sample group and 5 micron paraffin-embedded tissue sections which have contained normal lung tissues from the same patients have been used as control group in the study. Tumor histology has determined according to the World Health Organization (WHO) classification of lung tumors.

**Genomic DNA isolation from paraffin blocks.** For genomic DNA extraction from paraffin blocks, formalin fixed, paraffin wax embedded tissues have been cut into 5 micron sections. Microdissected tissues have been collected in 15-mL centrifuge tubes and deparaffinized by using xylene. Deparaffinized tissues have been homogenized overnight at 56ºC in proteinase K, and genomic DNA has been isolated by using Roche Pure Compact Nucleic Acid Isolation Kit I, by Roche Magna Pure Compact DNA Extraction Robot according to the manufacturer's instructions.

**MLPA analyses.** MLPA has performed by using the SALSA MLPA lung cancer P126/ P127 kit (MRC-Holland Amsterdam, The Netherlands). Genomic DNA, 200–300 ng, has been diluted in 5 µl Tris-EDTA buffers, and the MLPA analysis has been performed according to the manufacturer's instructions with minor modifications. In every MLPA run, 4 DNA samples of controls which were isolated from healthy, non smoker, no cancer history in family, no known carcinogen exposion people included to study as internal control as MLPA technique offers. The hybridization time was 16 hours, and the number of PCR cycles was 36. The samples were analyzed by using a capillary electrophoresis system (ABI 310 Genetic Analyzer, Applied Biosystems), and Genescan software (Applied Biosystems) was used to quantify the generated peaks. Specific peaks corresponding to the different telomeric regions were identified according to their migration relative to a size standard and subsequently exported to a Microsoft Excel spreadsheet. After all the results analyzed by an excel based Diagenalayzer programme.


### Acknowledgements

This study had been supported by Eskisehir Osmangazi University.


### References


1. Jerzy E. Tyczynski, Freddie Bray, Maxwell Parkin D. Lung cancer in Europe in 2000: epidemiology, prevention and early detection. Lancet Oncol 2003; 4:45-55.
2. Parkin DM, Bray FI, Devesa SS. Cancer burden in the year 2000. The global Picture. Eur J Cancer 2001; 37:4-66.
3. Angela Risch, Christoph Plass. Lung cancer epigenetics and genetics. Int J Cancer 2008; 123:1-7.
4. Mattson ME, Pollack ES, Cullen JW. What are the odds that smoking, will kill you? Am J Public Health 1987; 77:425-31.
5. Peto R, Darby S, Deo H, Silcocks P, Whitley E, Doll R. Smoking, smoking cessation and lung cancer in the UK since 1950: combination of national statistics with two case-control studies. BMJ 2000; 321:323-9.
6. Lung Cancer. Treatment Guidelines For Patients Version III/October 2006, American Cancer Society 2006.
7. Kerynne L. Braithwaite, Pamela H, Rabbitts U. Multistep evolution of lung cancer. Semin Cancer Biol 1999; 9:255-65.
8. Akbulut H, Akbulut KG. Karsinogenez, ANTIPAS. Tıp Kitapları ve Bilimsel Yayınlar, Ankara 1997; 23-38.
9. Fong KM, Minna JD. Molecular biology of lung cancer: clinical implication. Clinics In Chest Medicine 2002; 23:83-101.
10. Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. Nucleic Acids Res 2002; 30-57.
11. Yan WS, Song LY, Wei WD, Li A, Liang QW, Liu JH, et al. Chromosomal inbalance in primary lung squamous cell carcinoma and their relationship with smoking. Ai Zheng 2005; 24:47-52.
12. Dacic S, Ionescu DN, Finkelstein S, Yousem AS. Patterns of allelic loss of synchronous adenocarcinomas of the lung. Am J Surg Pathol 2005; 29:897-902.
13. Yoshino I, Osoegawa A, Yohena T, Kameyama T, Oki E, Maehara Y. Loss of Heterozygosity in non small cell lung cancer: differenece between adenocarcinoma and squamous cell carcinoma. Respir Med 2005; 99:308-12.
14. Pifarre A, Rosell R, Monzo M, De Anta JM, Moreno I, Sanchez JJ, et al. Prognostic Value of replication errors on chromosomes 2p and 3p in non small lung cancer. Br J Cancer 1997; 75:184-9.
15. Kwong FM, Wong SP, Lung ML. Genetic alterations detected on chromosomes 13 and 14 in Chinese non small cell lung carcinomas. Cancer Lett 2003; 192:189-98.
16. Reissmann PT, Koga H, Takahashi R, Figlin RA, Holmes EC, Piantadosi S, et al. Inactivation of the retinoblastoma susceptibility gene in non small cell lung cancer. Oncogen 1993; 8:1913-9.
17. Fong KW, Sekido Y, Minna JD. Molecular pathogenesis of lung cancer. J Thorac Cardiovasc Surg 1999; 118:1136-52.
18. Groeger AM, Odocha O, Mueller MR. Racial variation in lung cancer. Anticancer Res 1997; 17:2843-8.

19. Hussain SP, Haris CC. Molecular epidemiology and carcinogenesis: endogenous and exogenous carcinogens. Mutat Res 2000; 311-22.
20. Levin WJ, Casey G, Ramos JC. Tumor suppressor and immediate early transcription factor genes in nonsmall cell lung cancer. Chest 1994; 106:372-6.
21. Spivack SD, Fasco MJ, Walker VE, Kaminsky LS. The molecular epidemiology of lung cancer. Crit Rev Toxicol 1997; 27:319-65.
22. Tchia MM, Holmes MD, McLennan G. The molecular biology of lung cancer. Med J Aust 1991; 154:501-3.
23. Petzmann S, Ullmann R, Klemen H, Renner H, Popper HH. Losss of heterozygosity on chromosome arm 11q in lung carcinoids. Hum Pathol 2001; 32:333-8.
24. Manenti G, Gregorio DD, Pilotti S, Falvella FS, Incarbone M, Ravagnani F, et al. Association of chromosome 12p genetic polmorphism with lung adenocarcinoma risk and prognosis. Carcinogenesis 1997; 18:1917-20.
25. Sato S, Nakamura Y, Tsuchiya E. Difference of allelotype between squamous cell carcinoma and adenocarcinoma of the lung. Cancer Res 1994; 54:5652-5.
26. Testa JR, Siegfried JM, Lin Z, Hunt JD, Feder MM, Litwin S, et al. Cytogenetic analysis of 63 nonsmall cell lung carcinomas: Reccurent Chromosomes alterations amid frequent and widespread genomic upheaval. Genes Chromosomes Cancer 1994; 11:178-94.
27. Takashi K, Kawanishi M, Matsuda S, Ichikawa H, Takada M, Ohki M, et al. Homozygous deletion and frequent allelic loss of the 21q11.1-q21.1 region including the ANA gene in human lung carcinoma. Genes Chromosomes Cancer 1998; 21:236-43.

©2009 Landes Bioscience.
Do not distribute.