[Frontiers in Bioscience E4, 865-875, January 1, 2012]

Molecular biomarkers of lung carcinoma

Bozica Vrabec Branica[1], Srecko Gajovic[2]

[1]Department of Pathology and Cytology, University Hospital of Zagreb, Jordanovac 104, 10000 Zagreb, Croatia, [2] University of Zagreb School of Medicine, Croatian Institute for Brain Research, Salata 12, 10000 Zagreb, Croatia

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Types of lung carcinoma molecular biomarkers
3.1. DNA-based lung carcinoma molecular biomarkers
3.1.1. Genetic biomarkers
3.1.2. Epigenetic biomarkers
3.1.3. Chromosomal changes
3.2. RNA-based lug carcinoma biomarkers
3.3. Protein-based lung carcinoma biomarkers
3.4. Metabolomics
4. Applications of lung carcinoma molecular biomarkers
4.1. Lung carcinoma molecular biomarkers in detection of precancerous lesions and early carcinomas
4.2. Lung carcinoma molecular biomarkers as potential therapeutic targets
5. Lung carcinoma molecular biomarkers in various specimens
6. Various technologies in lung carcinoma molecular biomarkers
7. Our experience in PCR applications for detection of lung carcinoma biomarkers in cytology specimens
8. Perspective
9. Acknowledgements
10. References

1. ABSTRACT

Lung carcinoma is still the leading cause of cancer deaths in men and women. There is a constantly increasing need to find molecular biomarkers for lung cancer which can be used for risk stratification, early detection, treatment selection, prognosis and monitoring for recurrence. Recent advances in imaging and improved bronchoscopic techniques have intensified interest in lung carcinoma screening techniques, especially in new molecular markers which can help cytopathologists to make a definitive diagnosis on very small specimens in non-invasive, non-expensive, simple and efficient manner. Several decades of intensive research have originated numerous potential lung carcinoma molecular biomarkers but only few turned out to be useful in clinic. The review describes types of biomarkers, sources and techniques for their identification in cancer diagnosis and therapy. A deep understanding of each biomarker will be a key to efficiently diagnose lung carcinomas and direct patients toward beneficial drugs based on individual patient profile.

2. INTRODUCTION

The completion of human genome project expanded our knowledge of the pathogenesis of lung cancer at the molecular level while the recent advances in imaging and bronchoscopic techniques stirred intense interest for lung carcinoma molecular biomarkers (LCMB) in order to detect and recognize very small lung lesions. The cytopathologists find themselves being pressured to make a definitive diagnosis on very small specimens and to investigate different molecular markers which would serve as predictors of response to therapy in lung carcinomas.

3. TYPES OF LUNG CARCINOMA MOLECULAR BIOMARKERS

3.1. DNA-based lung carcinoma molecular biomarkers

DNA-based molecular biomarkers include detection of genetic, epigenetic and chromosomal changes as the main targets for biomarker discovery in tumor cells.

3.1.1. Genetic biomarkers

Detection of genetic alterations in the multistep process of carcinogenesis includes detection of mutations which lead to activation of oncogenes or inactivation of tumor-suppressor genes and subsequent uncontrolled cell growth development of cancerous cells (1). The genetic mutations most commonly studied as possible lung cancer biomarkers are located in *K-ras, p53, Rb* and *myc* gene. *K-ras* gene mediates signals from growth factor receptors and other signaling inputs. The mutations convert normal K-ras proto-oncogene to an oncogene, causing the oncoprotein to become overactive in transmitting growth factor-initiated signals (2). Mutations occur most often in exon 12. They are detected in 30% of lung adenocarcinoma in smokers and they are correlated with a poor prognosis (3-5). Both *Rb* and *p53* have critical roles in the control of cell cycle, and their abnormalities are the most frequently associated with cell cycle dysregulation in malignancy (6). The well-known „guardian of the genome", *p53* induces apoptosis and reduces phosphorylation of retinoblastoma (Rb) protein, which leads to cell cycle arrest at the G1-S check point. When this tumor-suppressor gene is deleted or inactivated, cells cannot control DNA repair and undergo aberrant growth. Mutations of *p53* are present in 50% of non-small cell lung carcinoma (NSCLC) and 75% of small cell lung carcinoma (SCLC) (7-11). *Rb* is inactivated in more than 80% of high-grade neuroendocrine lung tumors, especially SCLC (1). Myc is located on chromosome 8 and has transcriptional regulation activity. Gene amplification analysis showed it is overexpressed in 10-40% of SCLC.

The loss of one allele or loss of heterozygosity (LOH) is another predisposing factor to lung cancer (12,13) causing the inactivation or silencing of genes critical for growth regulation and homeostasis. More than 90% of small cell carcinomas and more than 70% of NSCLCs demonstrate loss of heterozygosity (14-16). In NSCLC loss of heterozygosity generally involves genetic loci on chromosome arms 1p, 3p, 8p, 9p, 13q, 17p, 19p, Xp and Xq. In small cell carcinomas, loss of heterozygosity generally involves chromosome arms 3p, 4q, 5q, 10q, 13q, 15q and 17p (14-19).

Telomerase activation represents a potential candidate for lung cancer molecular biomarker (20). Telomere shortening is the earliest and most prevalent genetic change identified in the precursor lesion (21). Telomere attrition is thought to predispose to chromosome fusion and the resulting translocations, followed by their missegregation during mitosis. Later during tumorigenesis, telomerase activity is resumed, compensating the telomere erosive processes while still permitting continued chromosomal instability. The ability to image and quantitavely assess telomeres makes these alterations a potential diagnostic tool, particularly to characterize early or preinvasive lesions (22). Telomerase activity has been detected in samples of abnormal bronchial epithelium: hyperplasia (in 71%), metaplasia (in 80%), in situ carcinoma (in 100%). High levels of telomerase activity are seen in nearly all small cell lung carcinoma and in 80% to 85% of all non-small cell lung carcinoma (20).

3.1.2. Epigenetic biomarkers

Epigenetic changes like hypermethylation of CpG (cytosine phosphate guanosine rich regions) islands is a common mechanism by which tumor suppressor genes are inactivated in most tumors including lung carcinomas (23-30). Hypermethylation of gene promoter regions is associated with silencing of transcription and it affects genes involved in various aspects of normal cell function. Therefore it is a critical trigger for malignant tranformation and progression. Aberrant methylation of *p16INK4a*, *APC, TMS1, RASSF1,* and *DAPK* has been reported in lung carcinoma (31-36).

3.1.3 Chromosomal changes

Inactivation of tumor suppressor genes can be induced by chromosomal changes like chromosomal deletions or rearrangements accidently happening during cell division. The most frequently abnormality is deletion of the short arm of chromosome 3 (3p) where several tumor suppressor genes are present (37-40).

3.2. RNA-based lung carcinoma molecular biomarkers

Compared to DNA biomarkers, which are mostly evaluated individually, most RNA-based biomarkers undergoing clinical evaluation are screened as multi-gene molecular patterns or „fingerprints". This could be more accurate but selection of genes to include in the pattern needs new developments in biostatistics, bioinformatics and data visualization (41).

Non-coding RNAs (ncRNAs) are functional transcripts that do not code for proteins but play a major role in regulating almost every level of gene expression (42). They play an important role in tumorigenesis and could represent biomarkers for carcinoma (43). Except abundant and functionally important transfer of ribosomal RNAs, ncRNAs include other RNAs such as small nucleolar RNAs (snoRNAs), micro RNAs (miRNAs), short interfering RNAs (siRNAs), piwi-associated RNAs, small Cajal body-specific RNAs (scaRNAs), small nuclear RNAs (snRNAs) and long ncRNAs that are still partially understood. From the small ncRNAs, miRNAs and siRNAs have been extensively been studied in carcinogenesis (42,44-46). The expression of specific populations of miRNAs in a tissue- and time-dependent manner correlates with clinical characteristics for several cancer types including lung cancer (47-49). miRNAs expression profiles can be used to classify human cancers and to suggest a correlation between disease prognosis and therapeutic outcome. Investigation of metastasis-associated miRNA markers is expanding and these markers were referred to as metastamirs (50). miRNA can act as a tumor suppressor as well as an oncogene (51). For example, let-7 is a suppressor for RAS in lung cancer and mir17 and mir21 clusters modulate PTEN, TGFβ-RII and are oncogenic in lung cancers (52).

3.3. Protein-based lung carcinoma molecular biomarkers

Protein-based biomarkers are very important because proteins are the main executioner bio-molecules in cells (53-54). The biomarkers currently of interest for

clinical use are single protein molecules and most are serum-derived. They are carcinoembryonic antigen (CEA), CYFRA-21-1 and tissue polypeptideantigen (TSA) for non-small cell lung carcinoma and progastrin-relasing peptide (ProGRP) and neuron–specific enolase (NSE) for neuroendocrine lung carcinoma. There are also many potential lung carcinoma molecular biomarkers not yet available in clinical usage: serum amyloid A, haptoglobin-α-2, a fragment of apolipoprotein A-1 (APOE) and plasma kalikrein B1 (KLKB1) (55). Some proteins are important for prognosis and selection of therapy, for example epidermal growth factor receptor (EGFR). The altered activation of EGFR activates the Ras-Raf-MEK-MAPK pathway. Together with diagnostic purpose it is important for therapy with several drugs which target this pathway and are specific for EGF receptor (EGFR) (56-57). Another example is bcl-2 protein, which acts as apoptosis inhibitor and indicates aberrant growth of cells. Its high expression is detected in 10-25% of NSCLC.

A different approach is provided by global protein assessment or proteomic analyses. Proteomic technologies analyze body fluids for protein constituents and identify biomarkers for early detection of cancers. Traditional approach for quantitative protein profiling is based on two-dimensional electrophoresis for protein separation, followed by mass spectrometric identification of selected proteins or all proteins detected (58). Another approach for disease biomarker discovery is using proteomic pattern analysis. This approach primarily compares the pattern of signals observed within a mass spectrum to identify differentially abundant peaks within normal and disease samples for distinguishing of two groups (target and control).

3.4. Metabolomics

Metabolomics detect low molecular weight molecules or metabolites such as amino acids, peptides, lipids and carbohydrates (1). During the progression of some cancers, the expression of certain N-linked and O-linked glycans changes. These altered glycoforms can serve as biomarkers for cancer detection (59-61).

4. APPLICATIONS OF LUNG CARCINOMA MOLECULAR BIOMARKERS

4.1. Lung carcinoma molecular biomarkers in detection of precancerous lesions and early carcinomas

Morphology is today a gold standard in diagnosing premalignant squamous lesions which are goblet cell hyperplasia, basal cell hyperplasia, immature squamous metaplasia, mature squamous metaplasia, mild squamous dysplasia, moderate squamous dysplasia, severe squamous dysplasia, squamous carcinoma *in situ*. Several studies detected molecular characterization of the premalignant changes. The earliest changes are loss of heterozygosity at multiple 3p chromosome sites and loss of heterozygosity at site 9p21 (62-64). Later changes are alterations at 8p21-23, 13q14 and 17p13 (62). The pattern of *p53* expression in precursor lesions suggests gene alteration, but the detection of gene mutation seems to be more important (65-66). Loss of heterozygosity and *p53* changes can be seen as well in normal bronchial mucosa of smokers, so it probably indicates the smoking related genetic damage, which with multiple stepwise accumulations of the mutations can still develop into lung cancer. A similar observation was made for methylation of *p16INK4a* which is demonstrated in 75% of *in situ* carcinoma adjacent to invasive squamous cell carcinoma of the lung. The frequency of this event increases from basal cell hyperplasia (17%) to squamous metaplasia (24%) to carcinoma *in situ* and invasive carcinoma (50-75%) (67-68).

In glandular lesions, recently, atypical adenomatous hyperplasia (AAH) is considered to represent the adenoma in a putative “adenoma-carcinoma" sequence in the lung periphery leading to development of nonmucinous bronchoalveolar carcinoma and invasive peripheral adenocarcinoma. *K-ras* mutations are detected in 24% to 50% of lung adenocarcinomas and represent an early event that precedes malignant growth (69). Recent studies suggest AAH could develop by either *K-ras* or *EGFR* gene mutation pathways, but some AAHs harboring *K-ras* gene mutation do not progress further to an invasive cancer (70). Loss of heterozygosity on chromosome 3p (*FHIT* gene) and 9p (*p16* gene) have been identified in 18% and 13% of AAH p53 protein expression has been demonstrated with increasing frequency in the progression from AAH through bronchioalveolar carcinoma (BAC) to invasive carcinoma (71-72).

In neuroendocrine carcinoma of the lung currently there are no genetic markers that could separate neoplastic from nonneoplastic proliferations.

4. 2. Lung carcinoma molecular biomarkers as potential therapeutic targets

The most known molecular biomarkers used as predictors for therapy with tyrosine kinase inhibitors (gefitinib and erlotinib) are *epidermal growth factor receptor* (*EGFR*) and *K-ras*. *EGFR* is frequently overexpressed in non-small cell carcinoma (NSCLC) and development of small molecule inhibitor of EGFR resulted in clinical trials that have shown a good response in lung adenocarcinomas with somatic mutations in exons 18 to 21 of the tyrosine kinase domain of EGFR together with clinical characteristics like female sex, Asian ethnicity and absent or low smoking history (73-76). The *K-ras* mutations have been shown as negative prognostic factor and as predictors of failure of EGFR tyrosine kinase inhibitors therapy (73, 74, 76). Testing for *EGFR* and *K-ras* status in lung carcinoma involves a variety of different methodologies including DNA mutational analysis, fluorescence *in situ* hybridization (FISH), and immunohistochemistry but still there is no standardization of the methods and the criteria for interpretation. Also there is a discussion about the most appropriate sample (surgical versus cytological) that should be submitted for analysis and whether to test primary tumor and/or metastasis as they could differ from each other (77). The *EML4-ALK* fusion gene is one of the most recently identified molecular targets for treatment of non small cell lung carcinoma. It has been identified in approximately 4% of patients with non small

cell lung carcinoma, commonly in younger males, never smokers and patients with adenocarcinoma. Its presence is strongly associated with resistance to EGFR-tyrosine kinase inhibitors and sensitivity to ALK inhibitors (78). One of the newest anticancer therapies is antiangiogenic therapy and there are now a large number of antiangiogenic drugs. Among the most advanced of these drugs are those that block the proangiogenic function of vascular endothelial growth factor (VEGF). Some studies suggest that plasma VEGF should be considered as a possible surrogate pharmacodynamic marker for determining the optimal biological dose of antibody drugs that block VEGFR-2 activity in a clinical setting (79).

5. LUNG CARCINOMA MOLECULAR BIOMARKERS IN VARIOUS SPECIMENS

Lung carcinoma molecular biomarkers can be detected in blood (serum), tissue specimens and cytology specimens. Blood is the most commonly used specimen obtained in clinics owing to the ease of its collection (1). Although blood contains a large number of proteins and represents a health status of a patient, it is very difficult biological sample to analyze. Six of the most abundant protein impede the detection of other protein signals in the serum or plasma and a very small amount of total proteins is cancer related (80, 81). There is no strict standard protocol for collection of blood and serum, therefore variations among many researchers may occur. Blood lung carcinoma molecular biomarkers are not specific only for lung carcinoma but are also associated with colorectal carcinoma and ovarian carcinoma (82). Currently, the most well-known markers are CEA, CA-125, cytokeratin 19, CYFRA 21-1, TPA, ProGRP, NSE and Tumor M2-pyruvate kinase (55).

Lung carcinoma tissue specimens the best represent the cancer constituents in human biological samples but the biopsy or resection is aggressive, sometimes impossible and expensive approach. Thorax puncture biopsy is also riskier than other cancer biopsies. There is no standardized method for collecting and handling biopsy samples, so variations among samples can occur (1). In biopsied lung tissue not only carcinoma cells but also nontumor cells and other molecules like immune cells, cytokines and derivates from immune or inflammatory response are present, which limits the value of sample as a source for biomarkers identification.

Diagnostics from cytology specimens has an advantage of non-invasive method, can be repeated several times and can be easily combined with other molecular techniques. In cytology, sputum was used for the longest time, since 1930s in diagnostic of lung carcinoma. Sputum cytology has high specificity (98%) but lower sensitivity (less than 65%). Similarly bronchoalveolar lavage cytology shows low sensitivity, about 27.5%. Several researches demonstrated that combination of several different cytohistological techniques increases sensitivity (83). Sputum contains biomaterial from the original site of the cancer which can be analyzed and the most representative biomarkers from sputum are *K-ras, EGFR, p53* mutations, *hnRNP* and *A2/B1* which can be also identified in pathological tissue samples.(81). Many recent studies have investigated the use of cytology samples for *EGFR* (84-89) and *K-ras* (77) testing. For example patients may only have metastatic disease documented on exfoliative or aspiration cytology material like pleural fluids, fine needle aspirate of the liver or of the pleura or fine needle aspirate of lymph nodes. Still even this cytology samples were suitable for *EGFR* FISH or mutational analysis (85,87,88,90-92) and for *K-ras* mutation analysis (77).

6. VARIOUS TECHNOLOGIES IN LUNG CARCINOMA MOLECULAR BIOMARKERS

For nucleic acid-based lung carcinoma molecular biomarkers identifications three of the most important techniques are FISH, PCR and recently also DNA-microarray. Fluorescence *In situ* Hybridization (FISH) is commonly used with cytological, biopsy and surgical specimens to detect tumor cells and certain microorganisms. It has prognostic value and it can be used to determine treatment response (93-99). *In situ* hybridization uses DNA or RNA probes to evaluate intact cells for genetic changes. Probes are visualized using fluorescent labels. In CISH (chromogenic *in situ* hybridization) probes are visualized with a chromogen that produces a colored chemical at the reaction site (5).

Polymerase Chain Reaction (PCR) is efficient and sensitive method for studying the molecular pathology of primary and metastatic neoplasms, inflammatory mechanisms and infectious diseases (100-107). This method is used to detect the modifications of cancer-related genes or chromosomal abnormalities in diagnosis or prediction of lung carcinomas (108). Due to its high availability it is expected to be useful in predicting individuals who are likely to develop lung carcinoma (109).

DNA-microarray is high-throughput technique used to study molecular alterations at the level of gene expression, DNA-protein interactions and whole genome analysis, as well as gene expression levels on thousands of genes simultaneously. Methods used to detect lung cancer biomarkers at the RNA expression level include Quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR), Serial Analysis of Gene Expression (SAGE), differential display, bead-based methods and microfluid card and micro-array analysis (110).

Identification of the biomarkers depends on accessibility to sufficient amount of material from different specimens. Another limitation is the level of RNA present in RT-PCR or microarray, which does not always indicate the level of present protein because mRNA and protein levels do not necessarily correspond. Therefore the nucleic acid- based biomarkers may not reflect the underlying characteristics of the cancer cell (55).

Protein-based “fingerprints” may outperform individual protein markers. Technologies as differential in-gel electrophoresis (DIGE), two- dimensional polyacrylamide gel electrophoresis (2D-PAGE) and

multidimensional protein-identification technology (MudPIT) can be used for higher-throughput profiling with microgram quantities of protein (111). Other high-throughput technologies as Mass Spectroscopy (MS), Matrix Associated Laser Absorption Desorption Ionization Time of Flight (MALDI-TOF), Surface Enhanced Laser Absorption Desorption Ionization Time of Flight (SELDI-TOF), and reverse phase microarray are more sensitive (47,54,112-114). Some other promising techniques are still are experimental: nanotechnology immuno-PCR, Field Effect Transistor (FET)-based Protein Detection and Quantum Dots (115-118).

Mass spectrometry can be used to detect disease-associated carbohydrate markers related to glycomics present in lung cancers (119,120).

7. OUR EXPERIENCE IN PCR APPLICATIONS FOR DETECTION OF LUNG CARCINOMA BIOMARKERS IN CYTOLOGY SPECIMENS

As it was mentioned before, genomic based biomarkers identification depends on sufficient amount of good quality DNA from specimen which is important for DNA amplification. Our primary interest was the analysis of lung cytological samples obtained during bronchoscopy. Obtaining genomic DNA for successful PCR amplification is critical for molecular analyses. Routinely used methods for DNA isolation include commercially available kits, or extensive procedures that usually involve cell lysis by proteinase K, followed by isopropanol and/or phenol/chloroform extraction as an additional step of DNA purification (121,122). The complexity and expenses of the procedure represent a burden in systematic large-scale DNA isolation; so every simplification in DNA isolation procedure is welcomed and saves money and time (123). We tested a simple method which combined alkaline and temperature lyses for isolation of DNA solution, and use it to extract DNA from cytology samples from bronchial aspirates. Alkaline lysis is a method for DNA isolation already used in the analysis of samples from human and mouse buccal swabs (124-126). It is based on solubilization of membrane and coagulation of proteins which enables the unaffected DNA to leave the cells and be available for further analysis by PCR in less than an hour. This method was a starting point to be adapted for use in cytology and it was tested for amplification of sex chromatin gene fragments in bronchial aspirates. The method was showed as highly reliable for DNA isolation, as it preserves high-quality DNA suitable for PCR-based assays. Its main advantages are noninvasive approach, simplicity of the procedure as only unsophisticated chemicals are used, and a high success rate of PCR (123). It was successfully used for testing by PCR molecular markers related to lung cancer, for example, the presence of "high-risk" human papilloma virus types which are associated with cervical cancers and subset of head and neck cancers and can be, according some authors, cocarcinogen for lung carcinoma development (52,123,127).

8. PERSPECTIVE

Early diagnosis is crucial for decreasing lung cancer mortality. Gene and protein expression profiling has advanced our understanding of lung carcinoma. The increasing knowledge of the pathway activation profile in lung carcinoma indicates new targets but also new markers to select patients and predict therapy efficiency. Many studies report numerous lung carcinoma molecular biomarkers but they are often made on small number of patients difficult to reproduce and not sensitive or specific enough for clinical use. It is clear that all lung carcinoma molecular biomarkers must be validated in a large set of clinical samples. Lung carcinomas are very heterogeneous and morphologic heterogeneity is reflected at the molecular level, so several lung carcinoma molecular biomarkers must be combined with different approaches according to the different lung carcinoma types. A deep biological understanding of each molecular biomarker is crucial and can be a key to correct diagnose and also a predictor for therapy efficacy. Larger new studies with clinical data and follow-up are required to achieve these goals.

9. ACKNOWLEDGEMENTS

This work was supported by grants of Ministry of Science and Technology, Republic of Croatia (108-1081870-1902), and Unity for Knowledge Fund, Republic of Croatia (UKF 35/08).

10. REFERENCES

1. J.Y. Cho and H.J. Sung: Proteomic Approaches in Lung Cancer Biomarker Development. *Expert Rev Proteomics* 6, 7-42 (2009)

2. S. Shiono, K. Omoe and A. Endo: K-ras gene mutation in sputum samples containing atypical cells and adenocarcinoma cells in the lung. *Carcinogenesis* 17, 1683-1686 (1996)

3. S. Aviel-Ronen, F.H. Blackhall, F.A. Shepherd and M.S. Tsao: K-ras mutations in non-small-cell lung carcinoma: a review. *Clin Lung Cancer* 8, 30-38 (2006)

4. K. Sugio, T. Ishida, H. Yokoyama, T. Inoue, K. Sugimachi and T. Sasazuki: Ras gene mutations as a prognostic marker in adenocarcinoma of the human lung without lymph node metastasis. *Cancer Res* 52, 2903-2906 (1992)

5. S. Rodenhuis and R.J. Slebos: Clinical significance of ras oncogene activation in human lung cancer. *Cancer Res* 52. Suppl 9, 2665s-2669s (1992)

6. T.C. Allen, P.T. Cagle and H.H. Popper: Basic Concept of Molecular Pathology. *Arch Pathol Lab Med* (132), 1551-1556 (2008)

7. J. Niklinski, W. Niklinska, L. Chyczewski, H.D. Becker and E. Pluygers: Molecular genetic abnormalities in premalignant lung lesions: biological and clinical implications. *Eur J Cancer Prev* 10, 213-226 (2001)

8. R. Salgia and A.T. Skarin: Molecular abnormalities in lung cancer. *J Clin Oncol* 16, 1207-1217 (1998)

9. S. Gazzeri, E. Brambilla, C. Caron de Fromentel, V. Gouyer, D. Moro, P. Perron, F. Berger and C. Brambilla: p53 genetic abnormalities and myc activation in human lung carcinoma. *Int J Cancer* 58, 24-32 (1994)

10. E. Brambilla, S. Gazzeri, S. Lantuejoul, J.L. Col, D. Moro, A. Negoescu and C. Brambilla: p53 mutant immunophenotype and deregulation of p53 transcription pathway (Bcl2, Bax and Warf1) in precursor bronchial lesions of lung cancer. *Clin Cancer Res* 4, 1609-1618 (1998)

11. A.J. Levine: p53, the cellular gatekeeper for growth and division. *Cell* 88, 323-331 (1997)

12. L. Mao, J.S. Lee, J.M. Kurie, Y.H. Fan, S.M. Lippman, J.J. Lee, J.Y. Ro, A. Broxson, R. Yu, R.C. Morice, B.L. Kemp, F.R. Khuri, G.L. Walsh, W.N. Hittelman and W.K. Hong: Clonal genetic alterations in the lungs of current and former smokers. *J Natl Cancer Inst* 89, 857-862 (1997)

13. I.I.Wistuba, C. Behrens, S. Milchgrub, D. Bryant, J. Hung, J.D. Minna and A.F. Gazdar: Sequential molecular abnormalities are involved in the multistage development of squamous cell lung carcinoma. *Oncogene* 18, 643-650 (1999)

14. L. Girard, S. Zochbauer-Muller, A.K. Virmani, A.F. Gazdar and J.D. Minna: Genome-wide allelotyping of lung cancer identifies new regions of allelic loss, differences between small cell lung cancer and non small cell lung cancer, and loci clustering. *Cancer Res* 60, 4894-4906 (2000)

15. M.T. Shiseki, J. Kohno, J. Adachi, T. Ozaki, T. Otsuka, H. Mizoguchi, M Noguchi, S Hirohashi and J. Yokota: Comparative allelotype of early and advanced stage non-small cell lung carcinomas. *Genes Chromosomes Cancer* 17, 71-77 (1996)

16. R.C. Tseng, J.W. Chang, F.J. Hsien, Y.H. Chang, C.F. Hsiao, J.T. Chen, C.Y. Chen, Y.S. Jou and Y.C. Wang: Genomewide loss of heterozygosity and its clinical associations in non small cell lung cancer. *Int J Cancer* 117, 241-247 (2005)

17. C.A. Powell, S. Klares, G. O'Connor and J.S. Brody: Loss of heterozygosity in epithelial cells obtained by bronchial brushing: clinical utility in lung cancer. *Clin Cancer Res* 5, 2025-2034 (1999)

18. C.A. Powell, R. Bueno, A.C. Borczuk, C.F. Caracta, W.G. Richards, D.J. Sugarbaker and J.S. Brody: Patterns of allelic loss differ in lung adenocarcinomas of smokers and nonsmokers. *Lung Cancer* 39, 23-29 (2003)

19. H. Pan, J. Califano, J.F. Ponte, A.L. Russo, K. Cheng, A. Thiagalingam, P. Nemani, D. Sidransky and S. Thiagalingam: Loss of heterozygosity patterns provide fingerprints for genetic heterogeneity in multistep cancer progression of tobacco smoke-induced non-small cell lung cancer. *Cancer Res* 65, 1664-1669 (2005)

20. R.L. De Menezes Duarte and M.E. Machado Paschoal. Molecular markers in lung cancer: prognostic role and relationship to smoking. *J Bras Pneumol* 32, 56-65 (2005)

21. A.K. Meeker, J.L. Hicks, C. Iacobuzio-Donahue, E.A. Montgomery, W.H. Westra, T.Y. Chan, B.M. Ronnett and A.M. De Marzo: Telomere length abnormalities occur early in the initiation of epithelial carcinognesis. *Clin Cancer Res* 10, 3317-3326 (2004)

22. J.N. O'Sullivan, J.C. Finley, R.A. Risques, W.T. Shen, K.A. Gollahon, A. H. Moskovitz, S. Gryaznov, C.B. Harley and P.S. Rabinovitch: Telomere length assessment in tissue sections by quntative FISH :image analysis algotithms. *Cytometry A* 58, 120-131 (2004)

23. G.T. Chung, V. Sundaresan, P. Hasleton, R. Rudd, R. Taylor and P.H. Rabbits: Sequential molecular genetic changes in lung cancer development. *Oncogene* 11, 2591-2598 (1995)

24. S.A. Belinsky, K.J. Nikula, W.A. Palmisano, R. Michels, G. Saccomanno, E. Gabrielson, S.B. Baylin and J.G. Herman. Aberrant methylation of p16(INK4a) is an early event in lung cancer and a potential biomarker for early diagnosis. *Proc Natl Acad Sci U.S.A.* 95,11891-1186 (1998)

25. L. Chaussade, B. Eymin, E. Brambilla and S. Gazzeri: Expression of p15 and p15.5 products in neuroendocrine lung tumours:relationship with p15(INK4b) methylation status. *Oncogene* 20,6587-6596 (2001)

26. M. Esteller, M. Sanchez-Cespedes, R. Rosell, D. Sidransky, S.B. Baylin and J.G. Herman: Detection of aberrant promoter hypermethylation of tumor suppresor genes in serum DNA from non-small cell lung cancer patients. *Cancer Res* 59, 67-70 (1999)

27. E. Kurakawa, T. Shimamoto, K. Utsumi, T. Hirano, H. Kato and K. Ohyashiki: Hypermethylation of p16(INK4a) and p15(INK4b) genes in non-small cell lung cancer. *Int J Oncol* 19, 277-281 (2001)

28. W.A. Palmisano, K.K. Divine, G. Saccomanno, F.D. Gilliland, S.B. Baylin, J.G. Herman and S.A. Belinsky. Predicting lung cancer by detecting aberrant promoter methylation in sputum. *Cancer Res* 60, 5954-5958 (2000)

29. A.K. Virmani, A. Rathi, S. Zochbauer-Muller, N. Sacchi, Y. Fukuyama, D. Bryant, A. Maitra, S. Heda, K.M. Fong, F. Thunnissen, J.D. Minna and A.F. Gazdar: Promoter methylation and silencing of the retinoic acid receptor-beta gene in lung carcinomas. *J Natl Cancer Inst* 92, 1303-1307 (2000)

30. S. Zochbauer-Muller, K.M. Fong, A.K. Virmani, J. Geradts, A.F. Gazdar and J.D. Minna: Aberrant promoter methylation of multiple genes in non-small cell lung cancers. *Cancer Res* 61, 249-255 (2001)

31. S. Belinsky: Gene-promoter hypermethylation as a biomarker in lung cancer. *Nat Rev Cancer* 4, 707-717 (2004)

32. X. Xue, Y.M. Zhu and P.J. Woll: Circulating DNA and lung cancer. *Ann N Y Acad Sci* 1075, 154-164 (2006)

33. H. Usadel, J. Brabender, K.D. Danenberg, C. Jeronimo, S. Harden, J. Engles, P.V. Danenberg, S. Yang and D. Sidransky: Quantitative adenomatous polyposis coli promoter methylation analysis in tumor tissue, serum and plasma DNA of patients with lung cancer. *Cancer Res* 62, 371-375 (2002)

34. J.L. Ramirez, C. Sarries, P.L. De Castro, B. Roig, C. Queralta, D. Escuin, I. De Aguirre, J.M. Sanchez, J.L. Manzano, M. Margeli, J.J. Sanchez, J. Astudillo, M. Taron and R. Rosell: Methylation patterns and K-ras mutations in tumor and paired serum of resected nonsmall cell lung cancer patients. *Cancer Lett* 193, 207-216 (2003)

35. M. Esteller, M. Sanchez-Cespedes, R. Rosell, D. Sidransky, S.B. Baylin and J.G. Herman: Detection of aberrant promoter hypermethylation of tumor suppressor genes in serum DNA from nonsmall cell lung cancer patients. *Cancer Res* 59, 67-70 (1999)

36. S.A. Belinsky, D.M. Klinge, J.D. Dekker, M.W. Smith, T.J. Bocklage, F.D. Gilliland, R.E. Crowell, D.D. Karp, C.A. Stidley and M.A. Picchi: Gene promoter methylation in plasma and sputum increases with lung cancer risk. *Clin Cancer Res* 11, 6505-6511 (2005)

37. V.N. Senchenko, J. Liu, W. Loginov, I. Bazov, D. Angeloni, Y. Seryogin, V. Ermilova, T. Kazubskaya, R. Garkavtseva, V.I. Zabarovska, V.I. Kashuba, L.L. Kisselev, J.D. Minna, M.I. Lerman, G. Klein, E.A. Braga and E.R. Zabarovsky: Discovery of frequent homozygous deletions in chromosome 3p21.3 LUCA and AP20 regions in renal, lung and breast carcinomas. *Oncogene* 23, 5719-5728 (2004)

38. J.S. Wiest, W.A. Franklin, H. Drabkin, R. Gemmill, D. Sidransky and M.W. Anderson: Genetic markers for early detection of lung cancer and outcome measures for response to chemoprevention. *J Cell Biochem* Suppl 28-29, 64-73 (1997)

39. E.R. Zabarovsky, M.I. Lerman and J.D. Minna: Tumor suppressor genes on chromosome 3p involved in the pathogenesis of lung and other cancers. *Oncogene* 21, 6915-6935 (2002)

40. X. Xue, Y.M. Zhu and P.J. Woll: Circulating DNA and lung cancer. *Ann N Y Acad Sci* 1075, 154-164 (2006)

41. J.A. Ludwig and J.N. Weinstein: Biomarkers in Cancer Staging, Prognosis and Treatment Selection. *Nat Rev Cancer* 5, 845-856 (2005)

42. M. Galasso, M. Elena Sana and S. Volinia: Non-coding RNAs: a key to future personalized molecular therapy? *Genome Med* 2, 12 (2010)

43. J. Liao, L. Yu, Y. Mei, M. Guarnera, J. Shen, R. Ruiyun, Z. Liu and F. Jiang: Small nucleolar RNA signatures as biomarkers for non-small-cell lung cancer. *Molecular Cancer* 9, 198 (2010)

44. V. Ambros: MicroRNA pathways in flies and worms: growth, death, fat, stress, and timing. *Cell* 6, 673-676 (2003)

45. R. Gillet and B. Felden: Emerging views on tmRNA-mediated protein tagging and ribosome rescue. *Mol Microbiol* 4, 879-885 (2001)

46. B.E. Jady and T. Kiss: A small nucleolar guide RNA functions both in 2'-O-ribose methylation and pseudouridylation of the U5 spliceosomal RNA. *EMBO J* 20, 541-551 (2001)

47. C.L. Bartels and G.J. Tsongalis: MicroRNAs: novel biomarkers for human cancer. *Clin Chem* 55, 623-631 (2009)

48. G.A. Calin and C.M. Croce: MicroRNA signaturs in human cancers. *Nat Rev Cancer* 11, 857-866 (2006)

49. J. Lu, G. Getz, E.A. Miska, E. Alvarez-Saavedra, J. Lamb, D. Peck, A. Sweet-Cordero, B.L. Ebert, R.H. Mak, A.A. Ferrando, J.R. Downing, T. Jacks, H.R. Horvitz and T.R. Golub: MicroRNA expression profiles classify human cancers. *Nature* 435, 834-838 (2005)

50. D.R. Hurst, M.D. Edmonds and D.R. Welch: Metastamir. The field of metastasis-regulatory microRNA is spreading. *Cancer Res* 69, 7495-7498 (2009)

51. S.K. Shenouda and S.K. Alahari: MicroRNA function in cancer: oncogene or a tumor suppressor? *Cancer Metastasis Rev* 28, 369-378 (2009)

52. A. Mishra and M. Verma: Cancer Biomarkers. Are We Ready for the Prime Time? *Cancer* 2, 190-208 (2010)

53. S. Srivastava, M. Verma and R. Gopal-Srivastava: Proteomic maps of the cancer-associated infectious agents. *J Proteome Res* 4, 1171-1180 (2005)

54. R. Ravichandran, G.B. Vasquez, S. Srivastava, M. Verma, E. Petricoin, J. Lubell, R.D. Sriram, P.E. Barker and G.L. Gilliland: Data Standards for Proteomics: Mitochondrial two-dimensional Polyacrylamide Gel Electrophoresis Data as a Model System. *Mitochondrion* 3, 327-336 (2004)

55. H.J. Sung and J.Y. Cho: Biomarkers for the lung cancer diagnosis and their advances in proteomics. *BMB reports* 41, 615-625 (2008)

56. T. Okano, T. Kondo, K. Fujii, T. Nishimura, T. Takano, Y. Ohe, K.Tsuta, Y. Matsuno, A. Gemma, H. Kato, S. Kudoh and S. Hirohashi: Proteomic signature corresponding to the response to gefitinib Iressa, ZD1839, an epidermal growth factor receptor tyrosine kinase inhibitor in lung adenocarcinoma. *Clin Cancer Res* 13, 799-805 (2007)

57. T. Hida, S. Ogawa, J.C. Park, J.Y. Park, J. Shimizu, Y. Horio and K. Yoshida: Gefitinib for the treatment of non-small-cell lung cancer. *Expert Rev Anticancer Ther* 9, 17-35 (2009)

58. S.P. Gygi, G.L. Corthals, Y. Zhang, Y. Rochon and R. Aebrsold: Evaluation of two-dimensional gel electrophoresis-based proteome analysis technology. *Proc Natl Acad Sci USA* 97, 9390-9395 (2000)

59. K.L. Abbott, A.V. Nairn, E.M. Hall, M.B. Horton, J.F. McDonald, K.W. Moremen, D.M. Dinulescu and M. Pierce: Focused glycomic analysis of the N-linked glycan biosynthetic pathway in ovarian cancer. *Proteomics* 8, 3210-3220 (2008)

60. T.F. Orntoft and E.M. Vestergaard: Clinical aspects of altered glycosylation of glycoproteins in cancer. *Electrophoresis* 20, 362-371 (1999)

61. R. Saldova, M.R. Wormald, R.A. Dwek and P.M. Rudd: Glycosylation changes on serum glycoproteins in ovarian cancer may contribute to disease pathogenesis. *Dis Markers* 25, 219-232 (2008)

62. I.I. Wistuba, C. Behrens, S. Milchgrub, D. Bryant, J. Hung, J.D. Minna and A.F. Gazdar: Sequential molecular abnormalities are involved in the multistage development of squamous cell lung carcinoma. *Oncogene* 18, 643-650 (1999)

63. I.I. Wistuba, S. Lam, C. Behrens, K. A.K. Virmani, K.M. Fong, J. LeRiche, J.M. Samet, S. Srivastava, J.D. Minna and A.F. Gazdar: Molecular damage in the bronchial epitehelium of current and former smokers. *J Natl Cancer Inst* 89, 1366-1373 (1997)

64. I.I. Wistuba, C. Behrens, A.K. Virmani, G. Mele, S. Milchgrub, L. Girard, J.W. Fondon III, H.R. Garner, B. McKay, F. Latif, M.I. Lerman, S. Lam, A.F. Gazdar and J.D. Minna: High resolution chromosome 3p allelotyping of human lung cancer and preneoplastic/preinvasive bronchial epithelium reveals multiple, discontinuous sites of 3p allele loss and three regions of frequent breakpoints. *Cancer Res* 60, 1949-1960 (2000)

65. V. Sundaresan, P. Ganly, P. Hasleton, R. Rudd, G. Sinha, N.M. Bleehen and P. Rabbitts: p53 and chromosome 3 abnormalities, characteristic of malignant lung tumours are detectable in preinvasive lesions of the bronchus. *Oncogene* 7, 1989-1997 (1992)

66. G. Sozzi, M. Miozzo, R. Donghi, S. Pilotti, C.T. Cariani, U. Pastorino, G. Della Porta and M.A. Pierotti: Deletions of 17p and p53 mutations in preneoplastic lesions of the lung. *Cancer Res* 52, 6079-6082 (1992)

67. S.A. Belinsky, W.A. Palmisano, F.D. Gilliland, L.A. Crooks, K.K. Divine, S.A. Winters, M.J. Grimes, H.J. Harms, C.S. Tellez, T.M. Smith, P.P. Moots, J.F. Lechner, C.A. Stidley and R.E. Crowell: Aberrant promoter methylation in bronchial epithelium and sputum from current and former smokers. *Cancer Res* 62, 2370-2377 (2002)

68. A. Lamy A, R. Sesboue, J. Bourguignon, B. Dautreaux, J. Metayer and T. Frebourg: Aberrant methylation of the CDKN2a/p16INK4a gene promoter region in preinvasive bronchial lesions: a prospective study in high-risk patients without invasive cancer. *Int J Cancer* 100, 189-193 (2002)

69. W.H. Westra, R.J. Slebos, G.J. Offerhaus, S. N. Goodman, S.G. Evers, T.W. Kensler, F.B. Askin, S. Rodenhuis and R. H. Hruban: K-ras oncogene activation in lung adenocarcinomas from former smokers: evidence that K-ras mutations are an early and irreversible event in the development of adenocarcinoma of the lung. *Cancer* 72, 432-438 (1993)

70. H. Sakamoto, J. Shimizu, Y. Horio, R. Ueda, T. Takahashi, T. Mitsudomi and Y. Yatabe: Disproportionate representation of KRAS gene mutation in atypical adenomatous hyperplasia, but even distribution of EGFR gene mutation from preinvasive to invasive adenocarcinomas. *J Pathol* 212, 287-294 (2007)

71 K.M. Kerr, F.A. Carey, G. King and D. Lamb: Atypical alveolar hyperplasia: relationship with pulmonary adenocarcinoma, p53 and c-erbB-2 expression. *J Pathol* 174, 249-256 (1994)

72. R.J. Slebos, I.O. Baas, M.J. Clement, G.J. Offerhaus, F.B. Askin, R.H. Hruban and W.H. Westra: p53 alterations in atypical alveolar hyperplasia of the human lung. *Hum Pathol* 29, 801-808 (1998)

73. G. Giaccone, R.M. Gallegos, T. Le Chevalier, N. Thatcher, E. Smith, J.A. Rodriguez, P. Janne, D. Oulid-Aissa and J. Soria: Erlotinib for frontline treatment of advanced non-small cell lung cancer: a phase II study. *Clin Cancer Res* 12, 6049-6055 (2006)

74. E. Massarelli, M. Varella-Garcia, X. Tang, A.C. Xavier, N.C. Ozburn, D.D. Liu, B.N. Bekele, R.S. Herbst and I.I. Wistuba: KRAS mutation in an important predictor of resistance to therapy with epidermal growth factor receptor tyrosine kinase inhibitors in non-small cell lung cancer. *Clin Cancer Res* 13, 2890-2896 (2007)

75. J.G. Paez , P.A. Janne, J.C. Lee, S. Tracy, H. Greulich, S. Gabriel, P. Herman, F.J. Kaye, N. Lindeman, T.J. Boggon, K. Naoki, H. Sasaki, Y. Fujii, M.J. Eck, W.R. Sellers, B.E. Johnson and M. Meyerson: EGFR mutations in lung cancer:correlation with clinical response to gefitinib thrapy. *Science* 304, 1497-1500 (2004)

76. W. Pao, T.Y. Wang, G.J. Riely, V. A. Miller, Q. Pan, M. Ladanyi, M.F. Zakowski, R.T. Heelan, M.G. Kris and H.E. Varmus: KRAS mutations and primary resistance of lung adenocarcinomas to gefitinib or erlotinib. *PloS Med* 21, e17 (2005)

77. S.E. Monaco, M.N. Nikiforova, K. Cieply, L.A. Teot , W.E. Khalbuss and S. Dacic: A comparison of EGFR and

KRAS status in primary lung carcinoma and matched metastases. *Hum Pathol* 41, 94-102 (2010)

78. P. Ulivi, D. Calistri, W. Zoli and D. Amadori: Predictive molecular markers for EGFR-TKI non-small cell lung cancer patients: new insights and critical aspects. *J Nucl Acid Invest* 1, e10 (2010)

79. G. Bocci, S. Man, S.K. Green, G. Francia, J.M.L. Ebos, J.M. du Manoir, A. Weinerman, U. Emmenegger, L.Ma, P. Thorpe, A. Dawidoff, J. Huber, D.J. Hicklin and R.S. Kerbel: Increased Plasma Vascular Endothelial Growth Factor (VEGF) as a Surrogate Marker for Optimal Therapeutic Dosing of VEGF Receptor-2 Monoclonal Antibodies. *Cancer Res* 64, 6616-6625 (2004)

80. A.F. Gazdar and J.D. Minna: Molecular detection of early lung cancer. *J Natl Cancer Inst* 91, 299-301 (1999)

81. S.M. Hanash, S.J. Pitteri and V.M. Faca: Mining the plasma proteome for cancer biomarkers. *Nature* 452, 571-579 (2008)

82. F. Barlesi, C. Tchouhadjian, C. Doddoli, J.P. Torre, P. Astoul and J.P. Kleisbauer: CYFRA21-1 level predicts survival in non-small-cell lung cancer patients receiving gefitinib as third-linr therapy. *Br J Cancer* 92, 13-14 (2005)

83. S. Garg, U. Handa, H. Monah and A.K. Janmeja: Comparative analysis of various cytohisological techniques in diagnosis of lung diseases. *Diagn Cytopathol* 35, 26-31 (2007)

84. A. Kalikaki, A. Koutsopoulos, M. Trypaki, J. Souglakos, E. Stathopoulos, V. Georgoulias, D. Mavroudis and A. Voutsina: Comparison of EGFR and KRAS gene status between primary tumours and coresponding metastases in NSCLC. *Br J Cancer* 99, 323-329 (2008)

85. C.H. Gow, Y.L. Chang, Y.C. Hsu, M.F. Tsai, C.T. Wu, C.J. Yu, C.H. Yang, Y.C. Lee, P.C. Yang and J.Y. Shih: Comparison of epidermal growth factor receptor mutations between primary and coresponding metastatic tumors in tyrosine kinase inhibitor-naive non-small-cell lung cancer. *Ann Oncol* 20, 696-702 (2009)

86. A. Horiike, H. Kimura, K. Nishio, F. Ohyanagi, Y. Satoh, S. Okumura, Y. Ishikawa, K. Nakagawa, T. Horai and M. Nishio: Detection of epidermal growth factor receptor mutation in transbronchial needle aspirates on non-small cell lung cancer. *Chest* 131, 1628-1634 (2007)

87. J.H. Smouse, E.S. Cibas, P.A. Jänne, V.A. Joshi, K.H. Zou and N.I. Lindeman: EGFR mutations are detected comparably in cytologic and surgical pathology specimens of nonsmall cell lung cancer. *Cancer Cytopathol* 117, 67-72 (2009)

88. M.F. Zakowski, S. Hussain, W. Pao, M. Ladanyi, M.S. Ginsberg, R. Heelan, V.A. Miller, V.W. Rusch and M.G. Kris: Morphologic features of adenocarcinoma of the lung predictive of response to the epidermal growth factor receptor kinase inhibitors erlotinib and gefitinib. *Arch Pathol Lab Med* 133, 470-477 (2009)

89. L. Boldrini, S. Gisfredi, S. Ursino, T. Camacci, E. Baldini, F. Melfi and G. Fontanini: Mutational analysis in cytological specimens of advanced lung adenocarcinoma: a sensitive method for molecular diagnosis. *J Thorac Oncol* 2, 1086-1090 (2007)

90. C. Bozetti, M. Tiseo, C. Lagranza, R. Nizzoli, A. Guazzi, F. Leonardi, D. Gasparo, E. Spiritelli, M. Rusca, P. Carbognani, M. Majori, V. Franciosi, G. Rindi and A. Ardizzoni: Comparison between epidermal growth factor receptor (EGFR) gene expression in primary non-small cell lung cancer (NSCLC) and in fine-needle aspirates from metastatic sites. *J Thor Oncol* 3, 18-22 (2008)

91. L. Daniele, L. Macri, M. Schena, D. Dongiovanni, L. Bonello, E. Armando, L. Ciuffreda, O. Bertetto, G. Bussolati and A. Sapino: Predicting gefitinib responsiveness in lung cancer by fluorescence *in situ* hybridization analysis of EGFR and HER2 in biopsy and cytology specimens. *Mol Cancer Ther* 6, 1223-1229 (2007)

92. E.X. Wei, A.A. Anga, S.S. Martin, J.G. Jackson, M.L. Nordberg, G.A. Herrera and E.A. Turbat-Herrera: EGFR expression as an acillary tool for diagnosing lung cancer in cytology specimns. *Mod Pathol* 20, 905-913 (2007)

93. L.A. Brown and D. Huntsman: Fluorescent *in situ* hybridization on tissue microarrays: challenges and solutions. *J Mol Histol* 38, 151-157 (2007)

94. K.C. Halling and B.R. Kipp: Fluorescence *in situ* hybridization in diagnostic cytology. *Hum Pathol* 38, 1137-1144 (2007)

95. Z. Theodosiou, I.N. Kasmpalidis, G. Livanos, M. Zervakis, I. Pitas and K. Lyroudiaz: Automated analysis of FISH and immunohistochemistry images: a review. *Cytometry* 71A, 439-450 (2007)

96. B.J. Dave and W.G. Sanger: Role of cytogenetics and molecular cytogenetics in the diagnosis of genetic imbalances. *Semin Pediatr Neurol* 14, 2-6 (2007)

97. S. Krishnamurthy: Applications of molecular techniques to fine-needle aspiration biopsy. *Cancer (Cancer Cytopathol)* 111, 106-122 (2007)

98. J. Bayani and J.A. Squire: Application and interpretation of FISH in biomarker studies. *Cancer Lett* 249, 97-109 (2007)

99. F.C. Tenover: Rapid detection and identification of bacterial pathogens using novel molecular technologies: infection control and beyond. *Clin Infect Dis* 44, 418-423 (2006)

100. I. Raad, H. Hanna, A. Huaringa, D. Sumoza, R. Hachem and M. Albitar: Diagnosis of invasive pulmonary aspergillosis using polymerase chain reaction-based

detection of aspergillus in BAL. *Chest* 121, 1171-1176 (2002)

101. L.M. Nogee, A.E. Dunbar III, S.E. Wert, F. Askin, A. Hamvas and J.A. Whitsett: A mutation in the surfactant protein C gene associated with familial interstitial lung disease. *N Engl J Med* 344, 573-579 (2001)

102. J.L. Lordan, F. Bucchieri, A. Richter, A. Konstantinidis, J.W. Halloway, M. Thornber, S.M. Puddicombe, D. Buchanan, S.J. Wilson, R. Djukanović, S.T. Holgate and D.E. Davies: Cooperative effects of Th2 cytokines and allergen on normal and asthmatic bronchial epithelial cells. *J Immunol* 169, 407-414 (2002)

103. W.H. Westra, I.O. Baas, R.H. Hruban, F.B. Askin, K. Wilson, G.J.A. Offerhaus and R.J.C. Slebos: K-ras oncogene activation in atypical alveolar hyperplasia of the human lung. *Cancer Res* 56, 2224-2228 (1996)

104. D. Pulte, E. Li, B.K. Crawford, E. Newman, A. Alexander, D.C. Mustalish and D.R. Jacobson: Sentinel lymph node mapping and molecular staging in nonsmall cell lung carcinoma. *Cancer* 104, 1453-1461 (2005)

105. R.M. Bremnes, R. Sirera and C. Camps: Circulating tumour derived DNA and RNA markers in blood: a tool for early detection, diagnostic and follow-up? *Lung Cancer* 49, 1-12 (2005)

106. Q. Pan, W. Pao and M. Ladanyi: Rapid polymerase chain reaction-based detection of epidermal growth factor receptor gene mutations in lung adenocarcinomas. *J Mol Diagn* 7, 396-403 (2005)

107. T. Bohlmeyer, T.N. Le, A.L. Shroyer, N. Markham and K.R. Shroyer: Detection of human papillomavirus in squamous cell carcinomas of the lung by polymerase chain reaction. *Am J Respir Cell Mol Biol* 18, 265-269 (1998)

108. R.P. Valle, C. Chavany, T.A. Zhukov and M. Jendoubi: New approaches for biomarker discovery in lung cancer. *Expert Rev Mol Diagn* 3, 55-67 (2003)

109. M.S. Tockman: Clinical detection of lung cancer progression markers. *J Cell Biochem* 25, 177-184 (1996)

110. V.E. Velculescu, L. Zhang, B. Vogelstein and K.W. Kinzler: Serial analysis of gene expression. *Science* 270, 484-487 (1995)

111. G. Zhou, H. Li, D. DeCamp, S. Chen, H. Shu, Y. Gong, M. Flaig, J.W. Gillespie, N. Hu, P.R. Taylor, M.R. Emmert-Buck, L.A. Liotta, E.F. Petricoin and Y. Zhao: 2D differential in-gel electrophoresis for the identification of esophageal scans cell cancer-specific protein markers. *Mol Cell Proteomics* 1, 117-124 (2002)

112. M. Verma, J. Kagan, S. Sidransky and S. Srivasatava: Proteomic analysis of the cancer cell mitochondria. *Nat Rev Cancer* 3, 789-795 (2003)

113. P.A. Everly, J. Krijgsveld, B.R. Zetter and S.P. Gaygi: Quantitative cancer proteomics: stable isotope labeling with amino acids in cell culture (SILAC) as a tool for prostate cancer research. *Mol Cell Proteomics* 3, 729-735 (2004)

114. C.P. Paweletz, L.Charboneau, V.E. Bichsel, N.L. Simone, T. Chen, J.W. Gillespie, M.R. Emmert-Buck, M.J. Roth, E.F. Petricoin III and L.A. Liotta: Reverse phase protein microarrays which capture disease progression show activation of pro-survival pathways at the cancer invasion front. *Oncogene* 20, 1981-1989 (2001)

115. T. Sano, C.L. Smith and C.R. Cantor: Immuno-PCR: very sensitive antigen detection by means of specific antibody-DNA conjugates. *Science* 258, 120-122 (1992)

116. J.M. Nam, C.S. Thaxton and C.A. Mirkin :Nanoparticle-based bio-bar codes for the ultrasensitive detection of proteins. *Science* 301, 1884-1886 (2003)

117. S.F. Service. American Chemical Society Meeting. Tiny transistors scout for cancer. *Science* 300, 242-243 (2003)

118. C. Seydel: Quantum dots get wet. *Science* 300, 80-81 (2003)

119. Y. Misonou, K.Shida, H. Korekane, Y. Seki, S. Noura, M. Ohue and Y. Miyamoto: Comprehensive clinico-glycomic study of 16 colorectal cancer specimens: elucidation of aberrant glycosylation and its mechanistic causes in colorectal cancer cells. *J Proteome Res* 8, 2990-3005 (2009)

120. A.S. Powlesland, P.G. Hitchen, S. Parry, S.A. Graham, M.M. Barrio, M.T. Elola, J. Mordoh, A. Dell, K. Drickamer and M.E. Taylor: Targeted glycoproteomic identification of cancer cell glycosylation. *Glycobiology* 19, 899-909 (2009)

121. W. Cao, M. Hashibe, J.Y. Rao, H. Morgenstern and Z.F. Zhang: Comparison of methods for DNA extraction from paraffin-embedded tissues and buccal cells. *Cancer Detect Prev* 27, 397-404 (2003)

122. C. Mulot, I. Stucker, J. Clavel, P. Beaune and M.A. Loriot: Collection of human genomic DNA from buccal cells for genetics studies: comparison between cytobrush, mouthwash and treated card. *J Biomed Biotechnol* 3, 291-296 (2005)

123. B. Vrabec Branica, D. Mitrecic, S. Grgic, S. Smojver Jezek, N. Chalfe and S. Gajovic: PCR Quality DNA Isolation from Human Bronchial Aspirates and Buccal and Eyelid Swabs by a Simple Procedure Based on Alkaline Lyses. *Genet Test and Mol Biom* 13, 799-802 (2009)

124. L. Rudbeck and J. Dissing: Rapid, simple alkaline extraction of human genomic DNA from whole blood, buccal epithelial cells, semen and forensic stains for PCR. *Biotechniques* 25, 588-592 (1998)

125. M. Meldegaard, P.J. Bollen and B. Finsen: Non-invasive method for sampling and extraction of mouse DNA for PCR. *Lab Anim* 25, 588-592 (2004)

126. D. Mitrecic, S. Mavric, B. Vrabec Branica and S. Gajovic: Mice genotyping using buccal swab samples-an improved mrthod. *Biochem Genet* 46, 105-112 (2008)

127. B. Vrabec Branica, S. Smojver-Jezek, Z. Juros, S. Grgic, N. Srpak, D. Mitrecic and S. Gajovic: Detection of Human Papillomaviruses Type 16,18 and 33 in Bronchial Aspirates of Lung Carcinoma Patients by Polymerase Chain Reaction: A Study of 84 Cases in Croatia. *Coll Antropol* 34, 159-162 (2010)

Key Words: Lung, Carcinoma, Molecular Biomarkers, Review

Send correspondence to: Srecko Gajovic, University of Zagreb School of Medicine, Croatian Institute for Brain Research, Salata 12, 10000 Zagreb, Croatia, Tel: 385 1 4566948, Fax: 385 1 4566795, E-mail: srecko.gajovic@hiim.hr

http://www.bioscience.org/current/vol4E.htm