

MINIREVIEW

Genomics of Lung Cancer may Change Diagnosis, Prognosis and Therapy

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Despite significant improvements in tumor management in general, the prognosis of lung cancer patients remains dismal. It is a hope that our increasing knowledge in molecular aspects of tumor development, growth and progression will open new targets for therapeutic interventions. In this review we discuss some of the more recent results of this field. This includes the susceptibility factors, an association between genetic changes in

EGFR pathway and tyrosine kinase inhibitors, the role of gene hypermethylation and genetic profiling, as well as different molecular aspects of tumor progression. Available data all support that lung cancer is a group of diseases with not only distinct histological but with similarly different genetic characters. Accordingly, the diagnosis, prognosis and therapy must accommodate this heterogeneity. (Pathology Oncology Research Vol 11, No 1, 5–10)

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Lung cancer is a leader in cancer mortality and one of the most fearful human malignancies due to the late discovery and early progression. It is a hope that a better understanding of the molecular events regulating the development and growth of lung cancer will improve all aspects of tumor management. The revolutionary changes in molecular technologies provide a basis to approach and fulfil this expectation. Today, lung cancer is classified according to histology; the four main subtypes are: small cell lung cancer (SCLC), squamous cell carcinoma (SC), adenocarcinoma (AC), and large cell carcinoma (LC). Clinically the last three are considered as non-small cell lung cancer (NSCLC). This classification reflects our very limited knowledge on the reasons of the heterogeneous clinical behavior of the individual tumors. This review will concentrate almost exclusively on the very recent data concerning the molecular or genetic/epigenetic characteristics of lung cancer.

Susceptibility genes/genetic predisposition

Smoking is related to lung cancer in 80-90% of cases, while only 10-15% of smokers will have lung cancer. The obvious individual differences could be resulted by genetic predisposition. Lung cancer genes with high penetrance and low frequency are still missing, although – rarely – accumulation of lung cancer in a family may occur. Among the genes with low penetrance and high frequency, those that are responsible for the metabolism of carcinogens or DNA-repair (e.g. CYP1A1, GSTM1, MPO, NQO1) express polymorphism as a potential sensitizing factor. Nevertheless, the results obtained so far are rather contradictory, with the possible exception that the genotype will influence the risk when the exposition is low (e.g. in moderate- or nonsmokers).¹ The study on the relationship between risk factors and susceptibility genes could be difficult, due to the infrequent occurrence of certain polymorphisms in a population, ethnic differences in the allele frequency, heterogeneity in lung cancer histology, etc. In spite of all these obstacles, the role of the susceptibility genes should not be undervalued, and their study must continue.

It has been shown by many studies – with some exceptions – that women are more susceptible to the carcinogens

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in tobacco than men. Women with lung cancer usually smoke less on average, are younger, and several times more frequently have never smoked. Moreover, adenocarcinoma occurs more often in females than in males, especially in nonsmokers and in young women, arguing in favor of hormonal influence in this subtype of lung cancer. Nevertheless, to identify the relevant hormonal, genetic, and metabolic differences between sexes require further data.²

Single genetic changes

Lung cancer is associated with numerous chromosomal regions, genes and pathways. Comparative genomic hybridization showed evidences of nonrandom increases (1p, 1q, 3q, 5p, 6p, 8q, 12, 17q, 19p, 19q, 20p, 20q, X) as well as decreases (2q, 3p, 4p, 5p, 8p, 9p, 10p, 11p, 11q, 13q, 17p) in chromosomal copy numbers. The former are indicative of the presence of oncogenes with potential overexpression and/or increased function (e.g. CMYC, KRAS, EGFR, cyclin D1, BCL2), while the latter suggest the contribution of tumor suppressor genes (e.g. p53, p16, pRb, FHIT, RASSF1A, SEMA3B, PTEN, hOGG1, BAP1).^{3,4}

Similarly to other tumors, a cascade of morphological changes is characterized during lung carcinogenesis. Attempts were made to identify genes that could be responsible for the sequential steps of progression. It seems that the change from normal alveolar or bronchiolar epithelial cells to squamous cell dysplasia or atypical adenomatous hyperplasia is due to – at least partly – by mutation of KRAS (18%) or β -catenin (<10%), as well as by 3p LOH (80%) (inactivation of FHIT, RASSF1, SEMA3B), and inactivation of p16 (70%) or pRb (15%). These changes were followed by p53 inactivation (50%) and 13q LOH (60%) to develop adenocarcinoma or squamous cell carcinoma. Further frequent changes are: 2q LOH (70%), 9p LOH (80%), 18q LOH (85%), 22q LOH (MYO18B) (75%), CMYC amplification (10%). The 3p LOH (80%) and pRb inactivation (90%) is probably important at the early stage of the transformation of epithelial cells with neuroendocrine character. These are followed by p53 inactivation (90%), 5q LOH (70%), 22q LOH (MYO18B?) (70%) to develop SCLC. The further progression of SCLC is associated with CMYC amplification (30%).⁵ The development of neuroendocrine cells, the niche of SCLC cells, is directed by ASH1 (Achate-Scute Homologue-I, an SCLC marker). In lung cancers the expression of GFI-I (a DNA-binding, transcription inhibitory protein) is related to ASH1, gastrin-releasing peptide, and other neuroendocrine markers (synaptophysin, chromogranin A).⁶

It is more than obvious that the malignant phenotype and its heterogeneity within a tumor subclass or even in a given tumor is caused by the interaction of many gene changes and pathways. Focusing on single genes to use as disease

markers is almost hopeless. However, it seems that the different gene changes are not equally important, since targeting single key gene lesions could cause therapeutic benefit. So far in lung cancer the best examples are members of the epidermal growth factor receptor family (EGFR/HER1, ERBB2/HER2, HER3, HER4), which accept different ligands and form homo- and heterodimers in order to be activated.

EGFR pathway

EGFR is occasionally amplified and/or mutated in NSCLC, and can be overexpressed with other members of the family, forming functional heterodimers. In a detailed study no mutations were found in 454 squamous cell carcinomas and 31 large cell carcinomas. Thirty-nine mutations (in exons 18, 19, 21) were detected in 375 adenocarcinomas (10%) (26% in 86 bronchioloalveolar carcinomas and 6% in 289 conventional adenocarcinomas). EGFR mutations and KRAS mutations were mutually exclusive.⁷ These mutations are point mutations (usually a replacement of leucine by arginine at codon 858 [L858R]), or small deletions that affect amino acids 747 through 750. In some cases – especially in adenocarcinomas – anilinoquinazoline EGFR inhibitors (gefitinib, erlotinib) achieved objective response, irrespectively from the previous chemotherapy regimens. Initial immunohistochemistry found no predictive value of pretreatment levels of intratumoral EGFR in the response to gefitinib. However, a close correlation was detected between the coding sequence mutations in the tyrosine kinase domain of EGFR with gefitinib response.^{8,9} It seems that mutations result in a rearrangement of critical residues surrounding the ATP-binding area of the tyrosine kinase domain of the receptor, thereby stabilizing their interactions with ATP-competitive inhibitors. This stabilizing effect can be ruined by further mutation(s), explaining the relapse after initially successful therapy. A further task is to explain the mechanism in those cases where gefitinib was effective without EGFR mutations. Amann et al¹⁰ found that in NSCLC the somatic deletions in the tyrosine kinase domain of EGFR were associated with increased EGFR copy numbers. Treatment with EGFR inhibitors (gefitinib, erlotinib, cetuximab) induced apoptosis in an NSCLC cell line with EGFR gene amplification and exon 19 deletion. Further data indicated that in addition to EGFR mutations, other factors in NSCLC cells, such as high expression of HER family members, may constitutively activate AKT and sensitize cells to EGFR inhibitors.

Sequencing another member of HER family, ERBB2 (HER2) in 120 lung cancers revealed mutations in the kinase domain in 4% of all cases and 10% of adenocarcinomas. In case of mutation of ERBB2 there was no mutation in KRAS, NRAS or BRAF (which could also be

involved in lung cancer development). Amplification of ERBB2 was rare (1/49 in adenocarcinomas, 1/14 in large cell carcinomas). In phase II and III studies Trastuzumab was not effective in NSCLC patients.¹¹

An important downstream pathway from EGFRs is the lipid-kinase route with members as PI3K, AKT, mTOR, eIF-4E. AKT (a serine/threonine kinase) could be a significant target in lung cancer. It was shown that AKT is constitutively active in NSCLC, and tobacco components (as NNK) activate AKT in primary cultures of human lung epithelial cells. Pharmacological inhibitors of PI3K, AKT or EGFR decreased AKT activity and increased apoptosis.¹² eIF-4E is a regulating factor in the initiation of translation of mRNA message. Its overexpression has been shown in many human malignancies, including lung adenocarcinomas, but not in squamous cell carcinomas.¹³ The exact role of eIF-4E is unknown, but it is highly possible that it can promote the synthesis of proteins that are key players in tumorigenesis.

Expression of a single gene sometimes could have a diagnostic value. Sugita et al¹⁴ suggested that the expression of MAGE-A (member of cancer/testis gene family) was associated with the histological classification of squamous cell lung cancer, and can be considered as a diagnostic marker.

Epigenetic changes

Epigenetic changes are those that do not interfere with the nucleotide sequence of DNA, but can influence gene activity. The switch on/off status of a gene is highly dependent on the methylation of the bases or the substitution of chromatin proteins (histones). The changes in the methylation pattern (which is also responsible for the gene imprinting) is related to aging, but it can accompany tumorigenesis. In cancer both hypo- and hypermethylation can cause trouble in the regulation of genetic programs (as cell proliferation or cell death). Methylation-specific PCR is available to perform an extended survey on the methylation of the promoter regions. It was found that many genes with various functions (cell cycle, DNA repair, apoptosis, RAS signaling, invasion markers as cadherins or LAMs) are hypermethylated in both SCLC and NSCLC groups. In certain genes the hypermethylation is astonishingly frequent (e.g. RASSF1A was methylated in all SCLC tumors.) Using various techniques, hypermethylated genes (CDKN2A, MGMT, DAPK, RASSF1A, H-cadherin, RAR β 2) were identified even in the sputum.¹⁵

Disturbances in WNT signaling pathway is rather common in human malignancies. (This pathway is considered as an important regulator of human somatic stem cells.) Inhibitory factor of WNT (WIF1) is a secreted antagonist, and binds to WNT extracellularly. Recently, a decreased production of WIF1 was observed in different tumors. Hypermethylation

of WIF1 promoter was found both in lung cancer cell cultures and in 15 out of 18 resected tumor samples.¹⁶ It seems that hypermethylation could serve as a biomarker to follow all stages of lung cancer growth and progression.

Gene expression profiles

Malignancies with identical morphology or stage can show a diversity of growth rate, invasive and metastatic capacity, and therapeutic response. It is a hope that the application of microarrays will help to identify gene activities associated with different clinical behavior of tumors. Some early studies showed that gene expression profiles distinguish tumor types using hierarchical and probabilistic clustering, and claimed an association between profiles and survival.¹⁷⁻¹⁹ Using strict evaluation criteria these results could be challenged.²⁰ In another and better designed study Beer et al²¹ described 50 genes in 86 primary lung adenocarcinomas that separated two groups of stage I tumors with statistically different survival rate: „good” and „bad”. While such reclassification of tumors on a genetic basis may have therapeutic implications, it is still unclear whether such molecular signatures will be more effective than a single or more prognostic markers.

If reclassification works, one can suggest that microarrays provide aids to diagnosis as well. It is known that differentiation of mesothelioma and metastasis of lung adenocarcinoma could be difficult. Gordon et al²² analyzing 181 samples (150 lung adenocarcinomas, 31 malignant mesotheliomas) identified a panel of 8 genes that made the distinction effective in a training set.

Genetic changes during tumor progression

Cancer progression can be *locoregional* with invasion of the surrounding tissue of the primary tumor, *lymphatic*, primarily to the regional lymph nodes, and *hematogenous*, using blood vessels of the primary tumor to reach distant organ sites. Although these three progression patterns require different genetic machineries of cancer cells, they also have common themes in the three progression pathways: invasiveness including matrix recognition, degradation and migration, and development of a degree of immunoresistance. Various cancer types can use similar tactics but apply different molecular tools to fulfil these tasks. Below, we intend to summarize our recent genetic knowledge on how lung cancers approach this problem. Since lung cancer is one of the most rapidly progressing malignancy to reach various organ sites, the discovery of the progression genes may shed some light on possible alternative pathways which may be applied by other tumor types as well. On the other hand, even our recent fragmented understanding may disclose potential therapeutic targets for a more successful clinical interference.

Prognostic genetic markers NSCLC

This histological entity would not live too long in the literature, however, data on their gene signature are still accumulating. The proliferative fraction of cancer is a common predictor of prognosis and it is relatively easy to assess histologically using Ki-67 immunohistochemistry. Meta-analysis of the available data from the literature indicated that positive and negative results occur in the literature in a relatively equal number, therefore the prognostic role of Ki-67 is controversial. Re-analysis of the data suggested that in a subset of tumors where Ki-67 labeling is high, this elevated level may be associated with poor prognosis.²³ DAPK and IL-10 methylation and the consequent decreased expression (100 patients),²⁴ as well as decreased BCL2 and increased Ki-67 were powerful markers of poor prognosis in a multivariate analysis (260 patients).²⁵ HGF/CMET autocrine loop may be important in NSCLC, since Masuya et al²⁶ found it a marker of poor prognosis (88 patients). On the other hand, PTEN and the downstream target RRM1 were found to be markers of good prognosis (RT-PCR, 120 patients). Along this line, genes involved in chromatin remodeling were found to have prognostic power (BRM and BRG1, 300 patients), since their expression was associated with better survival.²⁷ Immunohistochemical study on the metastasis suppressor genes, PTEN, KAI-1 and NM23-H indicated that the parallel expression of these three rather than the individual expression determines the low metastatic potential of NSCLC (100 patients).²⁸ In another study on 82 patients, microarray and RT-PCR studies revealed that metastatic NSCLC is characterized by S100P, S100A2 as well as MMP and trypsinogen-C/4B expression.²⁹ The role of MMP-9 in NSCLC progression was supported by another study.³⁰ Degradome of NSCLC involves heparanase as well, which serves as marker of poor prognosis.³¹ Interestingly, on the other hand, expression of a transmembrane heparan sulfate proteoglycan, syndecan-1 was found to be marker of good prognosis.³²

Squamous cell carcinoma (SC). Relatively few study focused on SC, although this type of lung cancer is quite frequent. A microarray analysis identified a 50-gene signature of SC, characterizing the progressive form.³³ Regulators of cell proliferation – cyclin E2 (associated with Ki-67) and aurora-2 – were found to be involved in mitotic machinery. The p53/p63 system is a clear characteristic of SC, and its expression is connected to poor prognosis.³⁴ This histological type of lung cancer is characterized also by BAX expression which correlates with that of p53.³⁵ The apoptotic index of SC is associated with BAX level, therefore raising the possibility that BAX is a marker of good prognosis. SC is characterized by the expression of

FHIT, an oncosuppressor in various cancers.³⁶ Interestingly, FHIT protein positivity in this tumor type can be considered to be a marker of poor prognosis associated with high Ki-67 labeling index. Concerning the invasive character of SC, the expression of TIMP1, an MMP inhibitor, seems to be a hallmark of this tumor associated with poor prognosis,^{30,37} together with the overexpression of the motility cytokine, autocrine motility factor (AMF).³³

Adenocarcinoma (AC). Based on pathologic and genetic analysis, the NSCLC group of lung cancer is heterogeneous, but the same is true for its subgroup, adenocarcinoma, containing papillary and bronchioloalveolar subtypes beside the classical form. Early microarray study on AC identified various subgroups based on clinical behavior where aggressive tumors were found to express p16/INK4, arachidonic acid metabolizing enzymes, COX2 and LTB4 dehydrogenase, as well as proteases (cathepsin-L and uPA).¹⁷ On the other hand, the good prognosis signature contained surfactant protein A,³⁸ TTF-1 and hepsin protease. Another microarray study on AC found that the poor prognosis signature contained p63 and caspase-4 involved in the regulation of the apoptotic potential, HER-2 and cytochrome p450,²¹ as well as matrix proteins (laminin and bone morphogenetic protein-2). A large-scale microarray study identified a 17-gene signature of the metastatic adenocarcinomas, but the majority of those genes were found to belong to the tumor stroma: collagen I, laminin, myosin and metallothionein.³⁹ It was revealed that progressing AC is characterized by MMP-9 overexpression.³⁰ Furthermore, COX2-positive AC seems to be a unique subset of ACs characterized by poor prognosis, since these tumors overexpress EGFR, p53 and MMP-9 (71 patients).⁴⁰ A contradictory study was reported on 117 patients where COX2 expression served as marker of good prognosis.⁴¹ Predominant expression of MUC1 mucin (EMA) in AC also serves as marker of poor prognosis.³⁸ The CKIT positive AC could well be another subset of this tumor, characterized by cyclin E2, HER2 and BCL2 co-expression.⁴²

Small cell lung cancer (SCLC). Microarray studies determined that the previously separated groups of neuroendocrine cancers (small- and large cell ones) are genetically highly similar if not identical.⁴³ Neuroectodermal cancers are characterized by CKIT expression (200 patients),⁴⁴ serving also as marker of better prognosis compared to CKIT negative cases. The good prognosis signature of SCLC is characterized by TTF-1, ELAV4 and CAPS gene expression, while the poor prognosis signature contained FOX-C1 and TSGA1 (38 patients).⁴³ A small immunohistochemical study on 17 patients suggested carboxypeptidase E as marker of good prognosis, while γ -glutamyl hydrolase as that of poor prognosis.⁴⁵

Angiogenic phenotype

Lung cancer is a genetically and clinically heterogeneous tumor entity, and the same heterogeneity applies to its vascularization and angiogenic geno/phenotype. Hypoxia-regulated mechanisms are critical in supporting the angiogenic phenotype of cancers including lung cancer, and are orchestrated by HIF1 α transcription factor. In NSCLC, tissue hypoxia is correlated with HIF1 α expression, and one of its target (and marker) gene turned out to be LDH-5. Interestingly, coexpression of HIF1 α and LDH-5 is correlated with poor prognosis.⁴⁶ Hypoxia or constitutive HIF1 α expression are considered to regulate the expression of angiogenic cytokines. In lung cancer various subtypes are characterized by various angiogenic cytokines: SCs overexpress VEGF-C,⁴⁷ adenocarcinomas overexpress VEGF-A as well as bFGF, HGF and even VEGF-C.^{48,49} Microvascular density in lung cancer was shown to have prognostic significance, but since this cancer frequently incorporate preexisting microvessels of the lung tissue, only the density of the newly formed vessels has significance, identified by the CD105 marker.^{50,51} The density of the CD105+ vessels correlates with VEGF-A expression and COX2 in NSCLC but not in SCLC.⁵² Overexpression of the lymphangiogenic cytokine, VEGF-C, both in SC and AC is a marker of poor prognosis,⁴⁹ which is even worse when it is combined with VEGF-A (the regulator of blood vessel formation).

Conclusion

Against all odds, the molecular targeting represents the new direction in every aspects of tumor management, especially in therapy. Advances in cytotoxic therapies are unlikely to result in more than marginal benefit in length or quality of survival. The early approaches designed against molecular targets still had no overwhelming results. However, a deeper understanding of the significance of each molecular change and altered signaling as well as executive pathways should lead us to reach the full potential of cancer management at molecular level. Lung cancer is obviously a group of diseases with heterogeneity in all pathological and clinical aspects, including histology, progression, therapeutic response, and gene expression. The contemporary molecular techniques will be required to tailor their clinical management to accommodate these profound differences.

References

1. *Schwartz AG*: Genetic predisposition to lung cancer. *Chest* 125: 86S-89S, 2004
2. *Olak J, Colson Y*: Gender differences in lung cancer: Have we really come a long way, baby? *J Thorac Cardiovasc Surg* 128: 346-351, 2004
3. *Minna JD, Roth JA, Gazdar AF*: Focus on lung cancer. *Cancer Cell* 1: 49-52, 2002
4. *Balsara BR, Testa JR*: Chromosomal imbalances in human lung cancer. *Oncogene* 21: 6877-6883, 2002
5. *Yokota J, Kohno T*: Molecular footprints of human lung cancer progression. *Cancer Sci* 95: 197-204, 2004
6. *Kazanjan A, Wallis D, Au N, et al*: Growth factor independence-1 is expressed in primary human neuroendocrine lung carcinomas and mediates the differentiation of murine pulmonary neuroendocrine cells. *Cancer Res* 64: 3874-6882, 2004
7. *Marchetti A, Martella C, Felicioni L, et al*: EGFR mutations in non-small-cell lung cancer: analysis of a large series of cases and development of a rapid and sensitive method for diagnostic screening with potential implications on pharmacologic treatment. *J Clin Oncol* 23: 857-865, 2005
8. *Lynch TJ, Bell DW, Sordella R, et al*: Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 350: 2129-2139, 2004
9. *Paez JG, Janne PA, Lee JC, et al*: EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 304: 1497-1500, 2004
10. *Amann J, Kalyankrishna S, Massion PP, et al*: Aberrant epidermal growth factor receptor signaling and enhanced sensitivity to EGFR inhibitors in lung cancer. *Cancer Res* 65: 226-235, 2005
11. Cancer Genome Project and Collaborative Group. *Nature* 431: 525-526, 2004
12. *West KA, Linnoila IR, Brognard J, et al*: Tobacco carcinogen-induced cellular transformation increases Akt activation in vitro and in vivo. *Chest* 125: 101S-102S, 2004
13. *De Benedetti A, Graff JR*: eIF expression and its role in malignancies and metastases. *Oncogene* 23: 3189-3199, 2004
14. *Sugita M, Geraci M, Gao B, et al*: Combined use of oligonucleotide and tissue microarray identifies cancer/testis antigens as biomarkers in lung carcinoma. *Cancer Res* 62: 3971-3979, 2002
15. *Belinsky SA*: Gene-promoter hypermethylation as a biomarker in lung cancer. *Nature Rev Cancer* 4: 707-717, 2004
16. *Mazieres J, He B, You L, et al*: Wnt inhibitory factor-1 is silenced by promoter hypermethylation in lung cancer. *Cancer Res* 64: 4717-4720, 2004
17. *Garber ME, Troyanskaya OG, Schluens K, et al*: Diversity of gene expression in adenocarcinoma of the lung. *Proc Natl Acad Sci USA* 98: 13784-13789, 2001
18. *Bhattacharjee A, Richards WG, Staunton J, et al*: Classification of human lung carcinomas by mRNA expression profiling reveals distinct adenocarcinoma subclasses. *Proc Natl Acad Sci USA* 98: 13790-13795, 2001
19. *Wigle DA, Jurisica I, Radulovich N, et al*: Molecular profiling of non-small cell lung cancer and correlation with disease-free survival. *Cancer Res* 62: 3005-3008, 2002
20. *Kaminski N, Krupsky M*: Gene expression patterns, prognostic and diagnostic markers, and lung cancer biology. *Chest* 125: 111S-115S, 2004
21. *Beer DG, Kardja SL, Huang CC, et al*: Gene expression profiles predict survival of patients with lung adenocarcinoma. *Nat Med* 8: 816-824, 2002
22. *Gordon GJ, Jensen RV, Hsiao LL, et al*: Translation of microarray data into clinically relevant cancer diagnostic tests using gene expression ratios in lung cancer and mesothelioma. *Cancer Res* 62: 4963-4967, 2002
23. *Martin B, Paesmans M, Mascaux C et al*: Ki-67 expression and patients survival in lung cancer: systematic review of the literature with meta-analysis. *Br J Cancer* 91:2018-2025, 2004

24. *Lu C, Soria J-C, Tang X, et al*: Prognostic factors in resected stage I non-small-cell lung cancer: a multivariate analysis of six molecular markers. *J Clin Oncol* 22:4575-4583, 2004
25. *Mehdi SA, Tatum AH, Newman NB et al*: Prognostic markers in resected stage I and II non-small-cell lung cancer: an analysis of 260 patients with 5 year follow-up. *Clin Lung Cancer* 1:59-67, 1999
26. *Masuya D, Huang C, Liu D, et al*: The tumour-stromal interaction between intratumoral c-Met and stromal hepatocyte growth factor associated with tumour growth and prognosis in non-small-cell lung cancer patients. *Br J Cancer* 90:1555-1562, 2004
27. *Fukuoka J, Fujii T, Shih JH et al*: Chromatin remodeling factors and BRM/BRG1 expression as prognostic indicators in non-small cell lung cancer. *Clin Cancer Res* 10:4314-4324, 2004
28. *Goncharuk VN, del-Rosario A, Kren L et al*: Co-downregulation of PTEN, KAI-1, and nm23-H1 tumor/metastasis suppressor proteins in non-small cell lung cancer. *Ann Diagn Pathol* 8:6-16, 2004
29. *Diederichs S, Bulk E, Steffen B et al*: S100 family members and trypsinogens are predictors of distant metastasis and survival in early-stage non-small cell lung cancer. *Cancer Res* 64:5564-5569, 2004
30. *Simi L, Andreani M, Davini F et al*: Simultaneous measurement of MMP9 and TIMP1 mRNA in human non small cell lung cancers by multiplex real time RT-PCR. *Lung Cancer* 45:171-179, 2004
31. *Takahashi H, Ebihara S, Okazaki T et al*: Clinical significance of heparanase activity in primary resected non-small cell lung cancer. *Lung Cancer* 45:207-214, 2004
32. *Shah L, Walter KL, Borczuk AC et al*: Expression of Syndecan-1 and expression of epidermal growth factor receptor are associated with survival in patients with nonsmall cell lung carcinoma. *Cancer* 101:1632-1638, 2004
33. *O-charoenrat P, Rusch V, Talbot SG et al*: Casein kinase II alpha subunit and C1-inhibitor are independent predictors of outcome in patients with squamous cell carcinoma of the lung. *Clin Cancer Res* 10:5709-5803, 2004
34. *Au NHC, Cheang M, Huntsman DG et al*: Evaluation of immunohistochemical markers in non-small cell lung cancer by unsupervised hierarchical clustering analysis: a tissue microarray study of 284 cases and 18 markers. *J Pathol* 204:101-109, 2004
35. *Shabman MS, Srinivasan R, Wali A et al*: Expression of p53 protein and the apoptotic regulatory molecules Bcl-2, Bcl-X_L, and Bax in locally advanced squamous cell carcinoma of the lung. *Lung Cancer* 45:181-188, 2004
36. *Toledo G, Sola JJ, Lorenzo MD et al*: Loss of FHIT protein expression is related to high proliferation, low apoptosis and worse prognosis in non-small-cell lung carcinoma. *Mod Pathol* 17:440-448, 2004
37. *Aljada IS, Rammath N, Donohue K et al*: Upregulation of the tissue inhibitor of metalloproteinase-1 protein is associated with progression of human non-small-cell lung cancer. *J Clin Oncol* 22:3218-3229, 2004
38. *Tsutsumida H, Goto M, Kitajima S et al*: Combined status of MUC1 mucin and surfactant apoprotein A expression can predict the outcome of patients with small-size lung adenocarcinoma. *Histopathology* 44: 147-155, 2004
39. *Ramaswamy S, Ross KN, Lander ES, Golub H*: A molecular signature of metastasis in primary solid tumors. *Nat Genet* 33:49-54, 2003
40. *Araki K, Hashimoto K, Ardyanto TD, et al*: Co-expression of Cox-2 and EGFR in stage I human bronchial adenocarcinomas. *Lung Cancer* 45:161-169, 2004
41. *Yamaguchi NH, Lichtens AJ, Dmarchi LM et al*: COX-2, MMP-9, and Noguchi classification provide additional prognostic information about adenocarcinoma of the lung. A study of 117 patients from Brazil. *Am J Clin Pathol* 121:78-86, 2004
42. *Pelosi G, Barisella M, Pasini F et al*: CD117 immunoreactivity in stage I adenocarcinoma and squamous cell carcinoma of the lung: relevance to prognosis in a subset of adenocarcinoma patients. *Mod Pathol* 17:711-721, 2004
43. *Jones MH, Virtanen C, Honjoh D et al*: Two prognostically significant subtypes of high-grade lung neuroendocrine tumours independent of small-cell and large-cell neuroendocrine carcinomas identified by gene expression profiles. *Lancet* 363:775-781, 2004
44. *Rohr U-P, Rehfeld N, Pflugfelder L et al*: Expression of the tyrosine kinase C-kit is an independent prognostic factor in patients with small cell lung cancer. *Int J Cancer* 111:159-263, 2004
45. *He P, Varticovski L, Bowman ED, et al*: Identification of carboxypeptidase E and γ -glutamyl hydrolase as biomarkers for pulmonary neuroendocrine tumors by cDNA microarray. *Hum Pathol* 35:1196-1209, 2004
46. *Koukourakis MI, Giatromanolaki A, Sivridis E et al*: Lactate dehydrogenase-5 (LDH-5) overexpression in non-small cell lung cancer tissues is linked to tumour hypoxia, angiogenic factor production and poor prognosis. *Br J Cancer* 89:877-885, 2003
47. *Nakashima T, Huan C, Liu D et al*: Expression of vascular endothelial growth factor-A and vascular endothelial growth factor-C as prognostic factors for non-small cell lung cancer. *Med Sci Monit* 10:BR157-165, 2004
48. *Iwasaki A, Kuwahara M, Yoshinaga Y, Shirakusa T*: Basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) levels, as prognostic indicators in NSCLC. *Eur J Cardio-thoracic Surg* 25:443-448, 2004
49. *Ogawa E, Takenaka K, Yanagihara K et al*: Clinical significance of VEGF-C status in tumor cells and stromal macrophages in non-small cell lung cancer patients. *Br J Cancer* 91:498-503, 2004
50. *Mineto TC, Ambrogi V, Baldi A et al*: Prognostic impact of VEGF, CD31, CD34, and CD105 expression and tumour vessel invasion after radical surgery for IB-IIA non-small cell lung carcinoma. *J Clin Pathol* 57:591-597, 2004
51. *Tanaka F, Yanagihara K, Otake Y et al*: Prognostic factors in resected pathologic (p-) stage IIIA-N2, non-small-cell lung carcinoma. *Ann Surg Oncol* 11:612-618, 2004
52. *Stefanou D, Batistatou A, Arkoumani E et al*: Expression of vascular endothelial growth factor (VEGF) and association with microvessel density in small-cell and non-small cell lung carcinoma. *Histol Histopathol* 19:37-42, 2004