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Review

Molecular Genetic Tumor Markers in Non-small Cell Lung Cancer

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Abstract. *Not only serum tumor markers, such as carcinoembryonic antigen (CEA), squamous cell carcinoma antigen (SCC) and carbohydrate antigen (CA) 125, but also serum growth factors have been examined to evaluate tumor stages and to predict the recurrence and metastasis in patients with non-small cell lung cancer (NSCLC) (1-5). In recent years, the analysis of the genome and proteome has advanced remarkably. An array of molecular genetic tumor markers (MGTMs) have been identified based on the biological characterization of tumors, such as tumor development, growth, invasion and metastasis. Molecular genetic tumor marker research has also entered a new era, since comprehensive gene profile analysis using cDNA microarrays and comprehensive protein expression analysis using proteomics technology have been developed. On the other hand, the frequency of lung cancer patients with which various tumor markers are associated is increasing in Japan (6-8). This paper reviews MGTMs characteristic of lung cancer and clarifies the clinical usefulness and applications of MGTM for cancer treatment.*

Clinical usefulness and applications of molecular genetic tumor markers in lung cancer patients (Table I)

MGTMs are useful since they permit the: i) diagnosis of tumor susceptibility, ii) diagnosis of tumor micrometastasis, including early diagnosis and molecular staging, iii) evaluation of tumor profiling, such as tumor malignancy and drug-sensitivity, and iv) molecular targeting therapy (9, 10).

Abbreviations: MGTM, molecular genetic tumor marker; NSCLC, non-small cell lung cancer.

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Diagnosis of tumor susceptibility

Certain types of genetic polymorphisms appear to be related to carcinogenesis. Single nucleotide polymorphism (SNP) loci occur once per approximately 1,000 nucleotides in an individual, and it is thought that 3 to 10 million SNP loci are scattered throughout the genome (11, 12). SNP loci also exist within enzyme genes, such as cytochrome P450 (CYP) and glutathione S-transferase (GST), resulting in individual differences in enzyme properties (structure), transcription and, ultimately, enzyme activity (11, 13). The metabolic activation of carcinogens by CYP1A1, CYP2A6 and CYP2E1 and their detoxication and inactivation by GSTM1 is altered due to their genetic polymorphisms (14-17). A relationship also exists between tumor susceptibility and some kinds of genetic polymorphisms (18), although the genes related directly to the carcinogenesis have not been identified in NSCLC.

Diagnosis of tumor micrometastasis

The presence of micrometastasis is helpful not only for the diagnosis of lung cancer (early diagnosis), but also for the molecular staging when occult micrometastatic tumor cells are detected by pathological, molecular and biological methods (6-8). This cancer is diagnosed as a tumor micrometastasis when gene alternations such as p53, K-ras and p16 mutations and telomerase activity are identified from samples of sputum, peripheral blood and bronchoalveolar lavage fluid (7, 10). It is also classified as a tumor micrometastasis when epithelial markers such as cytokeratin and CEA (carcinoembryonic

Table I. *Clinical usefulness and applications of molecular genetic tumor markers in lung cancer patients.*

Molecular genetic tumor marker	Frequency of abnormalities		
	SCLC*	NSCLC**	CUA***
Oncogene			
K-ras	0%	20%	B, C, D
erbB-1(EGFR)	0%	60%	B, E
erbB-2(HER-2/neu)	0%	30%	C, D, E
bcl-2	55 ~ 80%	10 ~ 35%	C, E
c-/N-/L-myc	15 ~ 30%	5 ~ 10%	C
c-kit	50%	<5%	C
Tumor suppressor gene			
p53	80%	50%	B, C, D, E
RB	95%	20%	C
p16	0%	70%	B, C
p27	100%	70%	C
FHIT	80%	40%	C
RASSF1A	100%	30 ~ 40%	C
Telomerase	100%	80%	B, C, E
Metastasis and invasion markers			
MMP, VEGF, COX-2			C, E
Cell adhesion factors			
E-cadherin, β-catenin			C
Epithelial markers			
cytokeratin, CEA			B
Single nucleotide polymorphism (SNP)			
CYP1A1, CYP2A6, GSTM1, GSTP1			A
TPMT, UGT1A1, TP, DPD			D
Anticancer drug susceptibility markers			
MRP, LRP, MDR, β-tubulin, ERCC1			D

SCLC: small cell lung cancer*
NSCLC: non-small cell lung cancer**
CUA: clinical usefulness and applications***
A. Diagnosis of tumor susceptibility
B. Diagnosis of tumor micrometastasis (early diagnosis and molecular staging)
C. Evaluation of tumor profiling (tumor malignancy)
D. Evaluation of tumor profiling (tumor drug sensitivity)
E. Molecular targeting therapy

antigen) are identified in samples of bone marrow, lymph nodes and peripheral blood, since there is no epithelial tissue normally present in these tissues (6, 8, 19). However, several problems are associated with the inspection of tumor micrometastasis, including its variability with regard to quasi-positivity in proportion to its sensitivity. Gene abnormalities in precancerous lesion can also be identified on inspection of the tumor micrometastasis.

Evaluation of tumor profiling

Recently, useful prognostic factors (tumor malignancy) such as abnormalities in oncogenes, tumor supressor genes and protein expression have been examined (3, 8, 9, 20-24). Cell adhesion factors such as E-cadherin and β-cadherin expression have also been inspected (25). There is no novel prognostic factor with a utility for determining the prognosis of lung cancer patients equal to staging. It is still difficult to predict the prognosis using a combination of multiple factors (7), although there are differences of protein expressions among NSCLC subtypes (26). Recently, it has become possible to estimate individual differences in susceptibility (tumor drug sensitivity) (27) and adverse effects toward anticancer drugs, since gene polymorophisms (SNPs) concerning drug metabolism have been investigated (12, 28). Studies on tumor drug sensitivity may enable us to develop the present order-made therapies into tailor-made therapies (29).

Molecular targeting therapy

In recent times, imatinib mesylate was found to have high antitumor activity against chronic myeloid leukemia (30) and gastrointestinal stromal tumors (31, 32). Trastuzumab is also highly effective against breast cancer (33, 34). Since molecular changes in malignant tissues continue to be characterized, approaches based on targeting the aberrant pathways as a treatment (molecular targeting therapy) have become important (35). For example, gefitinib (iressa), a tyrosine kinase inhibitor, targets the epidermal growth factor receptor (EGFR) gene (36). Lung tumor tissue is predicted to display a significant clinical response to gefitinib when clinically administered (37, 38). Furthermore, there is little correlation between the responses of individual cancers to gefitinib and the level of EGFR protein in lung cancer (39). Most lung cancers that respond to gefitinib possess an activating EGFR mutation (40, 41). Fewer than 10% of lung adenocarcinomas in patients in the United States have such a mutation, although the incidence may be higher than 30% in Japan (42). Phase III studies of matrix metalloproteinase inhibitors (MMPIs), such as neovastat, and antivascular endothelial growth factor monoclonals, such as bevacizumab (avastin), are ongoing in NSCLC (43, 44). Phase III trials have not yet found significant increases in overall survival and toxicity remains an issue.

Expression profiling of lung cancer-related genes, such as oncogenes, tumor suppressor genes and metabolic activation enzymes

Human gene expression profiles can be theoretically examined using recently developed technology such as cDNA microarrays, since human genes number around thirty-three thousand. Gene clusters, which are correlated

with the prognosis of patients and the chemosensitivity of tumors, have been identified in NSCLC patients using cDNA microarrays (45). Garber *et al*. examined 56 NSCLC patients using a 24,000-element cDNA microarray (46) and Bhattacharjee *et al*. examined 186 patients using a 12,600-element microarray (47). Tumor gene expression patterns are different for squamous cell carcinoma, large cell carcinoma (48), small cell carcinoma and adenocarcinoma. Gene expression patterns also make it possible to categorize adenocarcinomas into subgroups correlated with the degree of tumor differentiation as well as patient survival, although the gene cluster in these two reports is different with respect to patient prognosis (46, 47). Nakamura *et al*. found, using cDNA microarray analysis of 425 genes expressed in non-small cell lung carcinomas (NSCLC) with stage IA disease, that forty (9.4%) of the genes were overexpressed, while 74 (17.4%) were underexpressed (49). Currently the protein chip system is also being developed as a potential tool for assisting cancer diagnosis and for screening cancer in high-risk populations (50).

Much information has been obtained by comprehensively analyzing gene and protein expression profiles and it is necessary to effectively apply this information to clinical practice. In the near future, MGTM will probably be included in the NSCLC manual of medical therapeutics.

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