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Review

Prediction of Recurrence after Complete Resection in Patients with NSCLC

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Abstract. *A proportion of lung cancer patients develop recurrence, even after curative resection. This study reviewed the literature, focusing on the key words: recurrence, relapse, relapse-free survival, disease-free survival (DFS), surgery, and non-small cell lung cancer (NSCLC), not overall survival (OS) to evaluate the post-operative risk factors for tumor recurrence after surgery. This article reviews the current status and progress on this subject.*

Lung cancer has been the most common type of cancer world-wide for several decades (1). The standard treatment to cure this disease is surgery. A chest surgeon commonly performs complete resection based on radiographic findings. This type of resection is curative for many patients. However, there are cases that fail to achieve a cure, following surgery. In fact, nearly 50% of patients with non-small cell lung cancer (NSCLC) experience recurrence and have a poor prognosis despite curative resection (2, 3). Therefore, many patients eventually die of their disease (4). TNM staging indicates the level of disease progression and malignant potential of primary lung cancer (5). However, even patients with disease at the same stage are split between the recurrent and non-recurrent group after curative resection. Therefore, the current TNM staging system, which is based on clinical and pathological findings, may have reached the limit of its usefulness (6). Accurately predicting the cases in which disease is, likely to recur can help guide the administration of potentially harmful adjuvant therapies, not only to those most likely to benefit from them (7, 8), but also to those eligible for complete resection, since surgery itself possesses a certain amount of risk (9, 10). There are two methods for the identification of factors related to recurrence following surgery. One is the classical determination using clinical parameters. The other is based of molecular biological techniques. This article reviews the current findings and progress on this subject.

This study searched PubMed, a service of the National Library of Medicine (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi), to identify and extract information published since 2000, regarding factors predictive of recurrence of NSCLC following curative resection. The search focused on the key words recurrence, relapse, relapse-free survival, disease-free survival (DFS), surgery, and NSCLC, not overall survival (OS). The search identified 16 reports of prediction based on clinical parameters (Table I) and 34 based on molecular biological techniques (Table II).

The factors related to recurrence following surgery fall into two categories. One is the classical determination using clinical parameters in addition to the TNM classification. Tumor markers, such CEA were proven to be independent factors for recurrence (11). An extensive pathological investigation is also important. Five Japanese researchers reported that histological differentiation, vessel invasion, lymphatic permeation, and pleural invasion are poor prognostic factors for DFS (11-15). Rena *et al*. reported that patients with stage I pure bronchial alveolar carcinoma (BAC) have significantly longer DFS than those with similar stage adenocarcinoma (16). On the other hand, three East Asian groups identified the standard uptake value (SUV) as a significant independent factor for DFS (17-19). Furthermore, the physical examination becomes vastly more important because lower performance status (PS) and the presence of symptoms are unfavorable prognostic factors for DFS (20). Williams *et al*. concurred with these findings (21). Treatment is closely associated with tumor recurrence. Hung *et al*. reported that treatment for initial recurrence is a significant prognostic indicator in multivariate analyses (22). Surgical procedures other than lobectomy are independently associated

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with tumor recurrence (23). Another team reported that metastasectomy is a significant favorable factor in patients with distant metastases (24). Furthermore, complete mediastinal lymph node (MLN) dissection is associated with improved survival in comparison to random lymph node sampling for the patients with stage I NSCLC (25). Sugimura *et al*. reported that pre-operative chemotherapy and post-operative radiotherapy reduced DFS (26). In another study, adjuvant chemotherapy significantly improved DFS (27). Lee *et al*. used a multiple logistic regression analysis to identify the independent clinical predictors of recurrence and Cox's proportional hazard regression method to successfully develop a clinical prediction model (28). They also developed a predictive system by using an integrated model of clinical information and gene expression (29). The other method for prediction of recurrence uses molecular biology techniques. Lung cancer is a highly aggressive neoplastic disease that includes different histological subtypes with distinct clinicopathological and molecular features (4). Therefore, establishment of useful markers is necessary to accurately classify early- and advanced-stage disease (30). Tumor aggressiveness is related to many genetic alterations associated with cell proliferation, apoptosis, metastasis, and vascularization. An assessment of combination of the expression of cyclin E and p27, the cyclin E-negative/p27-positive group had a significantly higher DFS rate than did other groups (31). Woo *et al*. also reported that the combined use of KRAS status and the Ki-67 expression level was an excellent prognostic marker to predict the post-operative recurrence of stage I adenocarcinoma (32). The clinical usefulness of Ki-67, but not PCNA in primary lung adenocarcinoma was reported (33). There were also some findings from Western researchers. Poleri *et al*. showed that MIB-1 and Bcl-2 are independent prognostic factors of recurrence in stage IB but not for stage IA patients (34). Brock *et al*. reported that methylation of the promoter regions of p16 and CDH13 in both tumor and MLNs are associated with recurrence for patients with stage I NSCLC (35). On the other hand, there have been some negative findings. Baksh *et al*. reported no statistical significance in a comparison of allelic alterations in genes such as *L-myc, hOGG1, APC/MCC, c-fms, p53,* and DCC, and point- mutational changes in *K-ras-2,* with recurrence (36). With relation to metastasis, we previously reported the MACC as a predictive marker for recurrence in primary lung adenocarcinoma by immunohistochemical staining (37) and genetic methods (38). Kinch *et al*. reported that high levels of EPHA2, which has been linked to the regulation of cellular behavior, in the primary tumor predict brain metastases (39). Higher expression of CXCR7, which plays a role in cancer progression by enhancing tumor growth, is associated with poor DFS in patients with p-stage I NSCLC (40). Strong TS expression is a useful marker for predicting postoperative recurrence following surgery in adenocarcinoma of the lung (41). Several attempts have been made to detect micrometastatic tumor cells or disseminated tumor cells (DTC) in lymph nodes (LNs), bone marrow, and circulating tumor cells (CTCs) in peripheral blood (42,43). CTCs adhere to the endothelial lining and migrate through interstitial spaces in order to metastasize. Sienel *et al*. reported that MAGE-A transcripts, a marker for disseminated tumor cells, in bone marrow are associated with poor outcome (44). The DFS curves demonstrated that patients with CK cells in the pN0 lymph nodes had significantly shorter survival periods than those without CK cells (45). *CK19* mRNA in MLNs is significantly associated with an increased risk of rapid recurrence (46), which is consistent with recent data (4). Qiu *et al*. also reported that cytoplasmic keratins in lymph nodes are associated with the times-to- relapse (47). However, in contrast to previous published data, Poncelet *et al*. described that the presence of occult micrometastasis had no influence on DFS (48). Hsu *et al*.'s study also showed that occurrence of bone marrow microinvolvement is not a good predictor of long-term prognosis (49). D'Amico *et al*. reported that recurrence is predicted by decreasing levels of E-selectin, increasing levels of CD44, which is a transmembrane glycoprotein identified on cancer stem cells (CSC) as enhancig tumorigenicity (50), and increasing levels of uPAR (51). The importance of tumor-stromal interactions regulating cancer development has been noted. High MMP-2 expression in tumor and stromal cells is associated with poor survival in DFS analyses (52). We previously described a clinical application of dysadherin, a cancer-related cell membrane glycoprotein, and GalNAc-T3, which catalyzes O-glycosylation and has a role in tumor cell binding of cell adhesion molecules (53, 54). Tumor angiogenesis and the expression of angiogenic factors are closely associated with prognosis. The VEGF family of proteins modulates angiogenesis, which is essential for tumor growth and metastasis. However, positive expression of VEGF-C and VEGF were not independent factors associated with recurrence (4). EGFR stimulation activates intracellular signaling cascades that influence angiogenesis. A high co-expression of both IGF1R and EGFR is a significant prognostic factor of poor DFS (55). Activating mutations within the EGFR tyrosine kinase (TK) domain might predict the risk for recurrence in curatively resected pulmonary AD (56). However, two groups noted a lack of prognostic value of *EGFR* mutations in primary- resected NSCLC (57, 58). Therefore, there is no consensus concerning the role of *EGFR* mutations in predicting NSCLC recurrence.

IGF is the most important systemic mediator of growth hormone and the IGF pathway has been implicated in NSCLC pathogenesis (59). Shersher *et al*. reported that low serum IGFBP5 levels strongly correlate with a positive nodal status and any incidence of recurrence (60). There is new information concerning the relationship between IGF/IGF1R

Table I. *Clinical parameters predicting recurrence after complete resection for patients with NSCLC as seen in current literature.*

Parameter	Stage	Histology	No. of Pts	Significance
High CEA, lymphatic permeation, and pleural invasion	I	NSCLC	402	High CEA levels, lymphatic permeation, pleural invasion and, perioperative transfusion were proven to be independent factors for overall recurrence.
Histological differentiation, vessel invasion, and visceral pleural invasion	I-II	NSCLC	1967	Histological differentiation, vessel invasion, and visceral pleural invasion in stage I and AD histology and visceral pleural invasion in stage IIN0 and stage IIN1 were shown to be independent significant risk factors for recurrence.
Intratumoral vascular invasion and nodal involvement	I-III	NSCLC	819	Intratumoral vascular invasion and nodal involvement significantly influenced recurrence 5 years after complete resection.
Pleural invasion	IA	NSCLC	118	The 5-year DFS of patients with pleural invasion was significantly worse than that for patients without.
Intratumoral blood vessel invasion	IA	NSCLC	217	Independent prognostic factor in poor DFS.
Clinical prediction model	I-IV	NSCLC	1578	A multiple logistic regression analysis was used to identify the independent clinical predictors of recurrence and cox's proportional hazard regression method to develop a clinical prediction model.
SUV	I-III	NSCLC	57	SUV was the most significant independent factor for DFS.
SUV	I	NSCLC	201	Patients with high maxSUV and LVI were more likely to have recurrence.
SUV	I-III	NSCLC	53	A multivariate Cox proportional analysis identified maxSUV as determinant for DFS.
Peripheral nodular BAC	I	AD	1158	Patients with stage I pure BAC had significantly longer DFS than those with similar stage AD.
Number of LNs	I	NSCLC	442	Systematic sampling and complete MLNs dissection were associated with improved survival in comparison to random LNs sampling.
Treatment for initial recurrence	I	NSCLC	123	Treatment for initial recurrence was still a significant prognostic indicator in multivariate analyses.
Large cell histology, low PS and symptoms	I	NSCLC	110	Unfavorable prognostic factors for DFS.
Adjuvant chemotherapy	IB	NSCLC	145	Significantly improved DFS.
Treatment and PS	I-III	NSCLC	1361	Pre-operative chemotherapy and post-operative radiotherapy for the primary lung cancer, and poor PS, reduced DFS.
PS, and symptoms at recurence, liver recurrence, stage IIB or worse, and multiple recurrences	I-III	NSCLC	390	Strongly associated with post-recurrence survival.

Pts: Patients, BAC: bronchioloalveolar carcinoma, AD: adenocarcinoma, LN: lymph node, PS: performance status MLNs: mediastinal lymph nodes, SUV: standard uptake value.

and the epithelial-mesenchymal transition (EMT) for the response to IGF1R-inhibitors (61). A significant association was observed between the IGF1R and E-cadherin, γ-catenin, and Ki-67 (62) and, IGF1R predicts postoperative recurrence in patients with adenocarcinoma (59). EMT is an increasingly recognized mechanism to generate further CSC endowed with a more invasive phenotype (63, 64). In fact, the combination of stem cell markers CD133 and ABCG2 is reported to predict a relapse in stage I NSCLC (65).

Cancer is thought to arise from the multistage accumulation of not only genetic but also epigenetic alterations. Therefore, there are various techniques to investigate epigenetic changes to improve risk stratification. ALDH2, TPM3, ECH1, and IMMT were found by proteomic screening being able to predict tumor recurrence (66). Microarray analysis has made it possible to simultaneously measure the expression of thousands of genes (6). Tumor-specific genetic fingerprints/gene signatures can affect the prognosis (67). The deregulation of micro RNAs is linked to cancer initiation and progression, indicating that miRNAs may act as tumor suppressor genes or oncogenes (7). In fact, micro RNA expression in resected NSCLC could potentially identify those at high-risk of relapse after surgery (68). Single-nucleotide polymorphisms of DNA- and histone-modifying genes might be used as predictive biomarkers not only to identify patients with stage I NSCLC, who could benefit from adjuvant chemotherapy, but also to predict response to adjuvant chemotherapy (69). Despite these findings, it still remains a challenge to assign a prognostic value to these molecular pathways because of poor reproducibility of findings (70). Aberrant methylation of CpG islands acquired in tumor cells in promoter regions can

Table II. *Molecular parameters to predict recurrence*.

Parameter	Assay	Stage	Histology	No. of Pts	Significance
Cyclin E, p27, and Ki-67	IHC	I	NSCLC	62	The cyclin E-negative/p27-positive group had a significantly higher DFS rate than the other groups.
KRAS and Ki-67	PCR and IHC	I	AD	190	Excellent prognostic marker to predict the pos-toperative recurrence of stage I AD.
Ki-67	IHC	I-III	AD	183	Ki-67 expression was independently associated with an increased risk of poor DFS.
MIB-1 and Bcl-2	IHC	I	NSCLC	53	The mitosis count and MIB-1 expression significantly correlated with recurrence and Bcl-2 tumors had a poor outcome.
p16 and *CDH1*	MS-PCR	I	NSCLC	116	Methylation of the promoter regions of p16 and CDH13 in both tumor and MLNs were associated with recurrence for patients with stage I NSCLC.
Allelic imbalance	PCR-based analysis and sequencing	I	NSCLC	39	No statistical significance was seen 25 of comparison of the allelic alterations such as *L-myc, hOGG1, APC/MCC, c-fms, p53,* and *DCC,* and point mutational change in *K-ras-2* with recurrence.
MACC	real-time RT-PCR	I	AD	146	MACC1 gene amplification may be a useful marker for predicting post-operative recurrence.
MACC	IHC	I-III	AD	197	Positive staining for MACC1 expression in resected specimens was associated with a poorer DFS.
EphA2	IHC	I-IV	NSCLC	270	High levels of EphA2 in the primary tumor predict brain metastases.
CXCR7	RT-PCR	I	NSCLC	127	A higher expression of *CXCR7* is associated with poor DFS in patients with p-stage I NSCLC.
TS	IHC	I-III	AD	183	A strong TS expression may be a useful marker for predicting post-operative recurrence in patients with lung AD following surgery.
MAGE-A	RT-PCR	I-III	NSCLC	50	MAGE-A transcripts in the bone marrow were associated with a poor outcome.
CK	IHC	I	NSCLC	115	Presence of micrometastasis in the pN0 LNs was predictive of the pattern of recurrence.
CK/ p53	IHC	I	NSCLC	49	The detection of lymph nodal micrometastasis predicts the recurrence.
CK	IHC	I	NSCLC	107	The increased expression of CK in the LNs was significantly associated with recurrence.
CK	IHC	I	NSCLC	117	Micrometastasis was an independent relevant factor for recurrence.
CEA, p53, and AE1/AE3	FQ-PCR, IHC	I-III	NSCLC	28	The positive LNs for *CEA* mRNA, p53 protein, and AE1/AE3 were associated with the relapse time.
CK19	RT-PCR	I-III	NSCLC	57	*CK19* mRNA detected by RT-PCR in MLNS was significantly associated with an increased risk of rapid recurrence.
CK and Keratin	IHC	I-IV	NSCLC	99	The presence of occult micrometastasis had no influence on DFS.
CK, Ber-EP4, and MNF116	IHC	I-IV	NSCLC	192	Occurrence of bone marrow microinvolvement was not a good predictor of the long-term prognosis.
CK	IHC	I-III	NSCLC	351	The DFS curves demonstrated that the patients with CK cells in the pN0 LNs had significantly shorter survival periods than those without CK cells.
DAPK RARbetaP2	MS-PCR	I-III	AD	72	Demethylation of *DAPK* from normal tissue and hypermethylation of *RARbetaP2* from normal tissue were risk factors for DFS.
MMP-2	IHC		NSCLC	212	High MMP-2 expression in tumor cells was associated with poor DFS. In addition, high stromal MMP-2 expression was related to a poor outcome.
E-selectin, CD44, and uPAR	ELISA	I	NSCLC	196	Recurrence was predicted based on decreasing levels of E-selectin, increasing levels of CD44, and increasing levels of uPAR.
microRNA expression profiles	RT-PCR and microarray	I	NSCLC	77	microRNA expression profiles predicted recurrence of stage I NSCLC after surgical resection.
EGFR mutations	nested PCR amplification	I-III	AD	117	Activating mutations within the EGFR TK domain can be used to predict the risk of recurrence in curatively-resected pulmonary AD.
IGF1R and EGFR	IHC	I-III	NSCLC	125	A high co-expression of both IGF1R and EGFR was a significant prognostic factor of a worse DFS.

Table II. *Continued*

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Parameter	Assay	Stage	Histology	No. of Pts	Significance
IGF1R	IHC	I-III	AD	182	Positive staining for IGF1R was an independent factor associated with tumor recurrence.
IGFBP5	Immunobead assays	I-III	NSCLC	100	Low serum IGFBP5 levels strongly correlated with the incidence of disease recurrence.
CD133 and ABCG2	IHC	I	NSCLC	145	Patients with the dual expression of CD133 and ABCG2 had a high risk of early relapse.
EMT	IHC	I-III	AD	183	EMT does not provide any relevant prognostic information about lung adenocarcinoma.
micro-RNA	RT-PCR	I	NSCLC	46	The microRNA expression in resected NSCLC could potentially identify those at high risk of relapse after surgery.
ALDH2, TPM3, ECH1, and IMMT	Proteomic screening	I	AD	16	Some biomarkers can predict tumor recurrence in patients.
SNPs in epigenetic enzyme and MGMT	Genotyping	I-II	NSCLC	467	The set of genotypes may be used as predictive biomarkers to identify patients who could benefit from adjuvant chemotherapy, and might predict response to adjuvant chemotherapy.

miRNAs: microRNAs, FQ-PCR: fluorescent quantitation reverse transcription-polymerase chain reaction, ELISA: enzyme-linked immunosorbent assay, MS-PCR: methylation-specific PCR assay, MGMT: methylguanine DNA-methyltransferase.

cause the loss of gene function. Demethylation of DAPK from normal tissues and hypermethylation of RARbetaP2 from normal tissues are risk factors for a reduced DFS (71).

There are three limitations for the interpretation of these findings: [i] There is uncertainty differentiating between a second primary lung cancer and recurrent primary tumors. The molecular approaches might powerful methods to resolve this problem (72,73). [ii] Recurrent cases might include slow growth not leading to death, and non-recurrent cases may include the small lesions that cannot be detected (74). [iii] There is abundant heterogeneity in the findings in this review because most studies included various stages of NSCLC. Furthermore, individual studies are typically statistically underpowered with regard to their conclusion (5). Therefore, clinical trials with recurrence as a primary endpoint are needed in order to overcome these limitations.

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Conflict of Interest

The Authors declare no conflict of interest.

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