·Review·

The advancement of predictive diagnosis and molecular mechanism in multiple primary lung cancer

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[**Abstract**] Due to the advanced diagnostic technique and better understanding for multiple primary lung cancers (MPLC), the increasing incidence of MPLC has been reported. Very often, MPLC are misdiagnosed as metastasis because of lacking efficient molecular biomarkers for prediction and diagnosis. Studies on the molecular mechanism for tumorgenesis and progression of MPLC may therefore facilitate the discovery of biomarkers for disease diagnosis and prognosis, so that an individual and rational treatment can be achieved. We tried to further our understanding and improve the diagnostic skill for MPLC by reviewing the current status and the latest advancement of molecular markers related to MPLC.

Key words: Multiple primary lung cancer, molecular mechanism, diagnosis, predict

Surgery-based multi-discipline treatment has significantly prolonged the survival for patients with lung cancer, and at the same time increased risk for multiple primary cancers after the first treatment. At present, diagnostic criteria are simply based on radiology and pathology, as a result, the majority of multiple primary lung cancers are misdiagnosed as metastatic cancer; most patients are consequently misdiagnosed and mistreated. In recent years, a tendency of increasing incidence of multiple primary lung cancer is noticed. Therefore, investigation into the pathogenic mechanism and discovery of diagnostic and prognostic molecular markers for multiple primary lung cancer are of great practical implication in national treatment for patients with the disease. Herein, we summarized the updated definition of multiple primary lung cancer and the issues encountered in clinical setting, current fundamental and clinical studies, pathogenic mechanisms and diagnostic and prognostic molecular markers for the disease.

Updated Definition of Multiple Primary Lung Cancer and Issues Encountered in Clinical Setting

Dual primary lung cancer, also known as multiple primary lung cancer (MPLC), refers to two or more primary cancers in different sites of one or both lungs, with either consistent or different histology but no association between two cancers. Based on the time when the tumors are identified, the disease can be classified as synchronous or metachronous[1]. According to current histopathologic criteria, only dual tumors with different histology or in different organs will be diagnosed as multiple primary lung cancer. The problems with this in clinical setting includes: (1) all the tumors with consistent histopathology are diagnosed as metastatic cancer, but tumors with different genotypes should be actually diagnosed as multiple cancer; (2) synchronous lung tumors in one or different lobes are simply defined as satellite lesion (T4) or metastatic lesion (M1); (3) when the interval between the first and the second tumor is $\leqslant$ 2 years, metachronous multiple primary lung cancer with consistent histology is recommended to be diagnosed as metastatic cancer; when the interval is $>$ 4 years, it is to be diagnosed as multiple cancer; and when the interval is $>$ 2 years and $\leqslant$ 4 years, its diagnosis is still inconclusive; (4) even when histology of two tumors is different, the possibility of metastatic or recurrent tumors cannot be excluded given consideration to the heterogeneity of tumors; and (5) the differential diagnosis of intrapulmonary metastatic lung cancer (via bloodstream dissemination) verse primary lung cancer is also perplexing in clinical setting; however, tumors with consistent histology, and N2 and N3 lymph nodes or multiple-organ metastasis and those with consistent histology and < 2 years interval are often diagnosed as intrapulmonary metastasis[1]. A critical problem is that surgical treatment is a first-choice and effective strategy for multiple primary lung cancer, with a treatment efficacy comparable to that in first primary cancer; but in current clinical setting, the second tumor will usually be diagnosed and treated as metastatic cancer based on radiology, as a result, patients with multiple primary lung cancer lose the opportunity for surgical and other proper treatments. At the same time, it is difficult to obtain the specimens (or the specimens were not harvested at all) for histological classification. Such misdiagnosis poses huge

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damage on the patients, both physically and mentally, and probably in a fatal way. Therefore, one way to tackle this predicament is to explore new practical techniques and markers to diagnose and prognosis for multiple primary lung cancers.

Current Fundamental and Clinical Studies on Multiple Primary Lung Cancer

Multiple primary lung cancers have been identified for almost 60 years, yet relevant clinical and fundamental studies are making little progress. In the PubMed database, 334 and 40 relevant study reports were found as of April 2009 when using 'multiple primary tumors' and 'multiple primary lung cancers', respectively, as the searching keywords in the title. By analyzing these study reports, we found that: (1) since 2000, total numbers of study reports for both keywords significantly decreased, majorly due to greatly decreased clinical study reports; on the other hand, number of fundamental study reports has largely increased; (2) clinical reports published before 2000 were mainly individual case reports, and clinical studies were mostly retrospective; since 2000, clinical studies were mostly etiological and prospective; and (3) number of fundamental study reports was increased ever since 2000, more prominently after 2008. Several consensuses were established in these published literatures: (1) epidemically, the incidence of multiple primary lung cancer was significantly increased, mainly due to prolonged survival in the patients and progress in diagnostic techniques; and (2) surgical treatment was effective in treating multiple cancers as the first choice, with a treatment efficacy comparable to that for the first primary cancer. The common drawbacks in these studies were: (1) diagnosis of multiple primary lung cancer was mainly based on radiology and histopathology, resulting in much misdiagnosis and mistreatment; and (2) little fundamental studies discussing the molecular mechanisms and clinical diagnostic markers of multiple cancers were reports.

Pathogenic Mechanisms and Relevant Factors of Multiple Primary Lung Cancer

The pathogenic mechanisms of multiple primary lung cancer are rather complicated, but currently available clinical and fundamental study data are rare. The incidence of the disease was 1%–2% in previous clinical data and is 5%–6% in latest data. Domestically, incidence of multiple primary lung cancer is 0.5%–1.26%, which is lower than that reported in foreign literature[2]. Analyses on the main reasons for increased incidence of multiple primary lung cancer can help improve the understanding on the pathogenic mechanisms of the disease. Currently, widely accepted reasons for increased incidence of the disease are (1) new diagnostic tools (such as CT and PET) are capable for detecting malignant tumors at earlier stages; (2) with the progress in surgical skill, more and more primary lung cancer patients become appropriate candidates for surgical resection, and diagnostic rate is thus improved; (3) multi-discipline treatment is constantly prolonging the survival for lung cancer patients; (4) multiple primary tumors in one patient is related to prolonged human life-span; (5) increased use of radiotherapy and cytotoxic agents has led to increased risk for a new primary malignant tumor; and (6) with improved clinical awareness among thoracic surgeons, more and more multiple primary lung cancers are diagnosed.

Relationship between smoking and occurrence of multiple primary lung cancer

Cigarette smoke exposure rate in patients with multiple primary lung cancers is higher than that in those with single primary lung cancer, while patients who quit smoking within two years after the first diagnosis of lung cancer have significantly less risk for metachronous multiple primary lung cancer[3]. However, for patients with stage III non-small cell lung cancer who survive for more than three years after chemotherapy and radiotherapy, single variate analysis suggests that increased smoking amount and continued smoking are risk factors for multiple lung cancers. For patients with small cell lung cancer who quit smoking at or after the beginning of the first treatment, the relative hazard ratio for multiple primary lung cancer is significantly reduced; whereas those who continue smoking have significantly increased risk for metachronous multiple primary lung cancer. Patients with small cell lung cancer or non-small cell lung cancer who have successful treated are still at risk for smoking-related multiple lung cancers or other multiple cancers.

Influence of radiotherapy and chemotherapy on occurrence of multiple primary lung cancer

Patients with stage III NSCLC have higher incidence of multiple primary lung cancers after successful radiotherapy and chemotherapy, and the incidence increases with time[4]. Means-Markwell *et al*.[5] followed 29 patients who were at high risk for multiple primary lung cancers and suggested that radiotherapy might be a risk factor for precancerous lesion. The Eastern Cooperative of Oncology Group (ECOG) of the United States has followed up 588 patients who had radical surgery for stage-II or -IIIa non-small cell lung cancer; Among them, 242 patients had radiotherapy and 246 had chemotherapy and radiotherapy, with a median follow-up time of 73 months; a total of 30 patients had multiple primary lung cancers, of which 20 were in the radiotherapy alone group and ten in chemotherapy + radiotherapy group; the median interval between the first and the second cancer was 43 months in radiotherapy group and 36 months in chemotherapy + radiotherapy group; chemotherapy and radiotherapy had significantly shortened the interval from the first to the second primary cancer[6]. The increased risk for multiple primary lung cancers in patients with small cell lung cancer within ten years after the first treatment may derive from thoracic radiotherapy: relative hazard of developing multiple primary lung cancers is increased by about 2 times compared to those without radiotherapy, for those who have radiotherapy and continuous smoking, the risk is increased by about 4 times.

It was reported that patients who were diagnosed with lung cancer by sputum cytology with negative chest X-ray had higher incidence of metachronous multiple tumors. Saito *et al*.[7] reported an analysis on 127 NSCLC patients with negative imageology:

13 patients had metachronous tumors after the surgery; the 5-year cumulative incidence of metachronous tumors was 11%; the incidence of developing a second primary cancer in patients with non-small cell lung cancer after the first treatment over the study period was 2.2% per patient-year. Bechtel *et al.*[8] reported an analysis on 27 NSCLC patients with negative radiology: seven patients had metachronous cancers after the surgery. This was consistent with the high incidence of metachronous lung cancer observed in patients with central type lung cancer who had received sleeve resection.

Common sites and histological types of multiple primary lung cancers

In patients with non-small cell lung cancer, 70% of the second primary lung cancer had consistent histology with the first cancer, and 55% of them were seen in contra-lateral lung[9]. A large-scale multi-center study (with overlapped information) reported that 26 (51%) out of 51 cases of non-small cell lung cancer were squamous cell carcinoma[10]. While in small cell lung cancer, the second primary cancers were usually seen in different anatomic sites from the first primary cancer, and they were often squamous cell carcinomas in histology[11]. The major histology of multiple primary lung cancer has shifted from squamous cell carcinoma to adenocarcinoma.

Changes of incidence of multiple primary lung cancer with time

After the treatments, the incidences of multiple primary lung cancers in patients with both small and non-small cell lung cancers increased with time. Incidence of multiple primary lung cancer is increased after surgical treatment for lung cancer, with an incidence of 1% in the first five years, while the incidence was increased to 2% after five years. Some studies suggested that incidence of multiple primary lung cancer was increased from the fourth year after treatment. But contrary conclusion was also reached: incidence of multiple primary lung cancers within the first five years after surgical resection was 2.6% and was reduced to 1% after the sixth year[12]. By analyzing the prognosis in patients with small cell lung cancer who had survived for more than two years, we found that the risk of developing multiple primary lung cancer in these patients was 2%–14% per patient-year and increased by 2–7 times within ten years after the first diagnosis[13]. Recent study suggested that 10% of the patients with small cell lung cancer who survived for more than two years would eventually develop non-small cell lung cancer[12].

Molecular Mechanisms and Diagnostic and Prognostic Markers for Multiple Primary Lung Cancer

The exact pathogenic mechanism of multiple primary lung cancer is still unclear, but for the time being, field cancerization is one important hypothesis to explain the occurrence of multiple primary malignant tumors. According to the hypothesis, the organ systems exposed to the same carcinogen have increased chance for developing malignant tumors[14]. Examples are synchronous lung cancers, head-and-neck cancer or bladder cancer, because smoking as a carcinogen has important role in the etiology of these malignant tumors[15].

Besides environmental factors, genetic factors also have important role in the occurrence of malignant tumors. At present, more than 30 genes are known to be related to the occurrence of malignant tumors, and those closely related to increased risk for (multiple) tumors are tumor suppressor genes and DNA repairing genes[14]. Haraguchi *et al.*[16] demonstrated in their study that multiple primary lung cancers tended to be inherited in family; lung cancer patients with family history had higher chance for a second or third primary tumor than those without family history, and male smokers also had higher incidence than non-smokers. Latest studies found that genomic instability and changes in gene expression profile (such as tumor suppressor genes and DNA repairing genes[14]) and even mutation and deletion of chromosomes were closely related to the occurrence of multiple primary cancers. Froio *et al.*[17] labeled and analyzed the LOHs of the 40 chromosomes in a patient with three synchronous multiple primary cancers (adenocarcinoma and endobronchial squamous cell carcinoma in upper lobe and neuroendocrine carcinoma in lower lobe), and found different genetic labels for three tumors (genetic similarity of adenocarcinoma with squamous cell carcinoma was 0.28, while similarity with small cell lung cancer was 0.52), suggesting that three tumors were independent on each other and that genetic markers were useful in diagnosing multiple cancers. Mercer *et al.*[18] conducted a study on lung metastatic carcinoma from head-and-neck squamous cell carcinoma and primary lung carcinoma and found that they were hardly distinguishable in radiology and histology, but analysis on the microsatellite alterations and the deletion sites and manners in genomic DNA of the primary and metastatic cancers was helpful in distinguishing them, suggesting that detections of microsatellite alterations and deletion sites in tumor cell DNA could be used as diagnostic and prognostic markers for multiple cancers. Orlow *et al.*[19] analyzed the changes in DNA damage and repairing ability in peripheral monocytes in a group of 108 patients with multiple primary lung cancers and a group of 99 patients with single lung cancer as control, and revealed that the occurrence of the second primary cancer could be predicted by detecting DNA damage and repairing ability. Huang *et al.*[20] found in their study on multiple primary lung cancer and metastatic cancer that loss of heterogeneity was consistent among primary cancer and metastatic cancer lesion, but inconsistent among most synchronous or metachronous multiple primary lung cancers[2]; therefore, they provided strong evidence support for tumor molecular blotting as differential marker for primary cancer or metastatic cancer[20]. van Rens *et al.*[21] performed analyses on the mutant sites of p53 gene in the primary tumor lesions and the second tumor lesions of 64 patients with lung cancer (including 31 patients with multiple primary cancer) and found that difference in mutant sites of p53 gene was a useful molecular marker in diagnosing primary cancer and metastatic cancer.

Issues and Prospect

Since multiple primary lung cancerswere difficult to understan

and diagnose, its incidence has not been accurately calculated. In general, the incidence of multiple primary lung cancer may be underestimated because (1) most multiple primary lung cancers that occur after the treatment for lung cancer are treated as recurrence or metastasis, and it is currently impossible to identify multiple primary cancers among metastatic cancer; (2) the majority of metachronous cancers have consistent histology, but due to a larger anatomic distance, multiple primary cancers are less likely to be diagnosed or completely detected; and (3) available data merely involved multiple primary lung cancers that were confirmed after the second surgery, while those who were not given a second surgery for the second tumor did not have the opportunity to be confirmed whether it was multiple cancer. At present, second surgery for multiple primary lung cancers only accounts for 4.5% , or 0.8% –10% in other reports, of all surgeries for lung cancer, suggesting surgical treatment is given only in a limited number of patients. Although some progress has been made in the fundamental research of multiple primary lung cancers, the studies mainly focus on individual cases, retrospective data analysis and animal experiments, and there is still a long way to go before they are clinical applicable. Moreover, progress in diagnostic techniques for lung cancer and minimally invasive thoracic surgeries has enabled more patients with multiple primary lung cancers and metastatic cancer to benefit from surgical treatments, while providing adequate analyzable tumor specimens for clinical and fundamental studies. Therefore, it is necessary and urgent to study the molecular mechanisms underlying the occurrence and development of multiple primary lung cancers on DNA, protein and general levels, and to develop molecular markers to predict and diagnose multiple primary lung cancer using available molecular biological techniques and clinical (tumorous and hematological) specimens.

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