PROGNOSTIC CLINICOPATHOLOGICAL FACTORS AND BIOMARKERS IN LUNG CANCER WITH BRAIN METASTASIS

PhD thesis

Katalin Fábián, MD

Semmelweis University
Doctoral School of Clinical Medicine

Supervisor:
Judit Moldvay, MD, PhD

Official Reviewers:
Nóra Bittner, MD, PhD
Katalin Borka, MD, PhD

Head of the Final Examination Committee:
Péter Nagy, MD, DSc

Members of the Final Examination Committee:
Gabriella Gálffy, MD, PhD
József Tóvári, PhD

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INTRODUCTION

Lung cancer is the leading cause of cancer death both in Hungary and worldwide. At the time of lung cancer diagnosis brain metastasis can be found in 10-25% of the patients, and its incidence can increase up to 40-50% during the course of the disease. In advanced stage non-small cell lung cancer development of brain metastasis can be observed in 30-50% of the patients, while in small cell lung cancer its incidence is 15% at the time of diagnosis and can reach up to 50-60% at autopsies.

Patients with brain metastasis have poor prognosis; without oncological treatment the survival after the diagnosis of brain metastasis is about 1 month. This survival can be extended to about 4-6 months with whole brain radiotherapy. With the application of stereotaxic radiotherapy or surgical metastasectomy the overall survival can be improved to 6-12 months and to 9-14 months, respectively. Brain metastasis adversely affects the quality of life as well.

According to the literature, in lung cancer the risk of developing brain metastasis is increased in younger patients, in case of higher lymph node stage, in females, in non-squamous histological subtype, and in the use of neoadjuvant chemotherapy. Based on several survival studies, prognostic indices have been established. According to their data, the overall survival in case of brain metastasis is better in younger patients, with good performance status, and in solitary brain metastasis without extracranial tumor spread. In spite of these studies, the prognosis of lung cancer patients with brain

metastasis can't be determined properly yet. According to the literature there is hardly any data about the significance of the primary tumor location (lobar and/or bronchial), and there is no information about their influence on the development of brain metastasis and overall survival.

Peritumoral edema in primary brain tumors has prognostic value, however, in brain metastasis from lung cancer it was only sporadically investigated. Similarly, the role of peritumoral edema in survival has not been demonstrated yet.

In several studies higher lymph node status had an increased risk of brain metastasis. Some investigators also noted some cases with brain metastasis (sometimes even multiple metastases) without lymph node spread. This phenomenon, however, was not investigated in larger patient cohorts.

While significant number of data is available about histological biomarkers as predictive or prognostic factors, there is only few information about the changes in biomarker expression during development of brain metastasis. In our previous study, we examined the expression of several biomarkers of cell adhesion, differentiation, proliferation, and apoptosis in lung cancer patients using their primary tumor sample and the corresponding brain metastasis tissue. In this study, however, histological subtype analysis has not been performed.

In lung cancer with brain metastasis the correlation between peritumoral edema and histological biomarkers has not been widely studied.

The prognostic and/or predictive value of KRAS mutation in lung cancer is intensively investigated, however, it is not fully described. In colorectal carcinoma KRAS mutation incidence was found to be increased in patients with multiple organ metastases, however, no such data can be found in lung cancer. The incidence and the prognostic value of KRAS mutation was only sporadically studied in lung cancer with brain metastasis.

In summary, in my PhD thesis these scientific questions have been investigated.

OBJECTIVES

1. Our aim was to investigate a large cohort of lung cancer patients with brain metastasis. In this cohort we wanted to study the relationship between histological subtypes or the location of the primary tumor and brain metastasis development. We also wanted to investigate the influence of these parameters on overall survival and survival from the development of brain metastasis.

2. Our main objective was to characterize lung cancers metastasizing early and late into the brain. We wanted to study the clinicopathological features of these tumors and their metastasis, especially the relationship with peritumoral edema.

3. The prognostic value of the peritumoral edema in brain metastasis from lung cancer has not been intensively investigated so far. The aim was to determine its prognostic significance in our large cohort of lung cancer patients with brain metastasis.

4. In the pathologic WHO classification of lung cancer the importance of distinguishing adenocarcinoma from squamous cell lung cancer has been recently underlined. Accordingly, we

aimed to investigate the difference in biomarker expression in these histologic subtypes of lung cancer. Next, we wanted to study the expression of important biomarkers of cell adhesion, differentiation, proliferation, DNA repair and apoptosis in primary lung cancer tissues and their corresponding brain metastasis. We also aimed to correlate the studied biomarkers with the size of peritumoral brain edema.

5. Next, our objective was to investigate the KRAS mutation status in lung adenocarcinoma patients with distant organ metastasis, especially in tumors metastasizing to the brain. We also wanted to examine the prognostic value of KRAS mutation in lung cancer patient with brain metastasis.

PATIENTS AND METHODS

Patient cohorts

#1 cohort: We studied the clinicopathological data of 575 consecutive lung cancer patients with radiologically (by CT scan or MRI) detected brain metastasis.

#2 cohort: We investigated the expression of 29 biomarkers of cell adhesion, differentiation, proliferation, DNA repair and apoptosis in tumors of 52 non-small cell lung cancer patients. We studied the correlation between biomarker expression and clinicopathological parameters, such as the extension of peritumoral edema. In 26 patients the primary tumor and the matching brain metastasis were also studied. 26 patients, who had no evidence of brain metastasis during long term follow-up period served as a control group.

#3 cohort: In our study, 500 consecutive stage IV lung adenocarcinoma patients with known KRAS mutational status were analyzed. In 84 cases the distant metastasis was found in the brain.

Peritumoral edema

The thickness of peritumoral edema was measured on the images of CT scans and/or MRI. Typically, the axial slice where the enhancing tumor appeared largest was chosen for peritumoral edema measurement. We created three categories: 1: no edema (0 mm), 2: moderate edema (1-10 mm), 3: severe edema (>10 mm).

Tissue samples

Tissue microarray (TMA)

For further investigation we used formalin-fixed paraffin-embedded tissue blocks of surgically resected primary lung cancer and brain metastasis. Tissue cores were selected and punched out from the relevant tumor fields of donor blocks. With TMA method we embedded these samples in a recipient block, which could incorporate 70 samples.

Immunohistochemistry

Important molecules of cell adhesion, differentiation, proliferation, cell cycle regulation, and proteins of DNA replication, repair and apoptosis were investigated by immunohistochemistry. The immunostained sections were scored semiquantitatively using a 4-scale system (0-3).

KRAS mutation analysis

KRAS mutations were identified by microcapillary-based restriction fragment length analysis on formalin-fixed paraffin-embedded tissue blocks or cytological samples.

Statistical analysis

For statistical analysis we used SPSS software. The differences were considered statistically significant in case of $p<0.05$.

RESULTS

Clinicopathological patient cohort

According to our results, the frequency of the brain metastasis was higher in lung adenocarcinoma and small cell lung cancer. The risk of developing brain metastasis was higher in central airway primary tumors, in case of female gender and in younger patients (≤50 years).

The peritumoral edema was primarily characteristic for adenocarcinoma and squamous cell carcinoma, while in small cell lung cancer the extension of edema was significantly smaller ($p<0.001$ and $p <0.001$, respectively).

A positive correlation was observable between the size of brain metastasis and the thickness of peritumoral edema ($p<0.001$, $r=0.330$). Patients with wider peritumoral brain edema had longer median survival after brain metastasectomy ($p=0.007$).

Peritumoral edema was thicker in supratentorial tumors ($p=0.019$), in younger patients (≤50 years) ($p=0.042$) and in females ($p=0.016$). The time to brain metastasis was shorter in case of central airway primary tumors (5.3 vs. 9.0 months, $p=0.035$). Early metastasis was characteristic for adenocarcinoma (<0.001).

Out of 500 studied lung cancer patients with brain metastasis, there was no evidence of lymph node metastasis in 135 patients. These patients had mostly peripheral lung cancer ($p<0.001$) and

had longer time to brain metastasis and overall survival (both $p<0.001$).

The analysis of primary lung cancer and corresponding brain metastasis by immunohistochemistry

Our analysis for predicting brain metastasis showed that increased collagen XVII ($p=0.011$) in adenocarcinoma, increased caspase-9 ($p=0.008$), CD44v6 ($p=0.031$), and decreased cellular apoptosis susceptibility protein (CAS) ($p=0.029$) and Ki-67 ($p=0.032$) in squamous cell carcinoma correlated significantly with brain metastasis development.

In primary squamous cell carcinoma increased β-catenin ($p=0.026$) and E-cadherin ($p=0.026$), as well as decreased caspase-9 ($p=0.037$) expression showed significant correlation with the extension of peritumoral edema. In brain metastatic squamous cell carcinoma tissues decreased CD44v6 ($p=0.002$) expression was found in tumors with thicker peritumoral edema.

A significant positive correlation was found between smoking and the expression of caspase-8 ($p=0.045$) and p16 ($p=0.024$) in brain metastatic adenocarcinoma. In squamous cell carcinoma a negative correlation was demonstrated in the non-metastasizing group with caspase-3 ($p=0.039$) expression, and in brain metastatic tumors with p27 ($p=0.038$) expression.

KRAS mutation analysis in lung adenocarcinoma with brain metastasis

According to our results the incidence of brain metastasis was 16.8% in the whole cohort (n=500). These patients were significantly younger than the patients with lung metastasis (p=0.009). In the whole cohort the incidence of KRAS mutation was 28.6%. In patients with brain metastasis the KRAS mutation occurrence was similar, 29% (p=0.898). The overall survival of the brain metastatic patients with KRAS mutation showed no difference from patients with KRAS wild type tumor (p=0.504).

CONCLUSIONS

1. In our study we found that the development of brain metastasis is characteristic for adenocarcinoma and small cell lung cancer. The risk of brain metastasis development is higher in females, in younger (≤50 years) males and in case of central airway lung cancer. To the best of our knowledge this is the first study on airway location of the primary tumor and brain metastasis. We demonstrated that in case of central airway lung cancer the tumors are biologically more aggressive, these tumors have higher risk of brain metastasis development, therefore, these patients need closer observation and follow-up.

Early brain metastasis was characteristic for adenocarcinoma.

2. We proved, that lung cancers with brain metastasis but without lymph node spread, also known as “brain only” tumors, are different entities from the other lung cancer subtypes, because they have better prognosis. These tumors have mainly peripheral location and have longer time to brain metastasis and longer overall survival.

3. We first described the importance of peritumoral brain edema in large cohort of lung cancer patients. Peritumoral edema was characteristic for adenocarcinoma and squamous cell carcinoma, while it was rare in case of small cell lung cancer. The extension of edema correlated with the size and the location of brain

metastasis. In our whole cohort the peritumoral edema had no prognostic value, while in patients with brain metastasectomy the edema was associated with better prognosis.

4. In our next study we analyzed the expression of biomarkers of cell adhesion, proliferation and apoptosis in lung cancer. We found remarkable differences between adenocarcinoma and squamous cell carcinoma regarding protein expressions. In both primary tumor tissues and brain metastatic tissues we described important expression differences between these two histologic subtypes. The importance of smoking and gender in the biomarker expression could also be demonstrated. The peritumoral edema in brain metastasis is a rarely studied factor in lung cancer. We first described correlations between peritumoral edema and certain biomarker expressions.

5. Finally, we investigated the KRAS mutation status in a relatively homogenous cohort of lung adenocarcinoma patients with distant metastasis. Regarding the incidence of KRAS mutation, our results were in line with other studies and also with the data of Hungarian lung cancer patients. Although the development of brain metastasis itself is considered as a bad prognostic factor, in our brain metastatic cohort the prognostic role of KRAS mutation on survival was not proved.

BIBLIOGRAPHY OF PUBLICATIONS

Publications related to the theme of the PhD thesis

Fabian K, Gyulai M, Furak J, Varallyay P, Jackel M, Bogos K, Dome B, Papay J, Timar J, Szallasi Z, Moldvay J. Significance of Primary Tumor Location and Histology for Brain Metastasis Development and Peritumoral Brain Edema in Lung Cancer. ONCOLOGY 91:(5) pp. 237-242. (2016) (IF: 2,262)

Fábián K, Németh Zs, Furák J, Tiszlavicz L, Pápay J, Krenács T, Tímár J, Moldvay J: Protein expression differences between lung adenocarcinoma and squamous cell carcinoma with brain metastasis. ANTICANCER RESEARCH 34:(10) pp. 5593-5597. (2014) (IF: 1,826)

Lohinai Z, Klikovits T, Moldvay J, Ostoros G, Raso E, Tímár J, Fábián K, Kovalszky I, Kenessey I, Aigner C, Rényi-Vámos F, Klepetko W, Dome B, Hegedűs B: KRAS-mutation incidence and prognostic value are metastatic site-specific in lung adenocarcinoma: poor prognosis in patients with KRAS-mutation and bone metastasis. SCIENTIFIC REPORTS 7: Paper 39721. 8 p. (2017) (IF:4,122)

Publications not related to the theme of the PhD thesis

Fabian K, Puskas R, Kakuk T, Pres L, Fejes D, Szegedi Z, Rojko L, Szallasi Z, Dome B, Pipek O, Moldvay J: Renal Impairment Hampers Bisphosphonate Treatment in a Quarter of Lung Cancer Patients with Bone Metastasis. BASIC & CLINICAL PHARMACOLOGY & TOXICOLOGY 122:(1) pp. 126-132. (2018) (IF: 2,659 (2017))

Rojko L, Reiniger L, Teglasi V, Fabian K, Pipek O, Vagvolgyi A, Agocs L, Fillinger J, Kajdacsi Z, Timar J, Dome B, Szallasi Z, Moldvay J: Chemotherapy treatment is associated with altered PD-L1 expression in lung cancer patients. JOURNAL OF CANCER RESEARCH AND CLINICAL ONCOLOGY 144:(7) pp. 1219-1226. (2018) (IF: 3,282 (2017))

Krencz I, Sebestyen A, Fabian K, Mark A, Moldvay J, Khoor A, Kopper L, Papay J: Expression of mTORC1/2-related proteins in primary and brain metastatic lung adenocarcinoma. HUMAN PATHOLOGY 62: pp. 66-73. (2017) (IF: 3,125)

Moldvay J, Fábián K, Jäckel M, Németh Z, Bogos K, Furák J, Tiszlavicz L, Fillinger J, Döme B, Schaff Z: Claudin-1 Protein Expression Is a Good Prognostic Factor in Non-Small Cell Lung Cancer, but only in Squamous Cell Carcinoma Cases. PATHOLOGY AND ONCOLOGY RESEARCH 23:(1) pp. 151-156. (2017) (IF: 1,935)

Teglasi V, Reiniger L, Fabian K, Pipek O, Csala I, Bago AG, Varallyai P, Vizkeleti L, Rojko L, Timar J, Dome B, Szallasi Z, Swanton C, Moldvay J: Evaluating the significance of density, localization, and PD-1/PD-L1 immunopositivity of mononuclear cells in the clinical course of lung adenocarcinoma patients with brain metastasis. NEURO-ONCOLOGY 19:(8) pp. 1058-1067. (2017) (IF: 9,384)

Lohinai Z, Hoda MA, Fabian K, Ostoros G, Raso E, Barbai T, Timar J, Kovalszky I, Cserepes M, Rozsas A, Laszlo V, Grusch M, Berger W, Klepetko W, Moldvay J, Dome B, Hegedus B: Distinct epidemiology and clinical consequence of classic versus rare EGFR mutations in lung adenocarcinoma. JOURNAL OF THORACIC ONCOLOGY 10:(5) pp. 738-746. (2015) (IF: 5,040)

Moldvay J, Rokszin Gy, Abonyi-Tóth Zs, Katona L, Fábián K, Kovács G: Lung cancer drug therapy in Hungary – 3-year experience. ONCOTARGETS AND THERAPY 8: pp. 1031-1038. (2015) (IF: 2,272)

Moldvay J, Szallasi Z, Puskas R, Komaromi T, Fabian K, Kovalszky I, Papay J: Bone Metastasis with Histology Different from The Primary Lung Cancer and The Skin Metastasis. TRANSLATIONAL BIOIMEDICINE 6:(2) Paper 16. 3 p. (2015) (IF:0)

Podmaniczky E, Fábián K, Pápay J, Puskás R, Gyulai M, Furák J, Tiszlavicz L, Losonczy Gy, Tímár J, Moldvay J: Decreased ERCC1 expression after platinum-based neoadjuvant chemotherapy in non-small cell lung cancer. PATHOLOGY AND ONCOLOGY RESEARCH 21:(2) pp. 423-431. (2015) (IF: 1,940)

Cserepes M, Ostoros G, Lohinai Z, Raso E, Barbai T, Timar J, Rozsas A, Moldvay J, Kovalszky I, Fabian K, Gyulai M, Ghanim B, Laszlo V, Klikovits T, Hoda MA, Grusch M, Berger W, Klepetko W, Hegedus B, Dome B: Subtype-specific KRAS mutations in advanced lung adenocarcinoma: A retrospective study of patients treated with platinum-based chemotherapy. EUROPEAN JOURNAL OF CANCER 50:(10) pp. 1819-1828. (2014) (IF: 5,417)

Fábián K, Furák J, Gyulai M, Jackel M, Várallyay P, Pápay J, Losonczy Gy, Moldvay J: Agyi áttétet adó tüdőrákok klinikopatológiai analízise. MEDICINA THORACALIS (BUDAPEST) 66:(3) pp. 106-112. (2013) (IF: 0)

Fábián K, Moldvay J: Az agyi áttétek körül kialakuló peritumoralis ödéma jelentősége a tüdőrákban. MEDICINA THORACALIS (BUDAPEST) 65:(1) pp. 17-25. (2012) (IF: 0)

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