PhD thesis

Clinical relevance of KRAS mutational status and tumor location in bone-metastatic lung adenocarcinoma

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Budapest

2022

1. Introduction

Lung cancer, the leading cause of cancer-related deaths, is the most frequently diagnosed cancer worldwide in both sexes. The most common histologic subtype of lung cancer is lung adenocarcinoma (LADC), which comprises 40-50% of all lung cancer cases. With the development of molecular pathology and precision medicine, significant progress has been made in the treatment and prognosis of advanced non-small cell lung cancer (NSCLC) over the past two decades. In LADC, the most common gain-of-function alteration is the Kirsten rat sarcoma viral oncogenic homolog (KRAS) mutation, which accounts for about 25-30% of LADCs in Western countries and approximately 10-15% of Asian LADCs. The clinicopathological significance of different KRAS mutations is currently intensively studied, as both their prognostic and predictive role is controversial. The KRAS protein, encoded by the KRAS protooncogene, is a small guanine triphosphatase (GTPase) that serves as a binary linker in the signal transduction of most receptor tyrosine kinases, including epidermal growth factor receptor (EGFR), hepatocyte growth factor receptor (MET), or anaplastic lymphoma kinase (ALK), and thus plays a crucial role in tumor progression. Due to the frequency of LADC, several preclinical and clinical studies have been conducted to explore effective therapeutic options for KRAS mutation. Nevertheless, to date, no effective RAS inhibitors are used in routine clinical practice for LADC except for the KRAS G12C mutation.

2. Objectives

In a previous study, our research team demonstrated that KRAS mutation is associated with significantly shorter survival (compared to KRAS WT) in LADC patients with bone metastases. However, the therapeutic relevance of KRAS status is currently unknown in this patient population. Furthermore, we demonstrated in preclinical NSCLC models that KRAS WT LADC cell lines are more sensitive to zoledronic acid-induced prenylation inhibition and consequent inhibition of proliferation both in vitro and in vivo. At the same time, those carrying KRAS mutations are resistant to this inhibitory effect. Therefore, we aimed to investigate the significance of KRAS mutational status according to BTx and RTx in LADC patients diagnosed with bone metastases.

Furthermore, although the presence of distant organ metastases is a significant factor for an unfavorable prognosis in LADC patients, metastatic patterns and their influence on survival have not been extensively analyzed with regards to the localization of the primary tumor. Our group previously found that bone metastases were more frequent in patients with central tumors, whereas lung metastases in those with peripheral LADCs. Additionally, central LADCs were also associated with early metastatic spread. However, to date, the bone metastasis pattern is still largely unexplored in patients with bone-only metastases. Therefore, our cross-sectional study aimed to examine the impact of primary tumor location on bone metastasis site, type of affected bones and survival in a large comprehensive cohort of advanced-stage LADC patients diagnosed with skeletal metastases. This information

may help to guide early surveillance for bone metastasis detection or interventions in high-risk groups to improve the patients' survival and quality of life.

3. Results

The effects of bisphosphonate and radiation therapy in bone metastatic lung adenocarcinoma

A total of 134 patients diagnosed with LADC and simultaneous bone metastasis were included in this study. 93 patients of the full cohort were identified as KRAS WT (69.4%) and 41 (30.6%) as KRAS mutant patients. The mean age of patients with KRAS mutation was found to be significantly lower than those with WT KRAS (58.9 vs. 62.9, respectively; p=0.029). 83 patients (62%) received BTx and the mean age was significantly lower among patients with BTx than among those without BTx (mean age 60.3±9.2 vs. 64.0±10.3, respectively; p=0.03). With regards to specific bisphosphonate agents 37, 9 and 28 patients received clodronate, pamidronate and zoledronic acid, respectively. Of note, no data was available on the exact type of administered bisphosphonate agent in 9 cases. Our cohort consisted of 85 male and 49 female patients and no significant association was observed between gender and mutational status or therapeutic modality. KRAS mutation showed no association with ECOG score. The administration of RTx or BTx was also not significantly associated with KRAS mutational status. In contrast, patients receiving BTx were significantly more likely to have ECOG 0 and RTx.

The median OS for the entire cohort was 7.8 months. Patients with KRAS WT tumors had a significantly longer median OS compared to those with KRAS mutation (10.2 months vs. 5.1 months, respectively). Kaplan-Meyer curves demonstrated longer median OS in patients who received

BTx (10.1 months vs. 4.3 months in BTx-naive). Notably, patients receiving second-generation BTx exhibited significantly superior OS compared to those receiving first-generation BTx (median OSs were 13.2 months vs. 7.1 months, respectively; p=0.041). In regards with RTx, the median OS was higher among the patients receiving RTx compared to RTx-naive patients (11 months vs. 5.9 months). Importantly, the difference in survival between the groups dichotomized by therapeutic modalities disappears for the late events, accordingly only the Gehan-Breslow-Wilcoxon tests indicate significant differences. In contrast, KRAS mutational status curves remain separated for the entire survival range and thus KRAS status has a highly significant impact on survival both by Mantel-Cox and Gehan-Breslow-Wilcoxon tests. Following univariate analysis of the impact of KRAS mutation, RTx and BTx we performed a multivariate analysis using these three factors. The presence of KRAS mutation remained a significant predictor of shorter OS.

Next, we investigated whether KRAS mutation remains a significant prognosticator in the subgroups of patients receiving BTx or RTx. We found that the OS was significantly higher in the KRAS WT BTx group (vs. the KRAS mutant BTx group; the median OSs were 11 months vs. 5.8 months, respectively; p = 0.023). Similarly, KRAS mutation was a strong prognostic factor in the cohort of patients who received RTx (median OS KRAS WT vs. KRAS mutant were 13.5 months vs. 7 months, respectively; p = 0.0168).

Importantly, we also found that in the KRAS WT subgroup patients with BTx had significantly increased OS compared to patients without BTx

(median OSs were 11 months vs. 5.2 months, respectively; p=0.032, Gehan-Breslow-Wilcoxon test). As for patients with KRAS mutant tumors, the difference in median overall survival between patients with or without BTx did not reach statistical significance (median OSs were 5.8 months vs. 3.1 months, respectively; p=0.35).

Next, we evaluated the effects of RTx in the KRAS mutational status subgroups. In the KRAS WT subgroup, RTx conferred a significant benefit for OS when compared to patients not receiving RTx (median OS; 13.6 months vs. 7.4 months; p=0.032). As for the patients with KRAS mutation, the median OS difference was not statistically significant (7 months for RTx and 3 months for patients without RTx; p=0.12).

Finally, when evaluating the interaction between BTx and RTx irrespective of KRAS mutational status, we found that patients who received both BTx and RTx had a significantly longer OS compared to those who received only BTx or RTx or none of the aforementioned modalities (p=0.031).

Bone-specific metastasis pattern

In total, 209 LADC patients with synchronous isolated bone metastases were enrolled in this study. The median age was 62 years (range 34–84). All patients had Caucasian backgrounds and 113 of them were male (54%). Peripheral tumors occurred more frequently than centrally-located tumors (59% vs. 41%). Right-sided LADCs were found in 57% (vs. left-sided, 43%) and upper region tumor location in 70% (vs. lower region 30%) of the patients. The general clinicopathological

characteristics did not differ significantly with regards to the localization of the primary tumor. As for the localization of metastases, the most frequent metastatic sites were the spine (n=103), the ribs (n=60), the pelvis (n=36), and the femur (n=22), followed by humeral (n=17), skull (n=13), sternal (n=10), and clavicular or scapular (n=10) metastases. We identified 163 patients with single-bone metastatic disease and 46 with metastases affecting multiple bones at the time of diagnosis. With regards to specific bisphosphonate agents 67, 29 and 57 patients received clodronate, pamidronate and zoledronic acid, respectively (of note, no data was available on the exact type of administered bisphosphonate agent in case of 15 patients). Palliative external beam RTx was applied in case of 66 patients. Regarding major comorbidities 53 individuals had COPD, whereas hypertension was detected in 117 patients.

Investigating the impact of the primary tumors' localization on the metastatic site, we found that femoral (OR 3.486, 95%CI 1.09-14.71, p=0.022) and rib (OR 2.338, 95%CI 1.16-4.86, p=0.012) metastases were more frequently associated with peripheral tumors, whereas centrally located LADCs were associated with humeral metastases (OR 0.262, 95%CI 0.06-0.83, p=0.018). Importantly, we also found that left-sided tumors give rise to skull metastases more often than right-sided primary tumors (OR 4.836, 95%CI 1.19-28.19, p=0.018). These results remained significant at a 0.05 significance level with the use of Bonferroni correction. Of note, there was no significant association between the primary LADC region (i.e., lower vs. upper region tumors) and the bone-specific metastatic site. With regards to the type of affected bones,

metastases in flat bones were more commonly found in patients with peripheral tumors (vs. central LADCs), yet these results were not statistically significant (p=0.202). Likewise, the side- and region-specific localization of the primary tumor did not influence the type of bone metastases either. The localization of the primary tumors did not have an impact on the number of metastatic bones (i.e., single- vs. multiple-bone metastatic spread) at diagnosis (data not shown).

The median follow-up time for the total cohort of 209 bone-metastatic LADC patients was 33.7 weeks (of note, survival data was not available in case of 10 patients). Patients with centrally located primary LADCs had worse survival outcomes compared to those with peripheral tumors (median OS, 25.1 vs. 36.2 weeks, HR 1.359, 95%CI 1.020-1.810, p=0.035). No significant differences in OS have been observed for patients with right vs. left (p=0.941) or upper vs. lower region (p=0.238) located primary tumors. Next, we compared the number of metastatic sites with survival outcomes and found that the number of affected bones did not influence the median OS (p=0.436). When comparing the survival outcomes of LADC patients with solitary bone metastases, we found that the site of bone metastases did not influence survival significantly (p=0.307). Importantly, however, patients with femoral metastases tend to have better survival outcomes than those with other bone metastases (p=0.064). Although the median OS was visibly longer in patients with bone metastases affecting the long bones (vs. flat bones vs. irregular bones), this tendency does not appear to be statistically significant either (p=0.269). With regards to specific therapeutic approaches, as expected,

BTx-naive patients had significantly worse median OS than those receiving BTx (median OS, 12.0 vs. 40.2 weeks, HR 2.101, 95%CI 1.462-3.020, $p<0.001$). Similarly, CTx also conferred a significant benefit for OS when compared to CTx-naive patients (median OS, 50.2 vs. 17.4 weeks, HR 0.545, 95%CI 0.410-0.726, $p<0.001$). In order to assess if the prognostic value of tumor location (i.e., central vs. peripheral) was independent of other prognostic factors, we performed a multivariate Cox regression analysis. Importantly, we found that the peripheral location of primary LADCs was still significantly associated with a benefit in OS (HR 0.589, $p=0.001$). Besides, as expected, Cox regression analysis revealed that the specific therapeutic approaches (BTx and CTx) also influence the survival outcomes independently ($p<0.001$).

4. Conclusions

The present dissertation describes two projects. In the first part, we examined the prognostic relevance of KRAS mutation in a large and homogenous cohort of Caucasian LADC patients diagnosed with bone metastases. We also assessed how RTx and BTx affect the OS according to KRAS mutational status. In the second part, we evaluated the impact of primary tumor localization on bone metastasis pattern and survival outcomes. Based on our findings, we conclude that KRAS mutation is an independent negative prognosticator in bone metastatic LADC. In addition, we found that the use of RTx and BTx significantly increased the OS. Of note, however, the effects of the aforementioned therapeutic modalities were considerably higher in patients with KRAS WT tumors than in those with KRAS-mutant LADCs. Co-administration of BTx and RTx conferred a significant benefit for OS regardless of KRAS mutational status. In the second part, we revealed that peripheral tumors are significantly more likely to give rise to femoral and rib metastasis. In terms of survival, centrally-located tumors were associated with poorer survival than peripheral tumors. Our results may contribute to developing new therapeutic algorithms for early diagnosis, thus improving long-term survival.

5. Bibliography of the candidate's publications

5.1. The effects of bisphosphonate and radiation therapy in bone-metastatic lung adenocarcinoma: the impact of KRAS mutation.

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*shared first authorship

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* shared first authorship

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Acknowledgements

At the beginning of my professional career, I was offered an opportunity to start my training in thoracic surgery and gain insights into translational thoracic oncology research. I am grateful to a number of people who have helped me in the past years:

I will be always grateful to Ferenc Rényi-Vámos for offering me several opportunities to improve my surgical skills in thoracic cancer surgery, lung transplantation and everyday clinical practice.

I would like to thank Balázs Hegedűs, Balázs Döme, and Zsolt Megyesfalvi for helping and guiding me in the field of clinical and translational research. This dissertation would not have been possible without them.

I owe special thanks to all of my colleagues at the Department of Thoracic Surgery of the National Institute of Oncology, Budapest. It is a privilege for me to work together with such a talented and creative Team.

I would like to express might sincere gratitude to László Agócs and Ákos Kocsis who were my mentors in surgical training and helped me to become a better surgeon. I would also like to thank all my co-authors and collaborators from different thoracic oncology centers across Hungary, Austria and Germany.

And finally, I am grateful to my family and friends. They have always supported me in every decision I made. Therefore, I would like to say special thanks to:

my wife, Maria Steszli for her understanding and the encouragement she gave;

my children Emma, Márk, Jakab, and Adel for their tolerance;
my parents, Rozina Radeczky and Géza Radeczky for supporting and motivating me, and for all the opportunities they gave me in life;
my brother Kristof Radeczky and my sister Anna Radeczky, who helped me in every moment of my life.

Last but not least, I would like to thank all the people who have helped me to get to this point in life.