medical sciences forum

MDPI

Proceeding Paper

Novel Therapeutic Approaches for KRAS-Mutated Lung Cancer Involving LZTR1 Genetic Alteration †

Raj N. Sewduth *, Tonci Ivanisevic, Peihua Zhao and Anna A. Sablina

Laboratory for Mechanisms of Cell Transformation, Department of Oncology, Vlaams Instituut voor Biotechnologie (VIB)-Katholieke Universiteit (KU) Leuven Center for Cancer Biology, 49 Herestraat, 3000 Leuven, Belgium

* Correspondence: rajnayan.sewduth@kuleuven.vib.be

† Presented at the 3rd International Electronic Conference on Cancers: New Targets for Cancer Therapies, 16–30 March 2023; Available online: https://iecc2023.sciforum.net/.

Abstract: A total of 30% of lung adenocarcinoma are driven by activating KRAS mutations. The treatment options for KRAS-mutant lung cancer are still limited as a challenge for therapy is the high heterogeneity within *KRAS* mutant tumors. Co-existing genetic events alter RAS signaling, such as the genetic alteration of the ubiquitin ligase leucine zipper-like transcriptional regulator 1 (LZTR1). LZTR1 is an adaptor of CUL3 E3 ligase that controls the localization and expression levels of RAS proteins by regulating their ubiquitination. Recent studies demonstrated that the loss of *LZTR1* leads to resistance to the tyrosine kinase inhibitor and the multi-kinase inhibitor, suggesting that *LZTR1* loss might be associated with the drug resistance of KRAS-mutated lung tumors. TCGA analysis indicated that *LZTR1* loss affected progression survival in KRAS mutant LUAD patients, with a significant co-occurrence of *LZTR1* loss and KRAS mutations. While *LZTR1* depletion in LUAD cell lines did not affect proliferation in the cell culture, the knock-out (KO) of *Lztr1* in a mouse model with *Kras G12D* oncogenic mutations caused a clear and significant acceleration of tumor progression in the Lztr1 loss groups, indicating that Lztr1 can affect tumor onset and progression. To study the alterations of the RAS pathway triggered by *LZTR1* loss, we performed a global OMICS analysis on both in vitro and in vivo systems, identifying potential therapeutic targets. The characterization of immune populations in the tumors of flow cytometry also revealed changes in immune infiltrate in the KO mouse. We are now investigating how the changes caused by *Lztr1* deletion on KRAS signaling heterogeneity within tumor cells can affect the tumor microenvironment composition. Our results suggest that the dysregulation of KRAS function by *Lztr1* deletion contributes to cancer progression by affecting tumor cell communication with the microenvironment. Our work could explain how *Lztr1* loss can affect drug response and lead to therapy resistance.

Keywords: lung cancer; KRAS; LZTR1; ubitiquitination

Citation: Sewduth, R.N.; Ivanisevic, T.; Zhao, P.; Sablina, A.A. Novel Therapeutic Approaches for KRAS-Mutated Lung Cancer Involving LZTR1 Genetic Alteration. *Med. Sci. Forum* **2023**, *20*, 6. https://doi.org/10.3390/IECC2023-14221

Academic Editor: Carlos Moreno

Published: 16 March 2023

1. Introduction

Lung cancer is the most frequent cancer, with an aggressive clinical course and high mortality rates [1]. Almost 30% of adenocarcinomas in the lung are driven by an activating Kirsten rat sarcoma viral oncogene homolog (KRAS) mutation. Despite decades of research highlighting mutant KRAS as a central driver of tumorigenesis and clinical resistance, the development of therapeutics potently tackling KRAS aberrations has so far been unaccomplished. The treatment options for KRAS-mutant lung cancer are still limited, and chemotherapies remain the first-line recommendation. In recent years, a variety of efficient and specific chemicals have entered preclinical and early clinical settings. A striking breakthrough has been achieved with covalent inhibitors such as MRTX849 and AMG 510, as well as with LC-2, a degrader molecule against the endogenous protein, in patients with KRASG12C lung tumors. Rational combinations (e.g., combined chemotherapy with

targeted KRAS downstream agents) may further advance attempts to target KRAS-driven lung tumors. A critical point that challenges the design of such rational combinations is the high heterogeneity within KRAS mutant lung tumors [2].

Co-existing genetic events can alter RAS signaling, leading to the activation of a distinct set of downstream effectors to a different extent. In this study, we focused on a recently discovered the proteostatic regulator of KRAS: the ubiquitin ligase leucine zipper-like transcriptional regulator 1 (LZTR1). LZTR1, encoding a protein characterized by the KELCH-BTB-BACK-BTB-BACK domain architecture, is an adaptor of the CUL3-containing E3 ligase complex. We and others have also recently demonstrated that the LZTR1/CUL3 ubiquitin ligase complex controls localization and the expression levels of RAS proteins by regulating their ubiquitination [3–5]. It has now been demonstrated that *LZTR1* mutations can cause pediatric neoplasms, Noonan syndrome, glioblastoma, and schwannomatosis [6–11]. Recent studies have demonstrated how the loss of LZTR1 leads to resistance to the tyrosine kinase inhibitor imatinib [4] and the multi-kinase inhibitor sorafenib, which suppresses the activity of RAF and several transmembrane receptors [12]. These results suggest that LZTR1 loss might be associated with the drug resistance of KRAS-mutated lung tumors. To test this hypothesis, we tested several drug classes affecting cancer cell survival and fitness. This included a combination of cisplatin plus pemetrexed, which remains the best regimen for patients with KRAS-mutant lung cancer [2]. BI1701963 demonstrated promising antitumor activity against KRAS and was advanced into an ongoing phase I clinical trial as monotherapy or in combination with trametinib (NCT04111458).

A high heterogeneity within KRAS mutant lung tumors challenges the attempts to rationally design drug combinations for targeting this group of patients [2]. The loss of *LZTR1*, a gene coding a proteostatic regulator of KRAS, could impact the differential regulation of RAS signaling. While RAS signaling can classically activate the RAF1, MEK1/2, and ERK1/2 cascade, RAS also affects additional signaling pathways that are important for inflammation, but which are often overlooked. Several anti-inflammatory drugs were approved for the treatment of autoimmune diseases and could be repurposed quickly for anti-cancer therapy. More recently, anti-inflammatory drugs targeting RAS signaling complemented anti-MEK therapy in patient-derived tumors, overcoming drug resistance [13]. Such anti-inflammatory drugs present a clear advantage over cytokine blockade, as antibodies targeting cytokines directly appeared to have a paradoxical effect on tumor progression [14], making these non-viable anti-cancer options.

2. Results

Although *LZTR1* mutations were scattered through the whole gene, all characterized *LZTR1* missense mutations appeared to experience loss of function [4,5,9]. TCGA analysis indicated that *LZTR1* loss affected progression survival in KRAS mutant lung adenocarcinoma (LUAD) patients. The Genomic Identification of Significant Targets in Cancer (GISTIC) analysis also showed that focal deletions of *LZTR1* were commonly observed in lung adenocarcinoma and pancreatic adenocarcinoma. Furthermore, we observed a clear co-occurrence of *LZTR1* loss and *KRAS* mutations in lung adenocarcinoma, as indicated by the TCGA data.

To study LZTR1 function in vivo, our laboratory generated an *Lztr1* KO mouse model. Whereas the complete knock-out for Lztr1 is embryonically lethal, the heterozygous deletion of Lztr1 is viable and recapitulates Noonan syndrome phenotypes [5,11]. To assess the impact of *Lztr1* deletion on KRAS-driven lung cancer, we used the *Kras G12D lsl/wt* mouse model that forms lung tumors after the intratracheal injection of adenovirus coding for Cre-recombinase driven by the *Sftpc* promoter specific to the alveolar epithelium. We also used a floxed *Lztr1* allele system to induce the deletion of the gene in the same cells.

The computer tomography (CT) scan imaging of the lungs of animals of *Lztr1 flox/flox, Kras G12D lsl/wt* and *Lztr1 wt/wt*, *KrasG12D lsl/wt* backgrounds was used to measure tumor growth after 3D modeling of the tumor mass. A clear acceleration of tumor progression was observed in the *LZtr1 flox/flox* and *flox/wt* background, indicating that both homologous

and heterogenous deletion of Lztr1 can affect tumor growth. Hemalun Eosin of the lungs after induction was also performed, showing that tumors appeared more advanced in the *Lztr1 flox/flox* background.

Emerging evidence demonstrated that oncogenic RAS signaling is not homogenous but could activate a distinct set of downstream effectors to a different extent, causing drastic changes in the tumor environment and immune cell recruitment. To interrogate alterations in the RAS pathway triggered by *Lztr1* loss, we performed a global analysis of the proteome of wt-*Lztr1* and *Lztr1*-knockout tumor cells, as well H727 human lung cancer cells upon depletion of *LZTR1* using shRNA. An upstream analysis on differentially phosphorylated or expressed proteins identified regulators that were responsible for the changes observed in LUAD cells depleted for LZTR1. As these data strongly supported the potential of immunotherapy in our model, anti-inflammatory treatment efficiency was evaluated in the *Kras G12D*-driven lung adenocarcinoma model in the presence and absence of *Lztr1*. As indicated by CT scan analysis, anti-inflammatory drugs significantly reduced tumor progression in the *Kras G12D*, *Lztr1 flox* genetic background.

As a next step, we characterized the different immune populations in tumors within the different genetic backgrounds (*Kras G12D*, with wt-*Lztr1* or *Lztr1*-loss) using FACs. While most immune populations were not affected, an increase in neutrophils was observed in *Lztr1*-deleted lung tumors. An increase in neutrophils was also observed using histology. Finally, the Proximity ligation assay, as well as ubiquitin pulldown, indicated that KRAS-G12D ubiquitination was affected by *Lztr1* loss. This confirmed that LZTR1 could ubiquitinate the active G12D mutant variant of KRAS and suggests that this modulation of ubiquitination could affect tumor progression. In line with these findings, while we observed differential stabilization of wild-type or mutant KRAS upon *Lztr1* deletion, suggesting that LZTR1 regulates mutant and wild-type KRAS function differently.

3. Discussion

Here, we demonstrated for the first time that the RAS modifier LZTR1, can affect tumor progression through the activation of the pro-inflammatory pathway. Our work is a demonstration of the integration of multi-proteomics data, to understand alterations of signal transduction caused by the loss of a specific gene. We were also able to propose for the first time, targeted therapies targeting the alterations observed in *LZTR1* deleted-lung cancer. Our results show that the dysregulation of the KRAS function by *LZTR1* deletion contributes to lung cancer progression by promoting inflammatory pathways and causing increased immune infiltration, identifying promising therapeutic options.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/IECC2023-14221/s1, Conference Poster: The Role of the Kras Ubiquitination in Lung Cancer Heterogeneity.

Author Contributions: Conceptualization, R.N.S. and A.A.S.; methodology, R.N.S. and T.I.; bioinformatical analysis, P.Z.; writing—A.A.S., T.I. and R.N.S.; visualization, T.I. and R.N.S.; supervision, R.N.S.; project administration, A.A.S.; funding acquisition, A.A.S. and R.N.S. All authors have read and agreed to the published version of the manuscript.

Funding: ERC Consolidator grant Rasopathy, STK Fundamental mandate 2021-031.

Institutional Review Board Statement: Ethical agreement 204/2020 'Role of the ubiquitin system in lung cancer and associated cardiac disease' approved by ethical commission of KU Leuven.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Bray, F.; Ferlay, J.; Soerjomataram, I.; Siegel, R.L.; Torre, L.A.; Jemal, A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J. Clin.* **2018**, *68*, 394–424. [CrossRef] [PubMed]
2. Salgia, R.; Pharaon, R.; Mambetsariev, I.; Nam, A.; Sattler, M. The improbable targeted therapy: KRAS as an emerging target in non-small cell lung cancer (NSCLC). *Cell. Rep. Med.* **2021**, *2*, 100186. [CrossRef] [PubMed]
3. Abe, T.; Umeki, I.; Kanno, S.I.; Inoue, S.I.; Niihori, T.; Aoki, Y. LZTR1 facilitates polyubiquitination and degradation of RAS-GTPases. *Cell Death Differ.* **2019**, *27*, 1023–1035. [CrossRef] [PubMed]
4. Bigenzahn, J.W.; Collu, G.M.; Kartnig, F.; Pieraks, M.; Vladimer, G.I.; Heinz, L.X.; Sedlyarov, V.; Schischlik, F.; Fauster, A.; Rebsamen, M.; et al. LZTR1 is a regulator of RAS ubiquitination and signaling. *Science* **2018**, *362*, 1171–1177. [CrossRef] [PubMed]
5. Steklov, M.; Pandolfi, S.; Baietti, M.F.; Batiuk, A.; Carai, P.; Najm, P.; Zhang, M.; Jang, H.; Renzi, F.; Cai, Y.; et al. Mutations in LZTR1 drive human disease by dysregulating RAS ubiquitination. *Science* **2018**, *362*, 1177–1182. [CrossRef] [PubMed]
6. Chen, P.C.; Yin, J.I.; Yu, H.W.; Yuan, T.; Fernandez, M.; Yung, C.K.; Trinh, Q.M.; Peltekova, V.D.; Reid, J.G.; Tworog-Dube, E.; et al. Next-generation sequencing identifies rare variants associated with Noonan syndrome. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 11473–11478. [CrossRef] [PubMed]
7. Yamamoto, G.L.; Aguena, M.; Gos, M.; Hung, C.; Pilch, J.; Fahiminiya, S.; Abramowicz, A.; Cristian, I.; Buscarilli, M.; Naslavsky, M.S.; et al. Rare variants in SOS_2 and $LZTR_1$ are associated with Noonan syndrome. *J. Med. Genet.* **2015**, *52*, 413–421. [CrossRef] [PubMed]
8. Frattini, V.; Trifonov, V.; Chan, J.M.; Castano, A.; Lia, M.; Abate, F.; Keir, S.T.; Ji, A.X.; Zoppoli, P.; Niola, F.; et al. The integrated landscape of driver genomic alterations in glioblastoma. *Nat. Genet.* **2013**, *45*, 1141–1149. [CrossRef] [PubMed]
9. Motta, M.; Fidan, M.; Bellacchio, E.; Pantaleoni, F.; Schneider-Heieck, K.; Coppola, S.; Borck, G.; Salviati, L.; Zenker, M.; Cirstea, I.C.; et al. Dominant Noonan syndrome-causing LZTR1 mutations specifically affect the Kelch domain substrate-recognition surface and enhance RAS-MAPK signaling. *Hum. Mol. Genet.* **2019**, *28*, 1007–1022. [CrossRef] [PubMed]
10. Paganini, I.; Chang, V.Y.; Capone, G.L.; Vitte, J.; Benelli, M.; Barbetti, L.; Sestini, R.; Trevisson, E.; Hulsebos, T.J.; Giovannini, M.; et al. Expanding the mutational spectrum of LZTR1 in schwannomatosis. *Eur. J. Hum. Genet.* **2015**, *23*, 963–968. [CrossRef] [PubMed]
11. Sewduth, R.N.; Pandolfi, S.; Steklov, M.; Sheryazdanova, A.; Zhao, P.; Criem, N.; Baietti, M.F.; Lechat, B.; Quarck, R.; Impens, F.; et al. The Noonan Syndrome Gene Lztr1 Controls Cardiovascular Function by Regulating Vesicular Trafficking. *Circ. Res.* **2020**, *126*, 1379–1393. [CrossRef] [PubMed]
12. Damnernsawad, A.; Bottomly, D.; Kurtz, S.E.; Eide, C.A.; McWeeney, S.K.; Tyner, J.W.; Nechiporuk, T. Genome-wide CRISPR screen identifies regulators of MAPK and MTOR pathways mediating sorafenib resistance in acute myeloid leukemia. *Haematologica* **2020**, *107*, 77–85. [CrossRef] [PubMed]
13. Meeusen, B.; Cortesi, E.E.; Omella, J.D.; Sablina, A.; Ventura, J.-J.; Janssens, V. PPP2R4 dysfunction promotes KRAS-mutant lung adenocarcinoma development and mediates opposite responses to MEK and mTOR inhibition. *Cancer Lett.* **2021**, *520*, 57–67. [CrossRef] [PubMed]
14. Propper, D.J.; Balkwill, F.R. Harnessing cytokines and chemokines for cancer therapy. *Nat. Rev. Clin. Oncol.* **2022**, *19*, 237–253. [CrossRef] [PubMed]