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Proceeding Paper

Novel therapeutic approaches for KRAS mutated lung cancer involving LZTR1 genetic alteration

Raj Sewduth , Tonci Ivanisevic , Peihua Zhao and Anna Sablina

[1] VIB KU LEUVEN Center for cancer biology, 49 Herestraat, 3000 LEUVEN; rajnayan.sewduth@kuleuven.vib.be (R.S.); tonci.ivanisevic@kuleuven.be (T.V.); peihua.zhao@kuleuven.be; anna.sablina@kuleuven.vib.be

Abstract: 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 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 its 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 cell culture, the knock-out (KO) of *Lztr1* in a mouse model with *Kras G12D* oncogenic mutation 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 by 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 the tumor cells, can affect the tumor microenvironment composition. Our results suggest that 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 the drug response and lead to therapy resistance.

Keywords: Lung cancer; kras; lztr1; ubitiquitination

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1. Introduction

Lung cancer is the most frequent cancer with an aggressive clinical course and high mortality rates (1). Almost 30% of adenocarcinomas of 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 the attempts to target

KRAS-driven lung tumors. A critical point that challenges the design of such rational combinations is a high heterogeneity within KRAS mutant lung tumors (2).

Co-existing genetic events can alter RAS signaling, leading to activation of a distinct set of downstream effectors to a different extent. In this study, we focus on a recently discovered proteostatic regulator of KRAS, the ubiquitin ligase leucine zipper-like transcriptional regulator 1 (LZTR1). LZTR1, encoding a protein characterized by KELCH-BTB-BACK-BTB-BACK domain architecture, is an adaptor of CUL3-containing E3 ligase complex. We and others have also recently demonstrated that the LZTR1/CUL3 ubiquitin ligase complex controls localization and expression levels of RAS proteins by regulating its ubiquitination (3-5). It is now demonstrated that *LZTR1* mutations can cause pediatric neoplasms, Noonan syndrome, glioblastoma and schwannomatosis (6-10). Recent studies demonstrated that 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 includes 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 it 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 on the differential regulation of RAS signaling. While RAS signaling can classicaly activate the RAF1, MEK1/2, ERK1/2 cascade, RAS also affects additional signaling pathways important for inflammation that are often overlooked. Several anti-inflammatory drugs are approved for the treatment of autoimmune diseases and could be repurposed quickly for anti-cancer therapy. This drug has been shown to be efficient against neutrophil-driven disease (12) and was tested previously in mouse models of lung cancer (13) and breast cancer (14). More recently, anti-inflammatory drugs targeting RAS signaling complemented anti-MEK therapy in patients-derived tumors, overcoming drug resistance (15). Takinib, a drug targeting TAK1, also showed some promising effects in animal models of arthritis (16) and lymphoma (17). Such anti-inflammatory drugs present a clear advantage over cytokine blockade, as antibodies targeting cytokines directly appear to have a paradoxical effect on tumor progression (18), making those non-viable anti-cancer options.

2. Results

Although *LZTR1* mutations are scattered through the whole gene, all characterized *LZTR1* missense mutations appear to be 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* are 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 TCGA data.

To study LZTR1 function *in vivo*, our laboratory has 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 intratracheal injection of an 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 deletion of the gene in the same cells.

CT scan imaging of 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 onset. Hemalun Eosin of the lungs after induction was also performed, showing that adenoma nodules were larger, and the 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 of the RAS pathway triggered by *Lztr1* loss, we performed a global analysis of proteome of wt-*Lztr1* and *Lztr1*-knockout tumor cells. The analysis was performed on Human LUAD cell line H727 upon depletion of *LZTR1* using shRNA. An upstream analysis on differentially phosphorylated or expressed proteins, identified regulators that are responsible for the changes observed in LUAD cells depleted for LZTR1. As this 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 presence and absence of Lztr1. As indicated by the quantification of tumor volume on CT scans, 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 the 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, Proximity ligation assay, as well as ubiquitin pulldown indicated that KRAS-G12D ubiquitination was affected by *Lztr1* loss, as indicated by a significant decrease in both experiments. This confirms that LZTR1 can ubiquitinate the active G12D mutant variant of KRAS and suggests that this modulation of ubiquitination can affect tumor progression. In line with these findings, while we observed a stabilization of wild-type KRAS upon *Lztr1* deletion, we did not observe any changes in expression level of mutant G12D KRAS. This shows that LZTR1 mediated ubiquitination of KRAS G12D is not degradative, suggesting that LZTR1 affects mutant KRAS function differently.–

3. Discussion

Here, we demonstrate for the first time that the RAS modifier LZTR1, can affect tumor progression through activation of pro-inflammatory pathway. Our work is a clear 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, personalized therapies targeting the alterations observed in *LZTR1* deleted - lung cancer. Our results show that dysregulation of KRAS function by *LZTR1* deletion contributes to lung cancer progression by promoting the inflammatory pathways and causing increased immune infiltration, identifying promising therapeutic options.

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