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Loss of wild type *KRAS* in *KRAS*^{MUT} lung adenocarcinoma is associated with cancer mortality and confers sensitivity to FASN inhibitors

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Abstract

Objectives: Wild type RAS (RAS^{WT}) suppresses the function of oncogenic RAS mutants (RAS^{MUT}) in laboratory models. Loss of RAS^{WT}, which we termed loss of heterozygosity (LOH) for any *RAS* (LAR) or LAKR in the context of KRAS (LOH at KRAS), is found in patients with RAS^{MUT} cancers. However, the incidence and prognostic significance of LAR has not been studied in modern patient cohorts. LAR or LAKR in *RAS*^{MUT} cancers is attractive as a potential biomarker for targeted therapy.

Materials and methods: We evaluated for associations between LAKR and cancer mortality in patients with RAS^{MUT} lung adenocarcinoma (LUAD). We also evaluated for associations between LAKR and the metabolic state of cancer cell lines, given that KRAS has been shown to regulate fatty acid synthesis. In line with this, we investigated fatty acid synthase (FASN) inhibitors as potential therapies for RAS^{MUT} LAKR, including combination strategies involving clinical KRAS^{G12C} and FASN inhibitors.

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Results: 24% of patients with *KRAS*^{MUT} LUAD showed LAKR. *KRAS*^{MUT} LAKR cases had a median survival of 16 vs. 30 months in *KRAS*^{MUT} non-LAKR ($p = 0.017$) and LAKR was independently associated with death in this cohort ($p = 0.011$). We also found that *KRAS*^{MUT} LUAD cell lines with LAKR contained elevated levels of FASN and fatty acids relative to non-LAKR cell lines. KRAS^{G12C} LUAD cells with LAKR showed higher sensitivity to treatment with FASN inhibitors than those without. FASN inhibitors such as TVB-3664 showed synergistic effects with the KRAS^{G12C} inhibitor MRTX849 in LUAD cells with LAKR, including an *in vivo* trial using a xenograft model.

Conclusions: LAKR in *KRAS*^{MUT} cancers may represent an independent negative prognostic factor for patients with *KRAS*^{MUT} LUAD. It also predicts for response to treatment with FASN inhibitors. Prospective testing of combination therapies including KRAS^{G12C} and FASN inhibitors in patients with KRAS^{G12C} LAKR is warranted.

Keywords

Lung adenocarcinoma; *RAS*; *KRAS*; loss of heterozygosity

1. Introduction

RAS mutations are a common genetic feature in human tumors and are enriched in aggressive cancers such as lung adenocarcinoma, cholangiocarcinoma, colorectal cancer, melanoma, and pancreatic ductal adenocarcinoma [1]. However, changes in *RAS* zygosity, especially loss of *RAS*^{WT}, which we refer to hereafter as loss of heterozygosity (LOH) at any *RAS* (LAR), or LAKR (LOH at KRAS) in the context of KRAS, also occur in malignancy [2–4]. Presumably this is because in the setting of mutant RAS (*RAS*^{MUT}), *RAS*^{WT} behaves as a tumor suppressor and loss of *RAS*^{WT} enhances tumor fitness. The incidence of LAR in cancer patients has not been characterized in modern cohorts and the prognostic value of LAR for patient care is unknown. LAR is also assocaiated with changes in cellular responses to perturbagens [5] but LAR has not yet shown efficacy as a predictive biomarker for patient selection of targeted therapies.

The RAS subfamily of small GTPases consists of 4 isoforms with a high degree of sequence identity: HRAS, NRAS, and two splice variants of KRAS, KRAS4A and KRAS4B. RAS mutations are generally thought to operate by increasing the fraction of GTP-bound RAS, thereby enhancing signaling pathways mediated by protein-protein interactions between RAS and its effectors [6, 7]. Nevertheless, these isoforms are mutated at different rates and preferentially at different locations according to cancer type [1]. This, together with accumulating data showing functional differences of specific RAS mutants [8–10] suggests that individual RAS isoforms and specific mutations should be considered separately for mechanistic studies and therapeutic targeting strategies.

RAS copy number also varies non-randomly between cancer types and RAS isoforms [11–14]. Copy number changes in *RAS,* such as LAR, can impact RAS-mediated signaling and modulate sensitivity to certain drugs, such as MEK inhibitors [11, 15–17]. In the presence of *RAS*^{MUT}, *RAS*^{WT} often behaves similarly to tumor suppressors, such as RB and BRCA1, where loss of *RAS*^{WT} leads to tumor progression [18]. Accordingly, mouse models

demonstrated that LOH at *KRAS* (LAKR) increases the fitness of $KRAS^{MUT}$ LUAD [5]. Similarly, LAKR in $KRAS^{MUT}$ acute myeloid leukemia (AML) and colon adenocarcinoma cells enhances tumor aggressiveness [17]. Together these observations suggest that in the context of RAS^{MUT} cancer, LAR may be a clinically useful biomarker for prognostication and for patient selection of targeted therapies if appropriate therapies can be discovered.

In this study, we explored the relationship between LAKR and patient survival in $KRAS^{MUT}$ LUAD using data from public databases [19]. These data suggest that LAKR is an independent prognostic factor that may help guide clinical management of LUAD. We also searched for targetable biological effects associated with LAKR and found an association with lipid metabolism, specifically fatty acid synthase (FASN). This association is important because FASN inhibitors are currently in clinical trials for $KRAS^{MUT}$ LUAD, given that FASN is elevated in some RAS^{MUT} cancers [20]. We evaluated this potential therapeutic vulnerability for $KRAS^{MUT}$ LUAD with LAKR and believe it represents an opportunity to overcome known therapeutic resistance to clinical $KRAS^{G12C}$ inhibittors such as MRTX849.

2. Materials and method

2.1 Patients and clinical information

Patients with lung adenocarcinoma (LUAD), colon adenocarcinoma (COAD), pancreatic adenocarcinoma (PAAD) and skin cutaneous melanoma (SKCM) and their clinical information were obtained from the TCGA database. Samples lacking survival information, *RAS* status or LOH status were excluded from the study.

2.2 RAS and LOH status analysis

To determine *RAS* mutation status, “PASS” filter mutations in *KRAS*, *NRAS*, and *HRAS* were selected from the MC3 MAF file (v0.2.8) [21]. Intronic mutations, mutations in 3’ or 5’ UTRs or UTR flanking regions, and silent mutations were then further removed. LOH status of patient tumor tissues from TCGA was determined using the ABSOLUTE algorithm [22]. For each RAS gene and for each TCGA sample, the fraction of base pairs in each RAS gene composed of segments labeled with LOH by ABSOLUTE was computed. Cases where over 0.5 of the gene was labeled as LOH were considered to have undergone LOH.

2.3 Statistical methods

Descriptive statistics were used to characterize the patients at study entry. Chi-squared test was used to compare distributions of clinical characteristics across RAS^{MUT} patients with or without LAR. Fisher’s exact test was used when the sample size was smaller than 5. Two-sample t-test was used to compare the percentage of LAKR in wild type and mutated KRAS background. A p value < 0.05 was considered statistically significant. Cox Proportional Hazards Regression analysis was used to derive hazard ratios (HRs) for cancer-specific mortality in association with LAKR, with adjustment for age at diagnosis, gender, pathology stage, and smoking history. Kaplan-Meier survival curves were generated using “survival” and “survminer” R packages. Log Rank Test was used for the statistical analysis. All tests were two-tailed with a significance level of $p < 0.05$. All analyses were performed using R (version 3.6.0, R Foundation for Statistical Computing).

2.4 Cell lines and cell culture

Non-small lung cancer lines (NSCLC) H1150, H650, H2122, H2030, H23, HCC44, SW1573, H1373, H460, Calu-6, Calu-1, H358, H1792, H1573, and H441 were from the Hamon Center Cell Repository. Cells were cultured in RPMI-1640 supplemented with 10% FBS. Cell line identity was confirmed by DNA fingerprinting (PowerPlex 1.2 Kit, Promega) and mycoplasma-free status was verified by PCR (e-Myco Kit, Boca Scientific). The KRAS mutation status (from Cancer Cell Line Encylopedia, CCLE, data) and LAKR status of these cell lines are summarized in Table S1. LUAD cell lines with KRAS mutations that were homozygous for the mutations were scored as LAKR “positive”, while lines with $KRAS^{G12C}$ and KRAS wild type sequences (heterozygous for KRAS allele) were scored as LAKR “negative”.

2.5 Chemical compounds

FASN inhibitors,TVB-3116 and TVB-3664 were gifts from Sagimet Biosciences (San Mateo, CA). Cerulenin was purchased from Sigma-Aldrich (Burlington, MA) and Fasnall was purchased from Focus Biomolecules (Plymouth Meeting, PA). KRAS inhibitors, MRTX849 was purchased from DC Chemicals (Shanghai, China) and MRTX1257 were purchased from MedChemExpress (Monmouth Junction, NJ).

2.6 Cell proliferation assay

CellTiter-Glo was ordered from Promega (Madison, WI). Briefly, 50,000 cells were seeded per well in a 100 μl medium in 96-well format and were treated by a compound or transfected with a DNA construct. Assays were developed by addition of 100 μl of CellTiter-Glo reagent. Luminescence was measured using BioTek NEO plate reader.

2.7 Western Blotting

Whole cell lysates were separated by SDS-PAGE gel and protein was transferred onto Immobilon-P membrane. The membrane was blocked by 5% milk in PBS with 0.1% Tween-20. The blot was probed with the FASN antibody from Cell Signaling (Danvers, MA). Protein was detected by LumiGLO reagent and peroxide from Cell Signaling. Secondary antibody was from Cell Signaling.

2.8 Xenografts

8.5-week-old female nude mice (Jackson Laboratories Stock No: 007850 (J:NU), homozygous for Foxn1nu) were implanted at a single subcutaneous site with 5×10^6 H2122 NSCLC cells (0.1 ml). When tumors reached approximately 120 mm^3 (day 4 post tumor cell implantation), therapy was initiated with 5 mg/kg TVB3664 only, 10 mg/kg MRTX849 only, 5 mg/kg TVB3664 + 10 mg/kg MRTX849, or vehicle only (30% fresh PEG400 + 70% 20 mm pH 4.5 citrate buffer) 0.2 ml/mouse (8 mice/group). Compounds were administered daily as clear solutions orally. Mice were weighed three times per week and tumors measured with calipers three times per week. Tumor volume was calculated as $(L \times W^2 \times pi)/6$. When tumor volume reached >1250 mm^3 or if mice appeared moribund, they were euthanized. Weight loss did not exceed 20% in any animal and overall was comparable between groups. All mice not previously euthanized, were sacrificed on day 23. For

statistical analysis, TVB-3664 and MRTX849 arms were compared with the vehicle arm. The combination arm was compared with each single treatment arm. Statistical analyses were performed at day 19 (n = 8 mice/group). ANOVA was used to compare among multiple groups and each pair was analyzed by student t-test.

3. Results

3.1 LAKR is common in major cancer types

We evaluated four major cancer types, LUAD, COAD, PAAD, and SKCM, where *RAS* mutations are common and clinical data is available for associated TCGA specimens. Of the 501 evaluable patients with LUAD, 146 (29.1%) of them had mutations in *KRAS* (Table 1), consistent with other results [23]. Thirty-five (24%) of those had LAKR. As a control we also evaluated LAKR in the $KRAS^{WT}$ background and found a similar rate of LAKR (26%; Table 1). In the 171 evaluable patients with PAAD and the 399 evaluable patients with COAD, the rates of $KRAS^{MUT}$ LAKR were lower overall, at 14% for both. $KRAS^{MUT}$ PAAD showed statistically similar rates for LAKR in $KRAS^{WT}$ vs. $KRAS^{MUT}$ backgrounds (5.6% vs. 13.7%, $p = 0.12$). However, in COAD, LAKR was lower in $KRAS^{WT}$ (6.8% vs. 14.2%, $p = 0.01$; Table 1). Of the 469 evaluable patients with SKCM, 129 (27.5%) had mutations in *NRAS*. Twenty-eight (21.7%) of them had LANR, not statistically different from that observed in the $NRAS^{WT}$ background (18.8%, $p = 0.48$; Table 1). Based on this analysis we focused on LUAD because both KRAS mutations and LAKR were more common in LUAD than other three cancer types.

3.2 LAKR is associated with increased mortality in patients with $KRAS^{MUT}$ LUAD

We performed Kaplan-Meier analysis to determine if LAKR is associated with mortality in LUAD cases contained in the TCGA. When comparing $KRAS^{MUT}$ cancers with or without LAKR, the two populations were statistically balanced for age, gender, stage, and smoking history, although we noted that stage III-IV cases appeared numerically higher in the LAKR arm (Table 2). Kaplan-Meier analysis showed that LAKR was not associated with overall survival in the $KRAS^{WT}$ background, ($p = 0.96$; Fig. 1A), but was associated with shorter overall survival in the $KRAS^{MUT}$ background with a median survival of 16 vs. 30 months, ($p = 0.017$; Fig. 1B). The difference in survival suggests that LAKR-dependent effects on survival are mediated by an interaction between $KRAS^{MUT}$ and $KRAS^{WT}$. To determine if LAKR is an independent prognostic factor, we performed univariate and multivariate analyses using LAKR, age, gender, stage, and smoking history. Of the factors analyzed, both LAKR and stage were associated with an increased risk of death on univariate and multivariate analysis (Table 3). However, in the $KRAS^{WT}$ background, only tumor stage was associated with overall survival (Fig. S1), confirming that the association between survival and LAKR is dependent on $KRAS^{MUT}$. Overall, these results support that LAKR is associated with mortality in patients with $KRAS^{MUT}$ LUAD.

3.3 LAKR is associated with upregulated lipogenesis in $KRAS^{MUT}$ LUAD cell lines.

Previous studies showed that KRAS activation leads to lipogenesis through induction of fatty acid synthase (FASN) or acyl-coenzyme A synthetase long-chain family member 3 (ACSL3), and that $KRAS^{MUT}$ cancers are sensitive to inhibitors of these enzymes [20, 24].

We speculated that LAKR might contribute to this phenomenon in $KRAS^{MUT}$ LUAD if the suppressive effects of $KRAS^{WT}$ occur upstream of mechanisms that lead to upregulation of FASN by KRAS. We analyzed cellular metabolites from $KRAS^{MUT}$ LUAD lines and observed that $KRAS^{MUT}$ LUAD cell lines with LAKR had higher levels of long chain fatty acid and phospholipids compared to those without LAKR (Figure 2A and 2B). This is consistent with established models showing that that *KRAS* activation can stimulate expression of FASN, which catalyzes the condensation of acetyl-CoA and malonyl-CoA, an early step in the synthesis of long-chain fatty acids (Figure 2C). To confirm that LAKR upregulates FASN, we evaluated FASN expression in $KRAS^{MUT}$ LUAD cell lines with or without LAKR. FASN was almost uniformly upregulated in $KRAS^{MUT}$ LAKR cancer cell lines compared to LAKR-negative cell lines (Figure 2D).

3.4 $KRAS^{MUT}$ LUAD cells with LAKR are sensitive to FASN inhibition.

Inhibition of FASN in $KRAS^{G12D}$ driven mouse models of lung cancer decreases tumor formation, leading to the hypothesis that FASN inhibitors may have therapeutic implications for RAS^{MUT} lung cancer [25]. In light of our findings that $KRAS^{MUT}$ LAKR was associated with increased FASN expression, we considered that $KRAS^{MUT}$ LAKR might show vulnerability to FASN inhibitors. We treated eight LAKR $KRAS^{MUT}$ LUAD cell lines and seven non-LAKR $KRAS^{MUT}$ lines with multiple FASN inhibitors, including TVB-3116, TVB-3664, fasnall and cerulenin. On average, FASN inhibitors showed better anti-proliferative activity in $KRAS^{MUT}$ lung cancer cell lines with LAKR than lines without LAKR (Figures 3A, S2A and S2B).

$KRAS^{G12C}$ inhibitors have shown activity in clinical trials [26–28]. However, development of resistance is an area of immediate concern, given that acquired resistance is common for targeted therapies in general and because cases of resistance to $KRAS^{G12C}$ inhibitors have already been observed [29–32]. Although a prior report did not show clear associations between LAKR and sensitivity to $KRAS^{G12C}$ inhibitors, SW1573, HCC44 and H2122, all $KRAS^{G12C}$-bearing tumors with LAKR, show some degree of resistance to $KRAS^{G12C}$ inhibitors [33, 34]. We hypothesized that combining FASN and $KRAS^{G12C}$ inhibitors might be synergistic in $KRAS^{G12C}$ tumors with LAKR and provide an avenue to deal with therapeutic resistance. Combination treatment with MRTX849, a $KRAS^{G12C}$ inhibitor in human trials, and TVB-3664, a close analogue of a FASN inhibitor in human trials, showed synergy as measured by the Bliss metric in H2122, HCC44, and SW1573 cell lines (Figure 3B and Figure S3A). Of note, we did not observe synergy for H358, a $KRAS^{G12C}$ LUAD line without LAKR (Figure S3B) and H460, a $KRAS^{Q61H}$ LUAD line (Figure S3C).

To evaluate if these effects could translate to *in vivo* cancer models, we performed a trial using an H2122 mouse xenograft model. Of note, this line was categorized as partially sensitive when mice were dosed at 100 mg/kg of MRTX849 [34]. We dosed mice with 5 mg/kg of TVB-3664 and 10 mg/kg of MRTX849 for approximately 3 weeks. All treatment regimens were well tolerated by the mice which showed stable weights throughout (Figure S4). Combination treatment was substantially better than the single agent arms and was able to halt tumor growth (Figure 3D). Of note, this is similar to effects previously achieved by single arm treatment of 100 mg/kg (10-fold higher) of MRTX849 [34]. These results suggest

that concurrent inhibition of FASN and KRAS^{G12C} may be an effective strategy for overcoming resistance to KRAS^{G12C} inhibitors in patients with *KRAS^{G12C}* LAKR LUAD.

4. Discussion

Here we identify a high-risk lung cancer patient population, *KRAS^{MUT}* LUAD with LAKR. We also identify a potential strategy to intervene in this population, based on a new connection between LAKR and upregulation of lipid biogenesis at the level of FASN. Along these lines, we found that FASN dependence results in sensitivity to FASN inhibitors in *KRAS^{MUT}* LAKR. We further showed that inhibition of FASN was synergistic with direct KRAS^{G12C} inhibition in KRAS^{G12C} inhibitor-resistant cancer cells with *RAS^{G12C}* LAKR. These findings could quickly translate into a new targeted therapy paradigm for a subset of high-risk lung cancers but may have implications for other RAS-driven diseases as well.

Evaluating for LAKR in *KRAS^{MUT}* LUAD may improve the ability to prognosticate for LUAD, given the strong association between LAKR and mortality in our study, with an approximate doubling in median survival from 16 to 30 months for non-LAKR vs. LAKR KRAS^{MUT} NSCLC. If effective therapies targeted to *KRAS^{MUT}* LAKR can be discovered, it is conceivable this could alter clinical approaches to *KRAS^{MUT}* LUAD, especially given evolving attitudes and practices around other actionable molecular markers in LUAD, such as *EGFR* and *ALK.* Indeed, recent analysis showed that the presence of EGFR mutations or ALK rearrangements is associated with a quadrupling of median survival relative to no alterations (48 months vs. 12 months respectively) [35]. This has reshaped management of these patients with local therapies such as stereotactic radiation playing a more prominent role in the management of metastatic disease [36].

Based on our results, use of FASN inhibitors represents one possible therapeutic strategy for patients with *RAS^{MUT}* LAR. Using FASN inhibitors as an anti-cancer strategy is not a new idea. Cerulenin, an antifungal that irreversibly inhibits FASN, was identified in the 1970's [37], and has been widely studied in multiple cancer models. However, cerulenin is too toxic for use in humans [38]. A number of other FASN inhibitors have subsequently been developed, such as GSK2194069, benzimidazole, 4-hydroxyquinoline, piperazine diamide, fasnall, and TVB-2640 [39]. Early phase trials of TVB-2640 have shown safety, but generally modest responses in patients with advanced *KRAS^{MUT}* NSCLC, breast and ovarian cancers [40]. Trials designed to evaluate the efficacy of TVB-2640 in specific cohorts of colon cancer, breast cancer, high grade astrocytoma and lung cancer are ongoing [41–43]. These trials primarily consider metabolic biomarkers, although NCT03808558 uses any KRAS mutation as an enrollment criterion. However, LAKR is not considered. Based on our results, adding LAKR as an evaluation criterion may enable identification of a high-risk subgroup that is more likely to benefit from TVB-2640 treatment. Additionally, our results showed synergy of FASN with MRTX1257 in KRAS^{G12C} inhibitor-resistant lines, suggesting a potential treatment strategy for patients with KRAS^{G12C} tumors who are resistant to KRAS^{G12C} therapies. Additional study on the potential value of LAKR as a predictive biomarker in these populations is warranted.

Aside from FASN, other therapeutic approaches may also be effective for RAS^{MUT} LAR. LAR is associated with an increase in sensitivity to MEK inhibition in lung cancer [5] and myeloid leukemia (AML) [17]. Nevertheless, these associations have not been validated in large sample sets, and it is unknown if other variables, such as other aspects of genetic background or cancer type, are important. This may be a significant issue in diseases such as NSCLC where genetic heterogeneity is high [44]. It also may be possible to discover new therapeutic innovations related to the physical mechanisms that underlie the tumor suppressive effects of RAS^{WT}. Current data suggests that suppression by RAS^{WT} is rooted in RAS oligomerization [5]. Nevertheless, RAS complexes have been difficult to isolate and characterize leading to the assumption that these complexes are likely dynamic and may take multiple forms, each with a different biological function. [15, 16, 45]. Related to that concept, it is possible that different RAS mutations impact the state of RAS assemblies and related LAR-dependent phenomenon in different ways. If this is true, RAS mutation-specific strategies may be required. Regardless, multiple studies now suggest methods by which RAS complexes might be perturbed [46, 47]. It is possible some of these could have therapeutic utility in the setting of $KRAS^{MUT}$ LAKR.

In addition to finding LAKR in a sizable proportion of $KRAS^{MUT}$ LUAD, we also found a similar proportion of LAR within the context of $NRAS^{MUT}$ SKCM (melanoma). Whether LAR functions similarly in this setting will require additional study given that RAS isoforms and specific RAS mutations show biochemical and biological differences[48, 49]. Nevertheless, it is worth noting the potential to extend the utility of LAR-directed approaches beyond lung cancer. It is also worth noting that RAS genes are often amplified in cancers[17]. This phenomenon has the potential to show mechanistic similarities to LAKR, but further study is also required.

Because of its retrospective nature, the clinical associations observed in this study are subject to bias and should be verified by prospective study. Indeed, it is unclear what selection criteria may apply to cases within the clinical data set we evaluated. This study also suffers from small numbers, although this did not prevent us from finding statistical differences related to LAKR. Despite the above caveats, these results add evidence to the idea that $KRAS^{WT}$ plays a suppressive role in human $KRAS^{MUT}$ LUAD and that LAKR is a potentially useful predictive and prognostic biomarker [5, 12]. Moreover, these results could have immediate relevance in conjunction with $KRAS^{G12C}$ inhibitors. Prospective studies to validate these findings are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AML	acute myeloid leukemia
COAD	colon adenocarcinoma
FASN	fatty acid synthase
LAKR	LOH at KRAS
LAR	LOH at any RAS
LOH	loss of heterozygosity
LUAD	lung adenocarcinoma
NSCLC	Non-small lung cancer lines
PAAD	pancreatic adenocarcinoma
SKCM	skin cutaneous melanoma
TCGA	The Cancer Genome Atlas

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Highlights

- Loss of wild type $KRAS^{WT}$ (LAKR) in $KRAS^{MUT}$ lung adenocarcinoma is common.
- LAKR is associated with cancer mortality in $KRAS^{MUT}$ lung adenocarcinoma.
- FASN is upregulated in $KRAS^{MUT}$ lung adenocarcinoma with LAKR, but this confers sensitivity to FASN inhibitors.
- Combination treatment with FASN and $KRAS^{G12C}$ inhibitors is synergistic and overcomes resistance to $KRAS^{G12C}$ inhibitors *in vivo*.

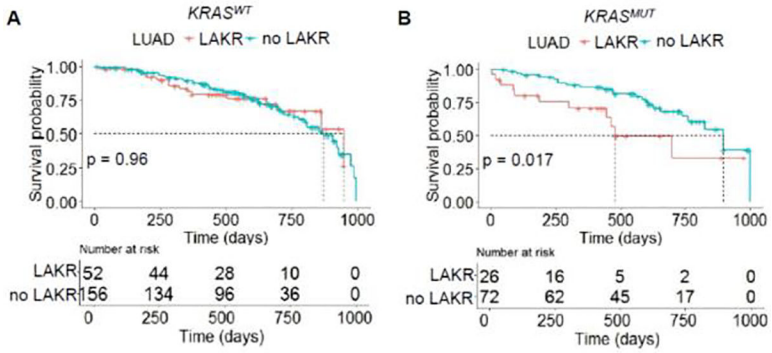

Figure 1.

LAKR is associated with cancer mortality in *KRAS*^{MUT} LUAD. Kaplan-Meier analysis of patients with (A) *KRAS*^{WT} or (B) *KRAS*^{MUT} LUAD stratified by LAKR (LOH) status.

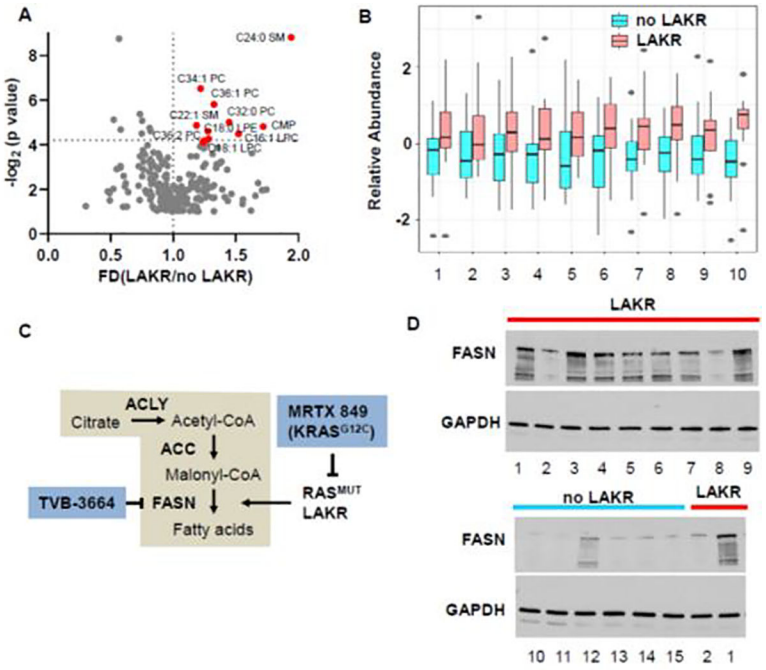

Figure 2.

LAKR correlates with increased lipogenesis in *KRAS*^{MUT} LUAD cell lines. (A) Metabolites in 31 *KRAS*^{MUT} LUAD cell lines were analyzed using the data from CCLE database. Mean fold change of metabolites in *KRAS*^{MUT} LUAD cell lines with LAKR (n = 15) vs. no LAKR (n = 16). Red circles represent significantly upregulated metabolites in LAKR positive cell lines. (B) Alternative view of metabolites shown in A. 1: aconitate; 2: CMP; 3: alpha-hydroxybutyrate; 4: C18:1 LPC; 5: C18: LPE; 6: C32:0 PC; 7: C34:1 PC; 8: C36:1 PC; 9: C16:1 SM; 10: C24:0 SM. (C) Schematic model of impact of KRAS^{MUT} LAKR on fatty acid (FA) synthesis. DAG, diacylglycerol; FA, fatty acid; LPA, lysophosphatidic acid; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine. (D) Expression of FASN is upregulated in *KRAS*^{MUT} LUAD cell lines with LAKR. H1155 and H650 were used as controls to compare FASN on two separate blots (lower blot). 1: H1155; 2: H650; 3: H2122; 4: H2030; 5: H23; 6: HCC44; 7: SW1573; 8: H1373; 9: H460;10: Calu-6; 11: Calu-1; 12: H358; 13: H1792; 14: H1573; 15: H441

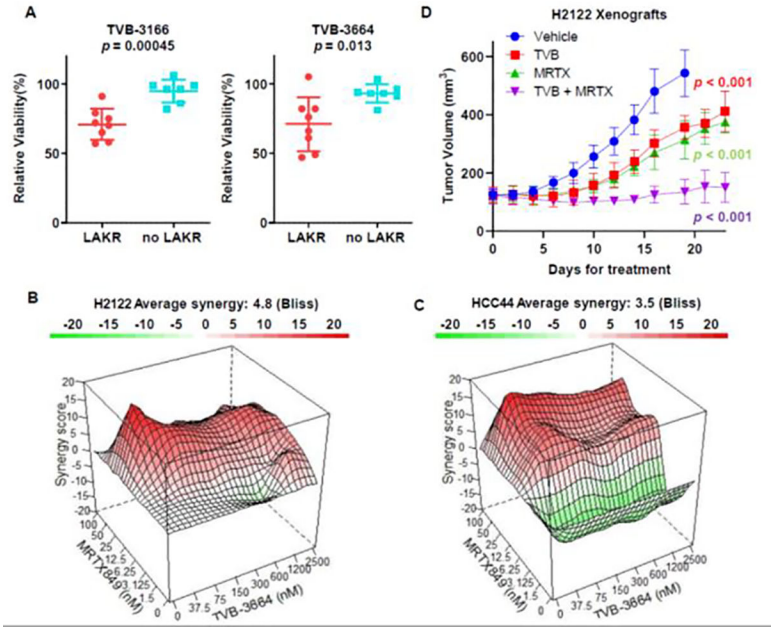

Figure 3.

KRAS^{G12C} LAKR LUAD cell lines show increased sensitivity to FASN inhibition and show synergy with direct inhibition of KRAS^{G12C}. (A) *KRAS*^{G12C} LUAD cell lines were treated by FASN inhibitors, TVB-3116 or TVB-3664 for 7 days. Then cell viability at the dose of 0.075 μM was measured by CellTiter-Glo. (B,C) The KRAS^{G12C} inhibitor MRTX849 and FASN inhibitor TVB-3664 are synergistic in LUAD cells lines with *KRAS*^{G12C} LAKR. Synergy between MRTX849 and TVB-3664 was assessed at 5 days. Cell viability was determined by CellTiter-Glo. Bliss scores were computed by R synergyfinder package. (D) H2122 xenograft tumor models show synergy between MRTX849 and TVB-3664 *in vivo*. Therapy started when tumors reached approximately 120 mm^3, therapy was initiated with 5 mg/kg TVB-3664, 10 mg/kg MRTX849 only, 5 mg/kg TVB-3664 + 10 mg/kg MRTX849, or vehicle (8 mice/group). Both inhibitors were delivered orally and were given daily. The results were shown as mean ± stdev.

Table 1.

Survey of LAR in four major cancer types.

Cancer Type	LOH Status	$KRAS^{MUT}$ (%)	$KRAS^{WT}$ (%)	*P*
LUAD	LOH	35 (24)	91 (26)	0.71
	no LOH	111 (76)	264 (74)	
	Total	146 (100)	355 (100)	
COAD	LOH	19 (14)	18 (7)	0.016
	no LOH	115 (86)	247 (93)	
	Total	134 (100)	265 (100)	
PAAD	LOH	16 (14)	3 (6)	0.12
	no LOH	101 (86)	51 (94)	
	Total	117 (100)	54 (100)	
		$NRAS^{MUT}$ (%)	$NRAS^{WT}$ (%)	
SKCM	LOH	28 (22)	64 (19)	0.48
	no LOH	101 (78)	276 (81)	
	Total	129 (100)	340 (100)	

Lung Cancer. Author manuscript; available in PMC 2022 March 01.

Table 2.

Distribution of $KRAS^{MUT}$ LUAD patients and treatment characteristics stratified by LAKR.

Baseline characteristics	LAKR (%)	no LAKR (%)	*p*
Total patients Age at diagnosis	26 (27)	72 (73)	
Age at diagnosis			0.75
<65	15 (58)	37 (51)	
≥ 65	11 (42)	35 (49)	
Gender			0.74
female	15 (58)	41 (57)	
male	11 (42)	31 (43)	
Stage			0.21
I	11 (42)	34 (47)	
II	5 (19)	23 (32)	
III–IV	9 (35)	14 (19)	
unknown	1 (4)	1 (1)	
Smoking history			0.70
<30 pack years	8 (31)	23 (32)	
≥ 30 pack years	14 (54)	33 (46)	
unknown	4 (15)	16 (22)	

Table 3.

HRs of mortality for $KRAS^{MUT}$ LUAD patients and clinical characteristics from univariate and multivariate Cox regression analysis.

Covariate	No. of patients	No. of events	Univariate analysis HR (95% CI)	*p*	Multivariate analysis HR (95% CI)	*p*
LAKR:				0.021		0.011
no	72	24	1 (reference)		1 (reference)	
yes	26	11	2.40 (1.1 – 4.90)		2.86 (1.28 – 6.40)	
Age at diagnosis				0.61		0.77
<65	46	17	1 (reference)		1 (reference)	
≥ 65	52	18	0.84 (0.43 – 1.60)		1.07 (0.50 – 2.30)	
Gender				0.14		0.24
female	56	17	1 (reference)		1 (reference)	
male	42	18	1.70 (0.85 – 3.30)		1.49 (0.70 – 3.20)	
Stage						
I	45	6	1 (reference)		1 (reference)	
II	28	16	3.50 (1.40 –8.50)	0.007	5.00 (1.86 – 13.50)	0.001
III–IV	23	12	5.10 (2.00 – 13.20)	< 0.001	6.25 (2.20 – 17.80)	< 0.001
Smoking history						
<30 pack years	31	10	1 (reference)		1 (reference)	
≥ 30 pack years	47	16	1.10 (0.51 – 2.50)	0.77	0.75 (0.32 – 1.80)	0.51
unknown	20	20	1.10 (0.43 – 2.70)	0.88	1.12 (0.43 – 2.90)	0.81