An integrative pharmacogenomics analysis identifies therapeutic targets in KRAS-mutant lung cancer

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(Article begins on next page)
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Abstract: Background
KRAS mutations are the most frequent oncogenic aberration in lung adenocarcinoma. Due to differences in protein structure and GTPase activity, KRAS mutant isoforms shape tumor biology and therefore may influence the treatment response in non-small-cell lung cancer. This heterogeneity challenges the development of effective targeted therapies for KRAS-driven lung cancer.

Methods
Here, we systematically investigated MEK/ERK inhibitors sensitivity for different KRAS mutant isoforms. Then we developed an integrative pharmacogenomics analysis to identify potential targets in lung cancer with KRAS(G12C) mutation, the most frequent aberration in patients with primary or metastatic KRAS mutant non-small cell lung cancer. We further validated our prediction by siRNA-mediated gene knockdown and TOPFlash reporter assay.

Findings
Our computational analysis identifies casein kinase 2A1 (CSNK2A1) as a mediator of MEK inhibitor resistance in KRAS(G12C) mutant cells which is not observed in cells with non-KRAS(G12C) mutations and in those harboring other oncogenic drivers as e.g. activating mutations in EGFR, BRAF, or NRAS. Knockdown of CSNK2A1 reduces proliferation, inhibits Wnt/β-catenin signalling and increases the anti-proliferative effect of selumetinib in KRAS(G12C) mutant lung cancer cells.

Interpretation
Our study suggested that accurate patients stratification will be necessary in order to observe significant benefit upon CK2 inhibition - alone or in combination - in a subset of patients with a favorable intratumoral genetic context. We provide a promising approach towards developing precision treatments for various subtypes of KRAS mutant lung cancer.

Fund
This work was supported by grants from National Natural Science Foundation of China (31571363, 31771469, and 81573023 to HW) the National key research and development program of China (2017YFC0908500 to HW), the
Lung Cancer Research Foundation (to CA) and a Mildred-Scheel postdoctoral fellowship from the German Cancer Aid Foundation (70111755 to JK).
An integrative pharmacogenomics analysis identifies therapeutic targets in KRAS-mutant lung cancer

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Abstract

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### Research in context

**Evidence before this study**

In NSCLC, different KRAS mutations have been identified according to the amino acid substitution which can affect drug sensitivity and tumor biology.

**Added value of this study**

We interrogated the publicly available pharmacogenomics dataset CGP to systematically unravel that cancer cells with different KRAS mutant isoforms differ in their drug sensitivities to MEK/ERK inhibitors. We further developed a computational pipeline to systematically identify novel therapeutic targets for KRAS(G12C) mutation, the most dominant KRAS mutation in lung cancer.

**Implications of all the available evidence**

Predicting novel therapeutic targets by considering the mutational heterogeneity of cancer histotypes will help to guide therapeutic decision-making and improve treatment outcomes. Our pipeline can potentially be extended to other mutant KRAS isoforms given that a large enough sample size is available for statistical analysis.
Introduction

The Kirsten rat sarcoma oncogene (KRAS) encodes for a small GTPase that couples growth factor signalling to the MAPK signalling cascade. Despite being an oncogene with a prevalence of 30% in non-small cell lung cancer (NSCLC), the development of KRAS targeted therapies has been largely unsuccessful in the past. This is mainly due to the higher affinity of RAS for GTP\,^{12}. Very recently, the pharmacokinetic and pharmacodynamic improvement of direct G12C inhibitors has raised great excitement\,\cite{3}, leading to two clinical studies that are currently on-going \citep{https://clinicaltrials.gov/ct2/results?cond=G12C&term=&cntry=&state=&city=&dist=}. As an alternative, inhibitors targeting kinases downstream of KRAS, such as BRAF and MEK, have been developed which showed promising activity in metastatic melanoma but were less active in KRAS mutant NSCLC. Furthermore, drug efficacy is limited by the development of acquired resistance or KRAS copy number variations\,\cite{4-12}. Hence, there is still an unmet need to develop more efficacious targeted treatment strategies for KRAS mutant lung cancer.

In NSCLC, different KRAS mutations have been identified according to the amino acid substitution which can affect drug sensitivity and tumor biology\,\cite{13,14}. The heterogenous behaviour of different KRAS mutations is due to differences in protein structure and GTPase activity\,\cite{15,17} which needs to be considered when investigating potential targets for KRAS mutant lung cancer.

Here, we perform a pan-cancer analysis to systematically investigate differences in treatment response to MEK inhibitors due to different KRAS mutational subtypes. An integrative pharmacogenomics analysis pipeline is then developed to identify potential targets in lung cancer with KRAS(G12C) mutation, the most frequent mutation (>40%) in patients with primary or metastatic KRAS mutant non-small cell lung cancer (NSCLC)\,\cite{17}. The most promising target predicted by this pipeline is casein kinase 2A1 (CSNK2A1) which encodes for the casein kinase 2 subunit alpha (CK2 alpha), a serine/threonine protein kinase that phosphorylates acidic proteins such as casein. Although there is strong evidence that CK2 plays a role in the pathogenesis of cancer\,\cite{18,20} and several CK2 inhibitors have entered clinical trials, the role of CSNK2A1 as a therapeutic target in KRAS mutant lung cancer remains unknown to date. Our study links
CSNK2A1 to Wnt/β-catenin signaling and explores its potential as therapeutic target for treating KRAS mutant lung cancer.

Materials & Methods

Key Resources Table

| KRAS(G12C) mutant cell lines for integrative pharmacogenomics analysis |
|---------------------------|-----------------|-----------------|
| Cell line                | KRAS            | Tissue          | TP53            |
| LU-65                    | G12C            | NSCLC_large cell| E11Q            |
| NCI-H2030                | G12C            | NSCLC_adenocarcinoma | Q16L |
| NCI-H2122                | G12C            | NSCLC_adenocarcinoma | C176F,Q16L |
| LU-99A                   | G12C            | NSCLC_large cell| wt              |
| NCI-H1792                | G12C            | NSCLC_adenocarcinoma | ess_splice |
| HCC-44                   | G12C            | NSCLC_adenocarcinoma | p.R175L,S94* |
| NCI-H23                  | G12C            | NSCLC_adenocarcinoma | M246I |
| NCI-H2291                | G12C;G12V       | NSCLC_adenocarcinoma | G154V |
| NCI-H358                 | G12C            | NSCLC_adenocarcinoma | wt |
| SW1573                   | G12C            | NSCLC_adenocarcinoma | wt |
| IA-LM                    | G12C            | NSCLC_large cell | Q192* |
| HOP-62                   | G12C            | NSCLC_adenocarcinoma | ess_splice |

| KRAS mutant cell lines for assays in vitro |
|----------------|-----------------|-----------------|
| Cell line       | KRAS            | Tissue          | TP53            |
| Calu1            | G12C            | NSCLC_adenocarcinoma | wt |
| H2030            | G12C            | NSCLC_adenocarcinoma | Q16L |
| A549             | G12S            | NSCLC_adenocarcinoma | wt |
Pharmacogenomics analysis to identify potential targets in KRAS(G12C) mutant lung cancer

The Cancer Genome Project (CGP) at the Wellcome Trust Sanger Institute resulted in a large-scale, high-throughput pharmacogenomic dataset for 1001 human cancer cell lines, including the mutation status of 19,100 genes, genome-wide DNA copy number variation (CNV) status, mRNA expression profiling of 17,419 genes, and pharmacological profiling for 267 anti-cancer drugs. The drug response is represented by the natural logarithm of the IC50 value, which corresponds to the half maximal inhibitory concentration of an anti-cancer drug. In this dataset, there are five MEK inhibitors including PD-0325901, selumetinib, CI-1040, trametinib, and refamtinib, and two ERK inhibitors including FR-180204 and VX-11e. Among 137 cancer cells harboring KRAS mutations, 35 cells are derived from lung cancer.

We developed a computational pipeline to identify novel therapeutic targets for KRAS mutant lung cancer (Fig. 1). First, we started the analysis with CGP dataset. With respect to tumor heterogeneity generated from the different KRAS mutation isoforms, here, we only focused on lung cancer cell lines with KRAS(G12C) mutation, the most frequent among KRAS mutation isoforms in lung cancer. In total, we included 12 cell lines into our analyses. MAPK signalling inhibitors, 5 types of MEK inhibitors including PD-0325901, selumetinib, CI-1040, trametinib, and refamtinib, and 2 types of ERK inhibitors including FR-180204 and VX-11e were included in this analysis. The expression of 17,420 genes was used to individually calculate their correlation with drug sensitivity. As expected, the high expression of some genes was significantly correlated with the decreased drug sensitivity of MEK inhibitors or ERK inhibitors. Of these, the genes with association with more than two MAPK signalling inhibitions were considered as the potential targets. Second, they were upregulated in KRAS(G12C) mutant Lung adenocarcinoma (LUAD) patients in comparison with normal samples in TCGA database. Optionally, the high expression of the genes has a poorer clinical prognosis. Third, we further filter the genes with selection criteria that required the genes to be part of cancer core pathways and to be known drug targets. Finally, by integrative analysis of the above-mentioned criteria, we identified potential targets for KRAS mutant lung cancer.
TCGA data analysis

The RNA-seq and clinical data of LUAD patients were downloaded from TCGA cBioProtal (http://www.cbioportal.org/index.do). The abundance of each gene was quantified as RSEM value, which was evaluated by a statistical method RSEM (RNA-Seq by Expectation Maximization). RSEM uses a generative model of RNA-seq reads and the EM algorithm, taking read mapping uncertainty into account and achieving the most accurate abundance estimates \(^{21}\). The statistical analysis of differentially expressed genes between cancer and normal samples was performed using DESeq2 \(^{22}\). LUAD patients were divided into high and low expressing group, based on the median value of gene expression across the patients. Kaplan-Meier test was used to compare the overall survival and cancer relapse between two groups.

Cell lines

The human lung cancer cell lines A549, H2030, H2009 and Calu1 were purchased from ATCC and grown at 37°C in RPMI medium supplemented with 10% fetal bovine serum (FBS), 100 \( \mu \) g/ml penicillin and 100 units/ml streptomycin (complete medium). The cell lines were authenticated using the Promega GenePrint 10 System at the RTSF Genomics Core at Michigan State University. All cell lines used in the study tested negative for Mycoplasma as determined by the Mycoplasma Plus PCR Primer Set (Agilent).

Assessment of cellular proliferation

Cells (1 \( \times \) 10\(^3\)) were seeded in 96-well plates in 100 \( \mu \) l RPMI media supplemented with 10% FBS and penicillin/streptomycin. The following day, plates were incubated in the IncuCyte ZOOM\(^\text{TM}\) (Essen BioScience) for real-time imaging, with three fields imaged per well under 10x magnification every two hours for a total of 120 hours. Data were analyzed using the IncuCyte Confluence version 1.5 software (Essen BioScience), which quantifies cell surface area coverage as confluence values. IncuCyte experiments were performed in triplicate. A representative growth curve is shown for each condition.

Western blot analysis

Cells from in vitro culture were lysed in RIPA lysis buffer (#89900 Thermo Fisher) supplemented with protease and phosphatase inhibitor cocktail tablets (Roche). The antibodies used for western blotting
included those against: HSP90 (H114) (Santa Cruz Biotech Cat#sc-7947), phosphorylated Akt (Ser473) (Cell Signaling Cat#4060), Akt (Cell Signaling Cat#9272), phosphorylated ERK1/2 (Cell Signaling Cat#4370), ERK1/2 (Cell Signaling Cat#4695), phosphorylated MEK (Cell Signaling Cat#9154), MEK (Cell Signaling Cat#8727), phosphorylated S6 (Ser235/236) (Cell Signaling Cat#4858), S6 ribosomal protein (Cell Signaling Cat#2217), β-catenin (Cell Signaling Cat#8480), p27 (Cell Signaling Cat#3688), cMyc (Cell Signaling Cat#2276), anti-rabbit IgG, HRP-linked secondary antibody Cell Signaling (Cat#7074P2), ECL Sheep anti-Mouse IgG, HRP-linked secondary antibody (GE Healthcare Cat#NA931V), ECL Donkey anti-Rabbit IgG, HRP-linked secondary antibody (GE Healthcare Cat#NA934V). Western blotting showed in the manuscript are representative of at least three independent experiments.

**SiRNA-mediated gene knockdown**

Cells (1.5x10^6) were seeded in a 10cm plate and incubated overnight at 37°C. On the next day, media was replaced by antibiotic free full media and the mixture of siRNA (scrambled, CSNK2A1, Dharmacon) at a final concentration of 20nM together with DharmaFECT 1 was added after allowing 30min of complex formation in serum-free media. Knockdown efficacy was assessed by Western blot and qRT-PCR after 48hrs of transfection. For treatment experiments cells were harvested and re-seeded after 48hrs of siRNA treatment and treated with selumetinib for another 24 to 96hrs.

**TOPFlash reporter assay**

Cells (1.5x10^6) were seeded in a 10cm plate and incubated overnight at 37°C. On the next day, cells were transiently transfected with 1μg of M50 Super 8x TOPFlash reporter plasmid, 100ng of a pRL Renilla Luciferase control reporter plasmid (Promega) and FuGENE HD (Promega). M50 Super 8x TOPFlash was a gift from Randall Moon (Addgene plasmid #12456). After 24hrs, cells were washed with PBS and full media was added for another 24hrs without or with Mek inhibitor (selumetinib, 1μm). Luciferase activity was measured with the Dual Luciferase reporter assay (Promega).

**Results**
Cancer cells with different KRAS mutant isoforms differ in their drug sensitivities to MEK/ERK inhibitors

We first interrogated the publicly available pharmacogenomics dataset CGP, which includes mutational and pharmacological profiles of >1000 human cancer cell lines treated with 265 anti-cancer drugs. Drug sensitivities are represented by the natural logarithm of the drug’s IC50 value. To investigate MEK/ERK inhibitors sensitivity for different KRAS mutant isoforms, we grouped all cancer cells based on their KRAS mutation status, and then used the Kruskal-Wallis H-test to compare drug sensitivities between multiple groups and the t-test to compare drug sensitivities between two groups.

KRAS mutant cancer cell lines were divided into 12 groups, respectively, with A146T, G12A, G12C, G12D, G12R, G12S, G12V, G13C, G13D, K117N, Q61H, or Q61L mutations. We found that MEK/ERK inhibitors drug sensitivities vary in cell lines with different KRAS mutations, including CI-1040, refametinib (RDEA119), PD-0325901, selumetinib, trametinib, and VX-11e (Fig. 2). Cells lines with G12R mutation were in general more sensitive to MEK inhibitors in comparison with other types of KRAS mutations (Fig. 2a-f). To address the question if also the tissue of origin influences response to MAPK pathway inhibition, we furthermore investigated the effect of different KRAS mutations on drug sensitivities in the two major cancer histotypes of lung and pancreatic cancer. Differences in MEK/ERK inhibitors sensitivities across the different types of KRAS mutations were observed in both cancer types, being pancreatic cancer cells with G12R mutation (Additional file 1: Figure S1a-c) and lung cancer cells with G12A mutation (Additional file 1: Figure S1d-e) most sensitive to MEK inhibition, respectively.

We surveyed the datasets of primary LUAD patients from TCGA and metastatic LUAD patients from MSK-IMPACT to investigate the prevalence of different KRAS mutational isoforms (Fig. 3). 75 (33%) of patients with primary LUAD and 241 (27%) of patients with metastatic LUAD patients harbor KRAS mutations, respectively. In total, we observed ten different types of KRAS mutations, including G12C, G12D, G12A, G12F, G12S, G12V, G12Y, Q61L, D33E, in the primary LUAD TCGA dataset (Additional file 2: Table S1). Whereas KRAS(G12C) is the dominant mutation in patients with primary KRAS mutant NSCLC (48.00%, Fig. 3a), patients with metastatic LUAD exhibit a more complex pattern
of KRAS mutations. Among 19 types of KRAS mutations, 11 types (A146T, A146V, A59T, AG59GV, G13C, G13D, G13E, G13R, G13V, Q61R, and T58I) are exclusively observed in patients with metastatic LUAD but not in primary tumors (Fig. 3b, Additional file3: Table S2). In metastatic LUAD patients, the KRAS(G12C) mutation is also the most prevalent one (42.74%), followed by G12V (15.35%) and G12D (15.35%) mutations.

**Pharmacogenomics analysis to identify potential targets in lung cancer harboring KRAS(G12C) mutations**

Our above-mentioned analysis of the CGP dataset suggests that cancer cell lines with different KRAS mutations exhibit different sensitivities to MAPK pathway inhibition. In the present analysis, we focused on lung cancer cell lines with KRAS(G12C) mutation, the most dominant KRAS mutation in lung cancer. In our analysis, a total of 12 cell lines were included (see Methods). We developed a computational pipeline to identify novel therapeutic targets for KRAS mutant lung cancer (Fig. 1). In a first step of this pipeline, 1212 genes with association with more than two inhibitors of MAPK signalling were considered as potential targets. In a second step, using the TCGA database, we selected 494 genes which are upregulated in LUAD patients and are associated with poor survival. Finally, we narrowed down the number of genes by requiring them to be part of core cancer pathways as well as to be known drug targets. This algorithm finally led to the identification of 14 potential therapeutic targets for KRAS mutant lung cancer (Fig. 1d), including AARS2, ALKBH2, CARS, CDK8, COMP, CSNK2A1, DARS, EPRS, HDAC1, IARS2, MAPK8, PARS2, RPL8 and YARS (Additional file 4: Table S3).

Among the 14 candidate genes identified by our pharmacogenomics analysis, CSNK2A1 was ranked as the most promising gene. CSNK2A1 encodes for a protein which is a component of the highly conserved serine/threonine protein kinase CK2 alpha. CK2 alpha itself is part of various pathways relevant for cancer cell biology among them Wnt (Fig. 4a) and NF-kappa B signaling. This is especially relevant as there is an increased interest for CSNK2 as a therapeutic target in ongoing clinical trials. The association of CSNK2A1 expression and reduced MEK/ERK inhibitors sensitivity was repeatedly observed for 4 different MEK inhibitors, including 2 replicate datasets for refametinib and selumetinib (Fig. 4b). Importantly,
LUAD patients or LUAD patients with KRAS(G12C) mutation showed an increased expression of CSNK2A1 in comparison with normal lung tissue (Fig. 4c, \( p=1.35e^{-18} \)). Moreover, LUAD patients with high CSNK2A1 expression had a trend towards poorer overall survival (Additional file 5: Figure S2).

Correlation of CSNK2A1 levels and MEK inhibitor resistance is neither observed in non-KRAS(G12C) mutant lung and pancreatic cancer cells, nor in lung cancer with EGFR, BRAF or NRAS mutations

We next investigated if the correlation between CSNK2A1 expression and MEK inhibitor resistance can also be observed in non-KRAS(G12C) mutant cancer cells. As KRAS(G12V) mutations represent the second most frequent mutation in LUAD, nine KRAS(G12V) mutant lung cancer cell lines from CGP were included in the statistical analysis. No correlation was found between CSNK2A1 expression and drug sensitivity to 7 MEK inhibitors in KRAS(G12V) mutant lung cancer cells (Fig. 5a). Due to the limited sample size for other non-KRAS(G12C) mutations, we pooled the remaining lung cancer cells with non-KRAS(G12C) mutations, for which no positive correlation between CSNK2A1 expression and drug sensitivity was observed (Fig. 5b). As KRAS(G12V) and KRAS(G12D) mutations occur more frequently in pancreatic cancer, we therefore also investigated the correlation of CSNK2A1 levels and MEK inhibitor resistance in KRAS(G12V) or KRAS(G12D) mutant pancreas cancer cells. This analysis also showed no correlation between CSNK2A1 expression and drug sensitivity (Fig. 5c,d).

We furthermore investigated if there is any correlation between CSNK2A1 expression and MEK inhibitor resistance in lung cancer cell lines with other oncogenic mutations affecting the MAPK signaling pathway as for example BRAF, EGFR, or NRAS. However, our analysis showed no correlation between CSNK2A1 expression and MEK inhibitor resistance for BRAF- (Fig. 6a), EGFR- (Fig. 6b), or NRAS-mutant (Fig. 6c) lung cancer cells. Cell lines we used for the analysis were in Additional file 6: Table S4.

CSNK2A1 knockdown reduces proliferation and Wnt/β-catenin reporter activity in KRAS(G12C) mutant lung cancer cells, and increases the anti-proliferative effect of selumetinib
We selected two KRAS(G12C) mutant lung cancer cell lines (Calu1 and H2030) and two non-KRAS(G12C) mutant cell lines (A549 and H2009) to investigate the effect of siRNA-mediated CSNK2A1 knockdown on cell proliferation and efficacy of MAPK pathway inhibition with 1µM of selumetinib.

Knockdown of CSNK2A1 (Fig. 7a) alone significantly decreased proliferation of KRAS(G12C) mutant cells Calu1 (Fig. 7b) and H2030 (Fig. 7c) and increased the anti-proliferative activity of simultaneous MEK inhibition in Calu1 cells (Fig. 7b). These effects were not observed in non-KRAS(G12C) mutant lung cancer cell lines (Fig. 7d-f).

As casein kinases have previously been connected to the drug resistance mediating Wnt/β-catenin pathway, we therefore investigated if CSNK2A1 influences Wnt/β-catenin signaling in KRAS(G12C) mutant lung cancer. We used gene expression profiles of LUAD patients (TCGA) and cancer cell lines (CCLE) and categorized samples into CSNK2A1 high and low expressing groups. Deseq2 was applied to call differentially expressed genes between the two groups. Gene set enrichment analysis (GSEA) was further employed to determine the pathways enriched by a pre-ranked list of all genes, which were sorted by the statistical significance of differential expression defined by the Deseq2 analysis. GSEA showed that the Wnt signaling pathway was significantly enriched in the CSNK2A1 high expressing group in CCLE (Fig. 8a, p=0.008) and TCGA (Fig. 8b, p=0.014).

To support our computational findings, we also investigated the differences in Wnt/β-catenin signaling parameters between KRAS(G12C) and KRAS(non-G12C) cell lines in vitro. After 24hrs of selumetinib treatment, accumulation of the cell cycle inhibitor p27 upon CSNK2A1 knockdown was relatively increased in KRAS(G12C) mutant Calu1 and H2030 cells compared to KRAS(non-G12C) A549 and H2009 cells (Fig. 8c). This suggests that Calu1 and H2030 cells are more dependent on CSNK2A1 to overcome MEK inhibitor induced growth arrest. This is also in agreement with the stronger anti-proliferative effect of CSNK2A1 knockdown itself in cells with KRAS(G12C) mutation (Additional file 7: Figure S3, Fig. 7b,e). Furthermore, transient transfection of the Wnt/TCF reporter plasmid 8xTOPFlash showed stronger reduction of reporter activity in Calu1 cells with KRAS(G12C) mutation than in
KRAS(G12S) mutant A549 cells upon CSNK2A1 knockdown and upon simultaneous treatment with selumetinib (Fig. 8d).

**Discussion**

In this study, we used pharmacogenomics data to systematically unravel the heterogeneity of responses to MAPK pathway inhibition due to different KRAS mutation isoforms. Subsequently, we developed a pharmacogenomics analysis pipeline to identify novel targets for the subgroup of KRAS(G12C) mutant lung cancer. Our computational pipeline identified a correlation between CSNK2A1 expression and MEK inhibitor resistance in KRAS(G12C) mutant cells, a finding that was exclusively observed in this mutational subset of lung cancer cells, but not in KRAS(non-G12C) mutant cells and neither in tumor cells harboring other oncogenic mutations as e.g. EGFR, BRAF, or NRAS. This suggests that the correlation between CSNK2A1 expression and MEK inhibitor resistance may depend on the context of KRAS(G12C) mutation in lung cancer. A pan-cancer analysis of the TCGA dataset showed that CSNK2A1 is upregulated in a wide range of cancers (Additional file 8: Figure S4) and that pancreatic cancer patients with high intratumoral CSNK2A1 expression have a worse overall and relapse-free survival (Additional file 9: Figure S5).

CSNK2A1 encodes for a protein that is a component of the highly conserved serine/threonine protein kinase CK2 alpha. Previous studies have identified CSNK2 as an oncogene when overexpressed in mice, playing a key role in the pathogenesis of cancer, including breast, lung, colon, and prostate cancer, as well as hematologic malignancies.\(^{19,20,30}\) Moreover, a cancer context-dependent effect of CK2 on signaling pathways such as Wnt signaling\(^{20}\), JAK/STAT\(^{31}\), NF-κB\(^{20}\), and PTEN/PI3K/Akt-PKB\(^{32,33}\) has been described in the past. In the Wnt pathway, CK2 acts by phosphorylating and stabilizing Dvl and β-catenin and promotes T-cell factor/lymphoid enhancer-binding factor (TCF) DNA binding in the nucleus (Fig. 4a). Based on these observations, CK2 has recently arisen as a promising candidate for targeted therapy, with two CK2 inhibitors in ongoing clinical trials (https://clinicaltrials.gov/ct2/results?cond=&term=CK2&cntry=&state=&city=&dist=). Our results suggest that accurate patients stratification will likely be necessary in order to observe significant benefit upon CK2.
inhibition - alone or in combination - in a subset of patients with a favorable intratumoral genetic context.

In agreement with our computational results, CSNK2A1 knockdown significantly reduced the proliferation of KRAS(G12C) mutant lung cancer cells, an effect that was not observed in cells with non-G12C KRAS mutations (Fig. 7). This identifies CSNK2A1 as an interesting target per se in KRAS(G12C) mutant NSCLC cell lines. Furthermore, simultaneous CSNK2A1 knockdown and MEK inhibition with selumetinib increased the anti-proliferative effect of selumetinib in KRAS(G12C) mutant but not in KRAS(non-G12C) mutant cells. Mechanistically, our analysis including transient 8xTOPFlash reporter transfection shows that CSNK2A1 mediates TCF transcriptional activity in KRAS(G12C) mutant lung cancer (Fig. 8d). The importance of CSKN2A1 for Wnt signaling is also supported by our gene-set enrichment analysis (GSEA) of the CCLE and TCGA datasets which show significant enrichment for the Wnt signaling pathway (Fig. 8a, b).

For a given oncogene, different types of nonsynonymous or indel variants impact differently on the biological function of the respective protein. This results in different oncogenic activities in cancer cells with different oncogenic mutations and challenges the development of effective targeted therapies. Therefore, predicting novel therapeutic targets by considering the mutational heterogeneity of cancer histotypes will help to guide therapeutic decision-making and improve treatment outcomes. Although the pharmacogenomics approach suggested by us here was applied to KRAS(G12C) mutant lung cancer cells, this pipeline can potentially be extended to other mutant KRAS isoforms given that a large enough sample size is available for statistical analysis.

Acknowledgements

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to HW), the Lung Cancer Research Foundation (to CA) and a Mildred-Scheel postdoctoral fellowship from the German Cancer Aid Foundation (70111755 to JK).

**Declarations of interests**

The authors declare no competing financial interests.

**Authors’ contributions**

HW, JK and CA conceived the hypothesis. HW and QL designed and performed the data analysis. YX, ZC, JZ, XC, and YD collected and preprocessed the data. JK and CA performed experimental validation. HW, JK and CA wrote the manuscript. PJ provided the resources of experimental validation and helpful suggestions on the validation.

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**Figures and Tables Legend**

**Figure 1. A pharmacogenomics analysis identifies the potential therapeutic targets in KRAS-mutant lung cancer cells.** A, pharmacogenomics data and analysis for identifying genes whose high expression associates with decreased drug sensitivity. B, TCGA data analysis for evaluating the expression of genes in lung adenocarcinoma (LUAD) patients as well as their correlation with clinical prognosis. C, Cancer core pathway and DrugBank information for further investigating the biological relevance of candidate genes. D, Overall integrative analysis for determining the potential therapeutic targets.

**Figure 2. Pan-cancer analysis of drug sensitivities to MEK/ERK inhibitors, including CI-1040, refametinib, PD-0325901, selumetinib, trametinib, and VX-11e, in cancer cells with different types of KRAS mutations.** The p value of the multiple-groups comparison is indicated. A symbol * denotes the pairwise comparison with p value smaller than 0.05.

**Figure 3. Frequencies of different KRAS mutations in the primary LUAD patients in TCGA dataset and the metastatic LUAD patients in MSK-IMPACT dataset.** KRAS(G12C) is the most common mutation (>40%) across LUAD patients.

**Figure 4. A pharmacogenomics analysis identifies CSNK2A1 as a potential therapeutic target in KRAS-mutant lung cancer cells.** A, The protein encoded by CSNK2A1 is a serine/threonine protein kinase, which is involved in various cellular processes, including the Wnt signalling pathway. B, High expression of CSNK2A1 is associated with decreased drug sensitivity to MEK inhibitors refametinib, selumetinib, CI-1040, and trametinib. C, CSNK2A1 expression is significantly higher in LUAD compared to normal lung tissue.
Figure 5. (A) Expression of CSNK2A1 in lung cancer cell lines with KRAS(G12V) mutation, the second most frequent mutation in lung cancer, is not correlated with drug sensitivity to MEK inhibitors. (B) Expression of CSNK2A1 in other non-G12C mutant lung cancer cell lines, is not positively correlated with drug sensitivity to MEK inhibitors. (C) Expression of CSNK2A1 in pancreatic cancer cell lines with KRAS(G12V) mutation, the most frequent mutation in pancreatic cancer, is not correlated with drug sensitivity to MEK inhibitors. (D) Expression of CSNK2A1 in pancreatic cancer cell lines with KRAS(G12D) mutation, the second most frequent mutation in pancreatic cancer, is not correlated with drug sensitivity to MEK inhibitors.

Figure 6. Expression of CSNK2A1 is not correlated with MEK inhibitor sensitivity in lung cancer cell lines with BRAF (A), EGFR (B) or NRAS (C) mutations.

Figure 7. CSNK2A1 Knockdown reduces the proliferation of KRAS(G12C) mutant lung cancer cells and increases the anti-proliferative effect of the MEK inhibitor selumetinib. (A) Western blot analysis of CSNK2A1 protein in KRAS(G12C) mutant cell lines Calu1 and H2030 transfected with scrambled or CSNK2A1 targeting siRNA without or with simultaneous selumetinib (1 μm) treatment. Growth rates of Calu1 (B) and H2030 (C) transfected with scrambled (black and purple) or CSNK2A1 targeting siRNA (red and grey) without or with simultaneous selumetinib (1 μm) treatment. Phase-contract images are shown at the bottom. (D) Western blot analysis of CSNK2A1 protein in KRAS(G12S) mutant cell line A549 and in KRAS(G12A) mutant cell lines H2009 transfected with scrambled or CSNK2A1 targeting siRNA without or with simultaneous selumetinib (1 μm) treatment. Growth rates of A549 (E) and H2009 (F) transfected with scrambled (black and purple) or CSNK2A1 targeting siRNA (red and grey) without or with simultaneous selumetinib (1 μm) treatment. Phase-contract images are shown at the bottom.

Figure 8. CSNK2A1 knockdown inhibits the activation of Wnt/β-catenin signaling in KRAS(G12C) mutant lung cancer cells. GSEA analysis based on CCLE lung cancer cells (A) and TCGA LUAD patients (B) with KRAS mutation shows that Wnt signaling pathway is enriched in tumors with high CSNK2A1 expression. (C) Western blot analysis shows increased p27 induction upon selumetinib
treatment in siCSNK2A1 treated Calu1 and H2030 compared to A549 and H2209. (D) Reporter activity of Wnt/TCF reporter assay in Calu1 KRAS(G12C) and A549 KRAS(G12S). Student t-test are performed, with a symbol *, **, and **** respectively representing the comparison with p value smaller than 0.05, 0.01 and 0.0001.

Additional file1: Figure S1. Pan-cancer analysis investigates the drug sensitivity to MEK/ERK inhibitors, including CI-1040, refametinib, PD-0325901, selumetinib, trametinib, and VX-11e, of the different types of KRAS mutations in pancreatic cancer and lung cancer cell lines. The p value of the multiple-groups comparison is given. A symbol * denotes the pairwise comparison with p value smaller than 0.05.

Additional file2: Table S1. The prevalence of different KRAS mutational isoforms in primary LUAD patients

Additional file3: Table S2. The prevalence of different KRAS mutational isoforms in metastatic LUAD patients

Additional file4: Table S3. Fourteen potential therapeutic targets for KRAS mutant lung cancer.

Additional file5: Figure S2. LUAD patients with higher expression of CSNK2A1 tend to have shorter overall survival.

Additional file6: Table S4. Information of cell lines with KRAS(G12C) mutation in lung cancer, with KRAS(G12V) mutation in lung cancer, with KRAS(non-G12C) mutation in lung cancer, with KRAS(G12V) mutation in pancreatic cancer, with KRAS(G12D) mutation in pancreatic cancer, with BRAF mutation in lung cancer, with EGFR mutation in lung cancer, and with NRAS mutation in lung cancer.
Additional file7: Figure S3. Growth rates of Calu1 KRAS(G12C) (a) and A549 KRAS(G12S) (b) transfected with scrambled or CSNK2A1 targeting siRNA without or with simultaneous selumetinib treatment. Student t-tests are performed, with a symbol *, ****, and n.s. respectively representing the comparison with p value smaller than 0.05, 0.0001 and greater than 0.05.

Additional file8: Figure S4. Expression of CSNK2A1 is upregulated in a wide range of cancers.

Additional file9: Figure S5. Pancreatic cancer patients with higher expression of CSNK2A1 significantly have poorer survival.
Pharmacogenomics data (CGP dataset)
* 12 KRAS(G12C) mutant lung cell lines
* 6 MEK inhibitors, 2 ERK inhibitors
* genome-wide gene expression

TCGA data (LUAD patients)
* 36 KRAS(G12C) mutant patients
* 576 samples (517 patients vs. 59 normal)
* genome-wide gene expression
* clinical prognosis

Cancer core pathway
* KEGG
* REACTOME
* BIOCARTA et al.
DrugBank
Primary LUAD (TCGA)

- G12C: 48.00%
- G12V: 26.67%
- G12A: 1.33%
- G12D: 1.33%
- G12F: 6.67%
- G12R: 2.67%
- G12S: 2.67%
- G12Y: 2.67%
- Q61L: 1.66%

Metastatic LUAD (MSK-IMPACT)

- G12C: 42.74%
- G12V: 15.35%
- G12A: 7.88%
- G12D: 4.98%
- G12F: 0.83%
- G12R: 0.41%
- G12S: 0.41%
- G12Y: 0.41%
- Q61L: 0.41%
Figure 4

Click here to download Figure: Fig4.CK1 as a candidate.pdf

A Wnt signaling pathway diagram

Expression of CSNK2A1

- Refametinib_ID1526 (MEK inhibitor)
  - Ln(C50) vs Expression of CSNK2A1
  - $r = 0.79$, $p = 0.002$

- Selumetinib_ID1062 (MEK inhibitor)
  - Ln(C50) vs Expression of CSNK2A1
  - $r = 0.881$, $p = 0.004$

- Refametinib_ID1014 (MEK inhibitor)
  - Ln(C50) vs Expression of CSNK2A1
  - $r = 0.782$, $p = 0.008$

- Selumetinib_ID1498 (MEK inhibitor)
  - Ln(C50) vs Expression of CSNK2A1
  - $r = 0.741$, $p = 0.006$

- CI-1040_ID1015 (MEK inhibitor)
  - Ln(C50) vs Expression of CSNK2A1
  - $r = 0.567$, $p = 0.112$

- Trametinib_ID1372 (MEK inhibitor)
  - Ln(C50) vs Expression of CSNK2A1
  - $r = 0.587$, $p = 0.045$

Expression of CSNK2A1
Figure 6

Click here to download Figure: Fig6_BRAF_EGFR_NRAS_mutation.pdf
Figure 7
Click here to download Figure: Fig7.cell_proliferation.pdf
Figure 8
Click here to download Figure: Fig8.Wnt_signaling.pdf

Enrichment plot: KEGG_WNT_SIGNALING_PATHWAY

- Enrichment score (ES)
- CCLE TCGA
- Enrichment score (ES)
- p = 0.008
- p = 0.014

|                | Calu1 | H2030 | A549 | H2009 |
|----------------|-------|-------|------|-------|
| siSCR          | +     | -     | +    | -     |
| siCSK          | -     | +     | -    | +     |
| selumet        | -     | +     | -    | +     |
| CSK            | -     | +     | -    | +     |
| CSK long exp.  | -     | +     | -    | +     |
| CyclinD1       | -     | +     | -    | +     |
| CyclinD1 long exp. | -   | +     | -    | +     |
| PARP           | -     | +     | -    | +     |
| cl. PARP       | -     | +     | -    | +     |
| p27            | -     | +     | -    | +     |
| c-MYC          | -     | +     | -    | +     |
| β-CAT          | -     | +     | -    | +     |
| HSP90          | -     | +     | -    | +     |

TCF

- RLU
- ****
- **
- A549
- Calu1
Supplementary Figure S2

Click here to download Figure: Additional file5_Figure S2.pdf

Survival Probability

Time (Months)

Low (253)

High (252)

CSNK2A1

p=0.07
Supplementary Figure S3

Click here to download Figure: Additional file7_Figure S3.pdf
Supplementary Figure S5

Overall survival probability
CSNK2A1

Relapse-free Probability
CSNK2A1

Low (69) vs High (69)
p=3.41e-04

Low (89) vs High (88)
p=9.12e-04

Click here to download Figure: Additional file9_Figure S5.pdf
| KRAS mutation | # of patients |
|---------------|--------------|
| D33E          | 1            |
| G12A          | 6            |
| G12C          | 36           |
| G12D          | 5            |
| G12F          | 2            |
| G12R          | 1            |
| G12S          | 2            |
| G12V          | 20           |
| G12Y          | 1            |
| Q61L          | 1            |
| total         | 75           |
| KRAS mutation | # of patients |
|---------------|---------------|
| A146T         | 1             |
| A146V         | 1             |
| A59T          | 1             |
| AG59GV        | 1             |
| G12A          | 19            |
| G12C          | 103           |
| G12D          | 37            |
| G12F          | 2             |
| G12R          | 2             |
| G12S          | 4             |
| G12V          | 37            |
| G13C          | 4             |
| G13D          | 12            |
| G13E          | 2             |
| G13R          | 1             |
| G13V          | 1             |
| Q61H          | 11            |
| Q61R          | 1             |
| T58I          | 1             |
| total         | 241           |
| drugId | drugName    | target_gene          | target_pathway gene | cells     | Spearman | pval  | adjPval |
|--------|-------------|----------------------|---------------------|-----------|----------|-------|---------|
| 1372   | Trametinib  | MEK1, MEK2 ERK MAPK si AARS2 | KRASG12C          | 0.657     | 0.02     | 0.397945292 |
| 1526   | RDEA119     | MEK1, MEK2 ERK MAPK si AARS2 | KRASG12C          | 0.615     | 0.033    | 0.533334017 |
| 1060   | PD-0325901  | MEK1, MEK2 ERK MAPK si ALKBH2 | KRASG12C          | 0.915     | 0        | 0      |
| 1014   | RDEA119     | MEK1, MEK2 ERK MAPK si ALKBH2 | KRASG12C          | 0.721     | 0.019    | 0.387727888 |
| 1498   | selumetinib | MEK1, MEK2 ERK MAPK si CARS | KRASG12C          | 0.811     | 0.001    | 0.031604522 |
| 1526   | RDEA119     | MEK1, MEK2 ERK MAPK si CARS | KRASG12C          | 0.79      | 0.002    | 0.061137987 |
| 1372   | Trametinib  | MEK1, MEK2 ERK MAPK si CARS | KRASG12C          | 0.748     | 0.005    | 0.138871421 |
| 1062   | selumetinib | MEK1, MEK2 ERK MAPK si CARS | KRASG12C          | 0.81      | 0.015    | 0.330350852 |
| 1498   | selumetinib | MEK1, MEK2 ERK MAPK si CARS | KRASG12C          | 0.673     | 0.033    | 0.533334017 |
| 1526   | RDEA119     | MEK1, MEK2 ERK MAPK si CDK8 | KRASG12C          | 0.769     | 0.003    | 0.088607594 |
| 1015   | CT-1040     | MEK1, MEK2 ERK MAPK si COMP | KRASG12C          | 0.601     | 0.039    | 0.584552683 |
| 1498   | selumetinib | MEK1, MEK2 ERK MAPK si COMP | KRASG12C          | 0.692     | 0.013    | 0.299435549 |
| 1526   | RDEA119     | MEK1, MEK2 ERK MAPK si CSNK2A1 | KRASG12C         | 0.79      | 0.002    | 0.061137987 |
| 1062   | selumetinib | MEK1, MEK2 ERK MAPK si CSNK2A1 | KRASG12C         | 0.881     | 0.004    | 0.115864071 |
| 1498   | selumetinib | MEK1, MEK2 ERK MAPK si CSNK2A1 | KRASG12C         | 0.741     | 0.006    | 0.163150558 |
| 1526   | RDEA119     | MEK1, MEK2 ERK MAPK si CSNK2A1 | KRASG12C         | 0.673     | 0.033    | 0.533334017 |
| 1372   | Trametinib  | MEK1, MEK2 ERK MAPK si CSNK2A1 | KRASG12C         | 0.769     | 0.003    | 0.088607594 |
| 1060   | PD-0325901  | MEK1, MEK2 ERK MAPK si DARS | KRASG12C          | 0.697     | 0.025    | 0.461067753 |
| 1014   | RDEA119     | MEK1, MEK2 ERK MAPK si DARS | KRASG12C          | 0.697     | 0.025    | 0.461067753 |
| 1498   | selumetinib | MEK1, MEK2 ERK MAPK si DARS | KRASG12C          | 0.881     | 0        | 0      |
| 1526   | RDEA119     | MEK1, MEK2 ERK MAPK si EPRS | KRASG12C          | 0.811     | 0.001    | 0.031604522 |
| 1014   | RDEA119     | MEK1, MEK2 ERK MAPK si EPRS | KRASG12C          | 0.818     | 0.004    | 0.115864071 |
| 1372   | Trametinib  | MEK1, MEK2 ERK MAPK si EPRS | KRASG12C          | 0.692     | 0.013    | 0.299435549 |
| 263    | FR-180204   | ERK ERK MAPK si HDAC1 | KRASG12C          | 0.72      | 0.008    | 0.20530223 |
| 262    | VX-11e      | ERK ERK MAPK si HDAC1 | KRASG12C          | 0.615     | 0.033    | 0.533334017 |
| 1060   | PD-0325901  | MEK1, MEK2 ERK MAPK si IARS2 | KRASG12C         | 0.818     | 0.004    | 0.115864071 |
| 1014   | RDEA119     | MEK1, MEK2 ERK MAPK si IARS2 | KRASG12C         | 0.721     | 0.019    | 0.387727888 |
| 262    | VX-11e      | ERK ERK MAPK si MAPK8 | KRASG12C          | 0.762     | 0.004    | 0.115864071 |
| 263    | FR-180204   | ERK ERK MAPK si MAPK8 | KRASG12C          | 0.699     | 0.011    | 0.261266107 |
| 1526   | RDEA119     | MEK1, MEK2 ERK MAPK si PARS2 | KRASG12C        | 0.72      | 0.008    | 0.20530223 |
| 1498   | selumetinib | MEK1, MEK2 ERK MAPK si PARS2 | KRASG12C        | 0.587     | 0.045    | 0.624014981 |
| 1526   | RDEA119     | MEK1, MEK2 ERK MAPK si RPL8 | KRASG12C        | 0.72      | 0.008    | 0.20530223 |
| 1498   | selumetinib | MEK1, MEK2 ERK MAPK si RPL8 | KRASG12C        | 0.636     | 0.026    | 0.467907815 |
| 1372   | Trametinib  | MEK1, MEK2 ERK MAPK si RPL8 | KRASG12C        | 0.608     | 0.036    | 0.56542678 |
| 1372   | Trametinib  | MEK1, MEK2 ERK MAPK si YARS | KRASG12C        | 0.678     | 0.015    | 0.330350852 |
| 1498   | selumetinib | MEK1, MEK2 ERK MAPK si YARS | KRASG12C        | 0.671     | 0.017    | 0.361519728 |
| 1014   | RDEA119     | MEK1, MEK2 ERK MAPK si YARS | KRASG12C        | 0.636     | 0.048    | 0.637656047 |
Table S4-1. Information of cell lines with KRAS(G12V) mutation in lung cancer

| Cell line  | COSMIC_ID&tissue | Gene | Mutation                                      | TP53                      |
|------------|-----------------|------|-----------------------------------------------|---------------------------|
| NCI-H727   | 724855_lung     | KRAS | (missense:c.35G>T:p.G12V)                     | (Missense:c.49)           |
| NCI-H2444  | 1298356_lung    | KRAS | (missense:c.35G>T:p.G12V)                     | (Missense:c.70)           |
| LCLC-97TM1 | 946361_lung     | KRAS | (missense:c.35G>T:p.G12V)                     | (frameshift:c.1)          |
| NCI-H2591  | 724874_lung     | KRAS | (missense:c.35G>T:p.G12V)(missense:c.49)     | (frameshift:c.1)          |
| SHP-77     | 724872_lung     | KRAS | (missense:c.35G>T:p.G12V)                     | (missense:c.46)           |
| COLO-668   | 910692_lung     | KRAS | (missense:c.35G>T:p.G12V)                     | (missense:c.52)           |
| SW900      | 724879_lung     | KRAS | (missense:c.35G>T:p.G12V)                     | (missense:c.10)           |
| NCI-H441   | 908460_lung     | KRAS | (missense:c.35G>T:p.G12V)                     | (missense:c.49)           |
| COR-L23    | 687780_lung     | KRAS | (missense:c.35G>T:p.G12V)                     | (missense:c.47)           |

Table S4-2. Information of cell lines with KRAS(non-G12C) mutation in lung cancer

| Cell line  | COSMIC_ID&tissue | Gene | Mutation                                      | TP53                      |
|------------|-----------------|------|-----------------------------------------------|---------------------------|
| NCI-H1355  | 724866_lung     | KRAS | (missense:c.37G>T:p.G13C)                     | (missense:c.85)           |
| Calu-6     | 724859_lung     | KRAS | (missense:c.181C>A:p.Q61K)                    | (nonsense:c.58)           |
| NCI-H727   | 724855_lung     | KRAS | (missense:c.35G>T:p.G12V)                     | (Missense:c.49)           |
| NCI-H1944  | 1240185_lung    | KRAS | (missense:c.38G>A:p.G13D)                     | wt                        |
| SK-LU-1    | 909721_lung     | KRAS | (missense:c.35G>A:p.G12D)                     | (Missense:c.57)           |
| NCI-H650   | 722066_lung     | KRAS | (missense:c.182A>T:p.Q61L)                    | (missense:c.49)           |
| A427       | 910851_lung     | KRAS | (missense:c.35G>A:p.G12D)                     | wt                        |
| EMC-BAC-2  | 1503370_lung    | KRAS | (missense:c.35G>C:p.G12A)                     | wt                        |
| NCI-H2444  | 1298356_lung    | KRAS | (missense:c.35G>T:p.G12V)                     | (missense:c.70)           |
| NCI-H1573  | 908472_lung     | KRAS | (missense:c.35G>C:p.G12A)                     | (missense:c.74)           |
| NCI-H647   | 1240191_lung    | KRAS | (missense:c.38G>A:p.G13D)                     | (miss splice:c.7)         |
| LCLC-97TM1 | 946361_lung     | KRAS | (missense:c.35G>T:p.G12V)                     | (frameshift:c.1)          |
| SHP-77     | 724872_lung     | KRAS | (missense:c.35G>T:p.G12V)                     | (missense:c.52)           |
| NCI-H460   | 905943_lung     | KRAS | (missense:c.183A>T:p.Q61H)                    | wt                        |
| A549       | 905949_lung     | KRAS | (missense:c.34G>A:p.G12S)                     | wt                        |
| COLO-668   | 910692_lung     | KRAS | (missense:c.35G>T:p.G12V)                     | (missense:c.10)           |
| NCI-H2009  | 724873_lung     | KRAS | (missense:c.35G>C:p.G12A)                     | (missense:c.81)           |
| SW900      | 724879_lung     | KRAS | (missense:c.35G>T:p.G12V)                     | (nonsense:c.49)           |
| NCI-H1734  | 722058_lung     | KRAS | (missense:c.37G>T:p.G13C)                     | (missense:c.81)           |
| NCI-H2347  | 687820_lung     | KRAS | (missense:c.57G>T:p.L19F)                     | wt                        |
| NCI-H1155  | 908467_lung     | KRAS | (missense:c.183A>T:p.Q61H)                    | (missense:c.81)           |
| NCI-H441   | 908460_lung     | KRAS | (missense:c.35G>T:p.G12V)                     | (missense:c.47)           |
| COR-L23    | 687780_lung     | KRAS | (missense:c.35G>T:p.G12V)                     | wt                        |
Table S4-3. Information of cell lines with KRAS(G12V) mutation in pancreatic cancer

| Cell line  | COSMIC_ID & tissue | Gene     | Mutation                                                                 | TP53               |
|------------|--------------------|----------|--------------------------------------------------------------------------|--------------------|
| DAN-G      | 1290797_pancreas   | KRAS     | (missense:c.35G>T:p.G12V)                                                | ess_splice:c.9     |
| QGP-1      | 1298534_pancreas   | KRAS     | (missense:c.35G>T:p.G12V)                                                | frameshift:c.2     |
| PANC-03-27 | 925346_pancreas    | KRAS     | (missense:c.35G>T:p.G12V)                                                | ess_splice:c.3     |
| KP-3       | 1298219_pancreas   | KRAS     | (missense:c.35G>T:p.G12V)                                                | frameshift:c.4     |
| MZ1-PC     | 755395_pancreas    | KRAS     | (missense:c.35G>T:p.G12V)                                                | frameshift:c.6     |
| CFPAC-1    | 906821_pancreas    | KRAS     | (missense:c.35G>T:p.G12V)                                                | (missense:c.72)    |
| PA-TU-8902 | 1298526_pancreas   | KRAS     | (missense:c.35G>T:p.G12V)                                                | (missense:c.72)    |
| CAPAN-1    | 753624_pancreas    | KRAS     | (missense:c.35G>T:p.G12V)                                                | (missense:c.47)    |
| Capan-2    | 910915_pancreas    | KRAS     | (missense:c.35G>T:p.G12V)                                                | wt                 |
| YAPC       | 909904_pancreas    | KRAS     | (missense:c.35G>T:p.G12V)                                                | (missense:c.53)    |
| HuP-T4     | 907286_pancreas    | KRAS     | (missense:c.35G>T:p.G12V)                                                | (missense:c.76)    |
| PA-TU-8988T| 1240201_pancreas   | KRAS     | (missense:c.35G>T:p.G12V)                                                | (missense:c.84)    |

Table S4-4. Information of cell lines with KRAS(G12D) mutation in pancreatic cancer

| Cell line  | COSMIC_ID & tissue | Gene     | Mutation                                                                 | TP53               |
|------------|--------------------|----------|--------------------------------------------------------------------------|--------------------|
| HPAF-II    | 724869_pancreas    | KRAS     | (missense:c.35G>A:p.G12D)                                                | (missense:c.45)    |
| PANC-02-03 | 1298475_pancreas   | KRAS     | (missense:c.35G>A:p.G12D)                                                | (missense:c.74)    |
| PANC-04-03 | 1298476_pancreas   | KRAS     | (missense:c.35G>A:p.G12D)                                                | (missense:c.73)    |
| PANC-08-13 | 925347_pancreas    | KRAS     | (missense:c.35G>A:p.G12D)                                                | wt                 |
| PL4        | 1298533_pancreas   | KRAS     | (missense:c.35G>A:p.G12D)                                                | (missense:c.79)    |
| KP-1N      | 1298216_pancreas   | KRAS     | (missense:c.35G>A:p.G12D)                                                | (missense:c.81)    |
| HPAC       | 1298136_pancreas   | KRAS     | (missense:c.35G>A:p.G12D)                                                | (missense:c.55)    |
| SW1990     | 910907_pancreas    | KRAS     | (missense:c.35G>A:p.G12D)                                                | (missense:c.73)    |
| PANC-10-05 | 925348_pancreas    | KRAS     | (missense:c.35G>A:p.G12D)                                                | (missense:c.76)    |
| AsPC-1     | 910702_pancreas    | KRAS     | (missense:c.35G>A:p.G12D)                                                | (frameshift:c.4)   |
| SUIT-2     | 1240219_pancreas   | KRAS     | (missense:c.35G>A:p.G12D)                                                | (missense:c.81)    |
| SU8686     | 1240218_pancreas   | KRAS     | (missense:c.35G>A:p.G12D)                                                | (missense:c.10)    |

Table S4-5. Information of cell lines with BRAF mutation in lung cancer

| Cell line  | COSMIC_ID & tissue | Gene     | Mutation                                                                 | TP53               |
|------------|--------------------|----------|--------------------------------------------------------------------------|--------------------|
| NCI-H1651  | 910900_lung        | BRAF     | (missense:c.1125A>T:p.E375D)                                            | (missense:c.52)    |
| NCI-H1395  | 680681_lung        | BRAF     | (missense:c.1406G>C:p.G469A)                                            | wt                 |
| NCI-H2227  | 688018_lung        | BRAF     | (missense:c.958G>T:p.A320S)                                             | ess_splice:c.7     |
| SW1271     | 1299062_lung       | BRAF     | (missense:c.2048G>A:p.S683N)                                            | (missense:c.83)    |
| IST-SL2    | 753565_lung        | BRAF     | (ess_splice:c.608+1G>T:p.?)                                             | (nonsense:c.88)    |
| NCI-H1666  | 908473_lung        | BRAF     | (missense:c.1397G>T:p.G466V)                                            | wt                 |
| NCI-H1755  | 908475_lung        | BRAF     | (missense:c.1406G>C:p.G469A)                                            | (missense:c.72)    |
| NCI-H2087  | 724834_lung        | BRAF     | (missense:c.1780C>G:p.L597V)                                            | (missense:c.46)    |
| NCI-H2405  | 687821_lung        | BRAF     | (inframe:c.1454_146916>A:p.L485_P490)                                    | (missense:c.81)    |
| CAL-12T    | 753540_lung        | BRAF     | (missense:c.1397G>T:p.G466V)                                            | (missense:c.40)    |
### Table S4-6. Information of cell lines with EGFR mutation in lung cancer

| Cell line | COSMIC_ID&tissue | Gene | Mutation |
|-----------|------------------|------|----------|
| NCI-H1355 | 724866_lung      | EGFR | (missense:c.3477G>C:p.Q1159H) (missense:c.2369C>T:p.T790M) |
| NCI-H1975 | 924244_lung      | EGFR | (missense:c.771A>T:p.E285K) |
| MS-1      | 753594_lung      | EGFR | (missense:c.818G>A:p.R273H) |
| NCI-H1650 | 687800_lung      | EGFR | (inframe:c.2235_2249del15:p.E746_A750del) (missense:c.853G>A:p.E285K) |
| PC-14     | 753608_lung      | EGFR | (inframe:c.2235_2249del15:p.E746_A750del) (missense:c.818G>A:p.R273H) |
| HCC-827   | 1240146_lung     | EGFR | (inframe:c.2236_2250del15:p.E746_A750del) |
| SHP-77    | 724872_lung      | EGFR | (inframe:c.2102A>G:p.Q701R) |
| NCI-H2291 | 724874_lung      | EGFR | (missense:c.2573T>G:p.L858R) |
| H3255     | 1247873_lung     | EGFR | (missense:c.844C>T:p.R282W) |
| NCI-H1793 | 908463_lung      | EGFR | (missense:c.2102A>G:p.Q701R) |
| NCI-H1568 | 1298348_lung     | EGFR | (inframe:c.2236_2244delGAATTAAGA:p.L747_E749delLRE) |
| PC-3 [JPC-3] | 1240202_lung | EGFR | (inframe:c.2236_2250del15:p.E746_A750del) |

### Table S4-7. Information of cell lines with NRAS mutation in lung cancer

| Cell line | COSMIC_ID&tissue | Gene | Mutation |
|-----------|------------------|------|----------|
| NCI-H1048 | 687995_lung      | NRAS | (frameshift:c.59_60delCA:p.T20fs*11) |
| COR-L279  | 910937_lung      | NRAS | (missense:c.14A>G:p.K5R) |
| SW1271    | 1299062_lung     | NRAS | (missense:c.182A>G:p.Q61R) |
| NCI-H1299 | 724831_lung      | NRAS | (missense:c.181C>A:p.Q61K) |
| NCI-H2087 | 724834_lung      | NRAS | (missense:c.181C>A:p.Q61K) |
| NCI-H2135 | 1298352_lung     | NRAS | (missense:c.181C>A:p.Q61K) |
| HCC-15    | 1240143_lung     | NRAS | (missense:c.557G>T:p.C186F) |
| NCI-H2347 | 687820_lung      | NRAS | (missense:c.182A>G:p.Q61R) |

Supplementary Table S4_3