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

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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).

1 An integrative pharmacogenomics analysis identifies therapeutic targets in KRAS-

2 mutant lung cancer

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29 Abstract

30 Background

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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.

41 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 antiproliferative effect of selumetinib in KRAS(G12C) mutant lung cancer cells.

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48 Our study suggested that accurate patients stratification will be necessary in order to observe significant 49 benefit upon CK2 inhibition - alone or in combination - in a subset of patients with a favorable intratumoral 50 genetic context. We provide a promising approach towards developing precision treatments for various 51 subtypes of KRAS mutant lung cancer.

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- 57 Keywords: Pharmacogenomic profiles, KRAS mutations, Lung adenocarcinoma, CSNK2A1
- 58

59 **Research in context**

60 Evidence before this study

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

63 Added value of this study

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

68 Implications of all the available evidence

69 Predicting novel therapeutic targets by considering the mutational heterogeneity of cancer histotypes will 70 help to guide therapeutic decision-making and improve treatment outcomes. Our pipeline can potentially be 71 extended to other mutant KRAS isoforms given that a large enough sample size is available for statistical 72 analysis.

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- 74
- 75

76

77 Introduction

78 The Kirsten rat sarcoma oncogene (KRAS) encodes for a small GTPase that couples growth factor 79 signalling to the MAPK signalling cascade. Despite being an oncogene with a prevalence of 30% in non-80 small cell lung cancer (NSCLC), the development of KRAS targeted therapies has been largely 81 unsuccessful in the past. This is mainly due to the higher affinity of RAS for GTP^{1,2}. Very recently, the 82 pharmacokinetic and pharmacodynamic improvement of direct G12C inhibitors has raised great excitement 83 3, leading clinical studies that are currently on-going to two 84 (https://clinicaltrials.gov/ct2/results?cond=G12C&term=&cntry=&state=&city=&dist=). As an alternative, 85 inhibitors targeting kinases downstream of KRAS, such as BRAF and MEK, have been developed which 86 showed promising activity in metastatic melanoma but were less active in KRAS mutant NSCLC. 87 Furthermore, drug efficacy is limited by the development of acquired resistance or KRAS copy number variations⁴⁻¹². Hence, there is still an unmet need to develop more efficacious targeted treatment strategies 88 89 for KRAS mutant lung cancer.

90

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

95

96 Here, we perform a pan-cancer analysis to systematically investigate differences in treatment response to 97 MEK inhibitors due to different KRAS mutational subtypes. An integrative pharmacogenomics analysis 98 pipeline is then developed to identify potential targets in lung cancer with KRAS(G12C) mutation, the most 99 frequent mutation (>40%) in patients with primary or metastatic KRAS mutant non-small cell lung cancer 100 (NSCLC)¹⁷. The most promising target predicted by this pipeline is casein kinase 2A1 (CSNK2A1) which 101 encodes for the casein kinase 2 subunit alpha (CK2 alpha), a serine/threonine protein kinase that 102 phosphorylates acidic proteins such as casein. Although there is strong evidence that CK2 plays a role in the pathogenesis of cancer ¹⁸⁻²⁰ and several CK2 inhibitors have entered clinical trials, the role of 103 104 CSNK2A1 as a therapeutic target in KRAS mutant lung cancer remains unknown to date. Our study links

- 105 CSNK2A1 to Wnt/β-catenin signaling and explores its potential as therapeutic target for treating KRAS
- 106 mutant lung cancer.
- 107
- 108 Materials & Methods

109 Key Resources Table

110 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

111

112 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

H2009

G12A

113

114 Pharmacogenomics analysis to identify potential targets in KRAS(G12C) mutant lung cancer

115 The Cancer Genome Project (CGP) at the Wellcome Trust Sanger Institute resulted in a large-scale, high-116 throughput pharmacogenomic dataset for 1001 human cancer cell lines, including the mutation status of 117 19,100 genes, genome-wide DNA copy number variation (CNV) status, mRNA expression profiling of 118 17,419 genes, and pharmacological profiling for 267 anti-cancer drugs. The drug response is represented by 119 the natural logarithm of the IC50 value, which corresponds to the half maximal inhibitory concentration of 120 an anti-cancer drug. In this dataset, there are five MEK inhibitors including PD-0325901, selumetinib, CI-121 1040, trametinib, and refamtinib, and two ERK inhibitors including FR-180204 and VX-11e. Among 137 122 cancer cells harboring KRAS mutations, 35 cells are derived from lung cancer.

123

124 We developed a computational pipeline to identify novel therapeutic targets for KRAS mutant lung cancer 125 (Fig. 1). First, we started the analysis with CGP dataset. With respect to tumor heterogeneity generated 126 from the different KRAS mutation isoforms, here, we only focused on lung cancer cell lines with 127 KRAS(G12C) mutation, the most frequent among KRAS mutation isoforms in lung cancer. In total, we 128 included 12 cell lines into our analyses. MAPK signalling inhibitors, 5 types of MEK inhibitors including 129 PD-0325901, selumetinib, CI-1040, trametinib, and refamtinib, and 2 types of ERK inhibitors including 130 FR-180204 and VX-11e were included in this analysis. The expression of 17,420 genes was used to 131 individually calculate their correlation with drug sensitivity. As expected, the high expression of some 132 genes was significantly correlated with the decreased drug sensitivity of MEK inhibitors or ERK inhibitors. 133 Of these, the genes with association with more than two MAPK signalling inhibitions were considered as 134 the potential targets. Second, they were upregulated in KRAS(G12C) mutant Lung adenocarcinoma 135 (LUAD) patients in comparison with normal samples in TCGA database. Optionally, the high expression of 136 the genes has a poorer clinical prognosis. Third, we further filter the genes with selection criteria that 137 required the genes to be part of cancer core pathways and to be known drug targets. Finally, by integrative 138 analysis of the above-mentioned criteria, we identified potential targets for KRAS mutant lung cancer.

139

140 TCGA data analysis

141 The RNA-seq and clinical data of LUAD patients were downloaded from TCGA cBioProtal 142 (http://www.cbioportal.org/index.do). The abundance of each gene was quantified as RSEM value, which 143 was evaluated by a statistical method RSEM (RNA-Seq by Expectation Maximization). RSEM uses a 144 generative model of RNA-seq reads and the EM algorithm, taking read mapping uncertainty into account 145 and achieving the most accurate abundance estimates²¹. The statistical analysis of differentially expressed 146 genes between cancer and normal samples was performed using DESeq2²². LUAD patients were divided 147 into high and low expressing group, based on the median value of gene expression across the patients. 148 Kaplan-Meier test was used to compare the overall survival and cancer relapse between two groups.

149

150 Cell lines

151 The human lung cancer cell lines A549, H2030, H2009 and Calu1 were purchased from ATCC and grown 152 at 37°C in RPMI medium supplemented with 10% fetal bovine serum (FBS), 100 μ g/ml penicillin and 100 153 units/ml streptomycin (complete medium). The cell lines were authenticated using the Promega GenePrint 154 10 System at the RTSF Genomics Core at Michigan State University. All cell lines used in the study tested

155 negative for Mycoplasma as determined by the Mycoplasma Plus PCR Primer Set (Agilent).

156

157 Assessment of cellular proliferation

158 Cells (1×10^3) were seeded in 96-well plates in 100 µ1 RPMI media supplemented with 10% FBS and 159 penicillin/streptomycin. The following day, plates were incubated in the IncuCyte ZOOMTM (Essen 160 BioScience) for real-time imaging, with three fields imaged per well under 10x magnification every two 161 hours for a total of 120 hours. Data were analyzed using the IncuCyte Confluence version 1.5 software 162 (Essen BioScience), which quantifies cell surface area coverage as confluence values. IncuCyte 163 experiments were performed in triplicate. A representative growth curve is shown for each condition.

164

165 Western blot analysis

166 Cells from *in vitro* culture were lysed in RIPA lysis buffer (#89900 Thermo Fisher) supplemented with167 protease and phosphatase inhibitor cocktail tablets (Roche). The antibodies used for western blotting

168 included those against: HSP90 (H114) (Santa Cruz Biotech Cat#sc-7947), phosphorylated Akt (Ser473) 169 (Cell Signaling Cat#4060), Akt (Cell Signaling Cat#9272), phosphorylated ERK1/2 (Cell Signaling 170 Cat#4370), ERK1/2 (Cell Signaling Cat#4695), phosphorylated MEK (Cell Signaling Cat#9154), MEK 171 (Cell Signaling Cat#8727), phosphorylated S6 (Ser235/236) (Cell Signaling Cat#4858), S6 ribosomal 172 protein (Cell Signaling Cat#2217), β-catenin (Cell Signaling Cat#8480), p27 (Cell Signaling Cat#3688), 173 cMyc (Cell Signaling Cat#2276), anti-rabbit IgG, HRP-linked secondary antibody Cell Signaling 174 (Cat#7074P2), ECL Sheep anti-Mouse IgG, HRP-linked secondary antibody (GE Healthcare 175 Cat#NA931V), ECL Donkey anti-Rabbit IgG, HRP-linked secondary antibody (GE Healthcare 176 Cat#NA934V). Western blotting showed in the manuscript are representative of at least three independent 177 experiments.

178

179 SiRNA-mediated gene knockdown

Cells (1,5x10⁶) 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.

186

187 TOPFlash reporter assay

188 Cells (1,5x10⁶) were seeded in a 10cm plate and incubated overnight at 37°C. On the next day, cells were 189 transiently transfected with 1µg of M50 Super 8x TOPFlash reporter plasmid, 100ng of a pRL Renilla 190 Luciferase control reporter plasmid (Promega) and FuGENE HD (Promega). M50 Super 8x TOPFlash was 191 a gift from Randall Moon (Addgene plasmid #12456). After 24hrs, cells were washed with PBS and full 192 media was added for another 24hrs without or with Mek inhibitor (selumetinib, 1µm). Luciferase activity 193 was measured with the Dual Luciferase reporter assay (Promega).

194

195 **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 ²³. 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.

204

205 KRAS mutant cancer cell lines were divided into 12 groups, respectively, with A146T, G12A, G12C, 206 G12D, G12R, G12S, G12V, G13C, G13D, K117N, Q61H, or Q61L mutations. We found that MEK/ERK 207 inhibitors drug sensitivities vary in cell lines with different KRAS mutations, including CI-1040, 208 refametinib (RDEA119), PD-0325901, selumetinib, trametinib, and VX-11e (Fig. 2). Cells lines with G12R 209 mutation were in general more sensitive to MEK inhibitors in comparison with other types of KRAS 210 mutations (Fig. 2a-f). To address the question if also the tissue of origin influences response to MAPK 211 pathway inhibition, we furthermore investigated the effect of different KRAS mutations on drug 212 sensitivities in the two major cancer histotypes of lung and pancreatic cancer. Differences in MEK/ERK 213 inhibitors sensitivities across the different types of KRAS mutations were observed in both cancer types, 214 being pancreatic cancer cells with G12R mutation (Additional file 1: Figure S1a-c) and lung cancer cells 215 with G12A mutation (Additional file 1: Figure S1d-e) most sensitive to MEK inhibition, respectively.

216

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, G12R, 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,

225 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.

229

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

232 Our above-mentioned analysis of the CGP dataset suggests that cancer cell lines with different KRAS 233 mutations exhibit different sensitivities to MAPK pathway inhibition. In the present analysis, we focused 234 on lung cancer cell lines with KRAS(G12C) mutation, the most dominant KRAS mutation in lung cancer. 235 In our analysis, a total of 12 cell lines were included (see Methods). We developed a computational 236 pipeline to identify novel therapeutic targets for KRAS mutant lung cancer (Fig. 1). In a first step of this 237 pipeline, 1212 genes with association with more than two inhibitors of MAPK signalling were considered 238 as potential targets. In a second step, using the TCGA database, we selected 494 genes which are 239 upregulated in LUAD patients and are associated with poor survival. Finally, we narrowed down the 240 number of genes by requiring them to be part of core cancer pathways as well as to be known drug targets. 241 This algorithm finally led to the identification of 14 potential therapeutic targets for KRAS mutant lung 242 cancer (Fig. 1d), including AARS2, ALKBH2, CARS, CDK8, COMP, CSNK2A1, DARS, EPRS, HDAC1, 243 IARS2, MAPK8, PARS2, RPL8 and YARS (Additional file 4: Table S3).

244

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 ²⁰. This is especially relevant as there is an increased interest for CSNK2 as a therapeutic target in ongoing clinical trials ²⁴. 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,

- 252 LUAD patients or LUAD patients with KRAS(G12C) mutation showed an increased expression of
- 253 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).
- 255

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

- 259 We next investigated if the correlation between CSNK2A1 expression and MEK inhibitor resistance can 260 also be observed in non-KRAS(G12C) mutant cancer cells. As KRAS(G12V) mutations represent the 261 second most frequent mutation in LUAD, nine KRAS(G12V) mutant lung cancer cell lines from CGP were 262 included in the statistical analysis. No correlation was found between CSNK2A1 expression and drug 263 sensitivity to 7 MEK inhibitors in KRAS(G12V) mutant lung cancer cells (Fig. 5a). Due to the limited 264 sample size for other non-KRAS(G12C) mutations, we pooled the remaining lung cancer cells with non-265 KRAS(G12C) mutations, for which no positive correlation between CSNK2A1 expression and drug 266 sensitivity was observed (Fig. 5b). As KRAS(G12V) and KRAS(G12D) mutations occur more frequently 267 in pancreatic cancer, we therefore also investigated the correlation of CSNK2A1 levels and MEK inhibitor 268 resistance in KRAS(G12V) or KRAS(G12D) mutant pancreas cancer cells. This analysis also showed no 269 correlation between CSNK2A1 expression and drug sensitivity (Fig. 5c,d).
- 270

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)
- 275 lung cancer cells. Cell lines we used for the analysis were in Additional file 6: Table S4.
- 276

277 CSNK2A1 knockdown reduces proliferation and Wnt/β-catenin reporter activity in KRAS(G12C) 278 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 nonKRAS(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).

286

287 As case in kinases have previously been connected to the drug resistance mediating Wnt/β -catenin pathway 288 25,26 , 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)²⁷ 289 290 and categorized samples into CSNK2A1 high and low expressing groups. Deseg2²⁸ was applied to call 291 differentially expressed genes between the two groups. Gene set enrichment analysis (GSEA)²⁹ was further 292 employed to determine the pathways enriched by a pre-ranked list of all genes, which were sorted by the 293 statistical significance of differential expression defined by the Deseq2 analysis. GSEA showed that the 294 What signaling pathway was significantly enriched in the CSNK2A1 high expressing group in CCLE (Fig. 295 8a, p=0.008) and TCGA (Fig. 8b, p=0.014).

296

297 To support our computational findings, we also investigated the differences in Wnt/ β -catenin signaling 298 parameters between KRAS(G12C) and KRAS(non-G12C) cell lines in vitro. After 24hrs of selumetinib 299 treatment, accumulation of the cell cycle inhibitor p27 upon CSNK2A1 knockdown was relatively 300 increased in KRAS(G12C) mutant Calu1 and H2030 cells compared to KRAS(non-G12C) A549 and 301 H2009 cells (Fig. 8c). This suggests that Calu1 and H2030 cells are more dependent on CSNK2A1 to 302 overcome MEK inhibitor induced growth arrest. This is also in agreement with the stronger anti-303 proliferative effect of CSNK2A1 knockdown itself in cells with KRAS(G12C) mutation (Additional file 7: 304 Figure S3, Fig. 7b,e). Furthermore, transient transfection of the Wnt/TCF reporter plasmid 8xTOPFlash 305 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 withselumetinib (Fig. 8d).

308

309 Discussion

310 In this study, we used pharmacogenomics data to systematically unravel the heterogeneity of responses to 311 MAPK pathway inhibition due to different KRAS mutation isoforms. Subsequently, we developed a 312 pharmacogenomics analysis pipeline to identify novel targets for the subgroup of KRAS(G12C) mutant 313 lung cancer. Our computational pipeline identified a correlation between CSNK2A1 expression and MEK 314 inhibitor resistance in KRAS(G12C) mutant cells, a finding that was exclusively observed in this 315 mutational subset of lung cancer cells, but not in KRAS(non-G12C) mutant cells and neither in tumor cells 316 harboring other oncogenic mutations as e.g. EGFR, BRAF, or NRAS. This suggests that the correlation 317 between CSNK2A1 expression and MEK inhibitor resistance may depend on the context of KRAS(G12C) 318 mutation in lung cancer. A pan-cancer analysis of the TCGA dataset showed that CSNK2A1 is upregulated 319 in a wide range of cancers (Additional file 8: Figure S4) and that pancreatic cancer patients with high 320 intratumoral CSNK2A1 expression have a worse overall and relapse-free survival (Additional file 9: Figure 321 S5).

322

323 CSNK2A1 encodes for a protein that is a component of the highly conserved serine/threonine protein 324 kinase CK2 alpha. Previous studies have identified CSNK2 as an oncogene when overexpressed in mice, 325 playing a key role in the pathogenesis of cancer, including breast, lung, colon, and prostate cancer, as well 326 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- κ B ²⁰, and PTEN/PI3K/Akt-PKB ^{32,33} has been 327 328 described in the past. In the Wnt pathway, CK2 acts by phosphorylating and stabilizing Dvl and β -catenin 329 and promotes T-cell factor/lymphoid enhancer-binding factor (TCF) DNA binding in the nucleus (Fig. 4a). 330 Based of these observations, CK2 has recently arisen as a promising candidate for targeted therapy, with 331 two CK2 inhibitors in ongoing clinical trials 332 (https://clinicaltrials.gov/ct2/results?cond=&term=CK2&cntry=&state=&city=&dist=). Our results suggest 333 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.

335

336 In agreement with our computational results, CSNK2A1 knockdown significantly reduced the proliferation 337 of KRAS(G12C) mutant lung cancer cells, an effect that was not observed in cells with non-G12C KRAS 338 mutations (Fig. 7). This identifies CSNK2A1 as an interesting target per se in KRAS(G12C) mutant 339 NSCLC cell lines. Furthermore, simultaneous CSNK2A1 knockdown and MEK inhibition with selumetinib 340 increased the anti-proliferative effect of selumetinib in KRAS(G12C) mutant but not in KRAS(non-G12C) 341 mutant cells. Mechanistically, our analysis including transient 8xTOPFlash reporter transfection shows that 342 CSNK2A1 mediates TCF transcriptional activity in KRAS(G12C) mutant lung cancer (Fig. 8d). The 343 importance of CSKN2A1 for Wnt signaling is also supported by our gene-set enrichment analysis (GSEA) 344 of the CCLE and TCGA datasets which show significant enrichment for the Wnt signaling pathway (Fig. 345 8a, b).

346

347 For a given oncogene, different types of nonsynonymous or indel variants impact differently on the 348 biological function of the respective protein. This results in different oncogenic activities in cancer cells 349 with different oncogenic mutations and challenges the development of effective targeted therapies. 350 Therefore, predicting novel therapeutic targets by considering the mutational heterogeneity of cancer 351 histotypes will help to guide therapeutic decision-making and improve treatment outcomes. Although the 352 pharmacogenomics approach suggested by us here was applied to KRAS(G12C) mutant lung cancer cells, 353 this pipeline can potentially be extended to other mutant KRAS isoforms given that a large enough sample 354 size is available for statistical analysis.

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358

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364

365 **Declarations of interests**

- 366 The authors declare no competing financial interests.
- 367

368 Authors' contributions

- 369 HW, JK and CA conceived the hypothesis. HW and QL designed and performed the data analysis. YX, ZC,
- 370 JZ, XC, and YD collected and preprocessed the data. JK and CA performed experimental validation. HW,
- 371 JK and CA wrote the manuscript. PJ provided the resources of experimental validation and helpful
- 372 suggestions on the validation.
- 373

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447

- 448 Figures and Tables Legend
- 449 Figure 1. A pharmacogenomics analysis identifies the potential therapeutic targets in KRAS-mutant

450 lung cancer cells. A, pharmacogenomics data and analysis for identifying genes whose high expression 451 associates with decreased drug sensitivity. B, TCGA data analysis for evaluating the expression of genes in 452 lung adenocarcinoma (LUAD) patients as well as their correlation with clinical prognosis. C, Cancer core 453 pathway and DrugBank information for further investigating the biological relevance of candidate genes. D,

- 454 Overall integrative analysis for determining the potential therapeutic targets.
- 455

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.

460

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.

464

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.

471

472 Figure 5. (A) Expression of CSNK2A1 in lung cancer cell lines with KRAS(G12V) mutation, the second 473 most frequent mutation in lung cancer, is not correlated with drug sensitivity to MEK inhibitors. (B) 474 Expression of CSNK2A1 in other non-G12C mutant lung cancer cell lines, is not positively correlated with 475 drug sensitivity to MEK inhibitors. (C) Expression of CSNK2A1 in pancreatic cancer cell lines with 476 KRAS(G12V) mutation, the most frequent mutation in pancreatic cancer, is not correlated with drug 477 sensitivity to MEK inhibitors. (D) Expression of CSNK2A1 in pancreatic cancer cell lines with 478 KRAS(G12D) mutation, the second most frequent mutation in pancreatic cancer, is not correlated with 479 drug sensitivity to MEK inhibitors.

480

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.

483

484 Figure 7. CSNK2A1 Knockdown reduces the proliferation of KRAS(G12C) mutant lung cancer cells 485 and increases the anti-proliferative effect of the MEK inhibitor selumetinib. (A) Western blot analysis 486 of CSNK2A1 protein in KRAS(G12C) mutant cell lines Calu1 and H2030 transfected with scrambled or 487 CSNK2A1 targeting siRNA without or with simultaneous selumetinib (1 µ m) treatment. Growth rates of 488 Calu1 (B) and H2030 (C) transfected with scrambled (black and purple) or CSNK2A1 targeting siRNA 489 (red and grey) without or with simultaneous selumetinib $(1 \mu m)$ treatment. Phase-contract images are 490 shown at the bottom. (D) Western blot analysis of CSNK2A1 protein in KRAS(G12S) mutant cell line 491 A549 and in KRAS(G12A) mutant cell lines H2009 transfected with scrambled or CSNK2A1 targeting 492 siRNA without or with simultaneous selumetinib (1 µ m) treatment. Growth rates of A549 (E) and H2009 493 (F) transfected with scrambled (black and purple) or CSNK2A1 targeting siRNA (red and grey) without or 494 with simultaneous selumetinib $(1 \mu m)$ treatment. Phase-contract images are shown at the bottom.

495

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

500	treatment in siCSNK2A1 treated Calu1 and H2030 compared to A549 and H2209. (D) Reporter activity of
501	Wnt/TCF reporter assay in Calu1 KRAS(G12C) and A549 KRAS(G12S). Student t-test are performed,
502	with a symbol *, **, and **** respectively representing the comparison with p value smaller than 0.05,
503	0.01 and 0.0001.
504	
505	Additional file1: Figure S1. Pan-cancer analysis investigates the drug sensitivity to MEK/ERK inhibitors,
506	including CI-1040, refametinib, PD-0325901, selumetinib, trametinib, and VX-11e, of the different types
507	of KRAS mutations in pancreatic cancer and lung cancer cell lines. The p value of the multiple-groups
508	comparison is given. A symbol * denotes the pairwise comparison with p value smaller than 0.05.
509	
510	Additional file2: Table S1. The prevalence of different KRAS mutational isoforms in primary LUAD
511	patients
512	
513	Additional file3: Table S2. The prevalence of different KRAS mutational isoforms in metastatic
514	LUAD patients
515	
516	Additional file4: Table S3. Fourteen potential therapeutic targets for KRAS mutant lung cancer.
517	
518	Additional file5: Figure S2. LUAD patients with higher expression of CSNK2A1 tend to have shorter
519	overall survival.
520	
521	Additional file6: Table S4. Information of cell lines with KRAS(G12C) mutation in lung cancer, with
522	KRAS(G12V) mutation in lung cancer, with KRAS(non-G12C) mutation in lung cancer, with
523	KRAS(G12V) mutation in pancreatic cancer, with KRAS(G12D) mutation in pancreatic cancer, with
524	BRAF mutation in lung cancer, with EGFR mutation in lung cancer, and with NRAS mutation in lung
525	cancer.
526	

527	Additional file7: Figure S3. Growth rates of Calu1 KRAS(G12C) (a) and A549 KRAS(G12S) (b)
528	transfected with scrambled or CSNK2A1 targeting siRNA without or with simultaneous selumetinib
529	(1umettreatment. Student t-test are performed, with a symbol *, ****, and n.s. respectively representing the
530	comparison with p value smaller than 0.05, 0.0001 and greater than 0.05.
531	
532	Additional file8: Figure S4. Expression of CSNK2A1 is upregulated in a wide range of cancers.
533	
534	Additional file9: Figure S5. Pancreatic cancer patients with higher expression of CSNK2A1
535	significantly have poorer survival.
536	
537	
538	



Figure2 Click here to download Figure: SFig2noGPEalhhlmi@50_DNA.pdf

b

Refametinib (MEK inhibitor)

p=8.004e-10

wt







KRAS_isoform





KRAS isoform





Figure3 Click here to download Figure: Fig3.kras_isoforms.pdf ^b





D33E
G12A
G12C
G12D
G12F
G12R
G12S
G12V
G12Y
Q61L







Expression of CSNK2A1



Expression of CSNK2A1



Figure8 Click here to download (Figgere: Fig8.Wnt_signaling.pdf





TCGA



d

TCF



Supplementary Figure S1 Click here to download Figure: Additional file1_Figure S1.pdf

с



e







d



Refametinib (MEK inhibitor) Lung





Supplementary Figure S3 b Click here to download Figure: Additional file7_Figure S3.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

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

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

Table S2. The prevalence of different KRAS mutational isoforms in metastatic

drugId dru	gName target_gen	e target_pathws gene	cells	Spearman py	val	adjPval
1372 Trai	metinib MEK1, MI	EK2 ERK MAPK si AARS2	KRASG12C	0.657	0.02	0.397945292
1526 RDI	EA119 MEK1, MI	EK2 ERK MAPK si AARS2	KRASG12C	0.615	0.033	0.533334017
1060 PD-	-0325901 MEK1, MI	EK2 ERK MAPK si ALKBH2	KRASG12C	0.915	0	0
1014 RDI	EA119 MEK1, MI	EK2 ERK MAPK si ALKBH2	KRASG12C	0.721	0.019	0.387727888
1498 selu	metinib MEK1, MI	EK2 ERK MAPK si CARS	KRASG12C	0.811	0.001	0.031604522
1526 RDI	EA119 MEK1, MI	EK2 ERK MAPK si CARS	KRASG12C	0.79	0.002	0.061137987
1372 Trai	metinib MEK1, MI	EK2 ERK MAPK si CARS	KRASG12C	0.748	0.005	0.138871421
1062 selu	metinib MEK1, MI	EK2 ERK MAPK si CARS	KRASG12C	0.81	0.015	0.330350852
1014 RDI	EA119 MEK1, MI	EK2 ERK MAPK si CARS	KRASG12C	0.673	0.033	0.533334017
1498 selu	metinib MEK1, MI	EK2 ERK MAPK si CDK8	KRASG12C	0.769	0.003	0.088607594
1526 RDI	EA119 MEK1, MI	EK2 ERK MAPK si CDK8	KRASG12C	0.601	0.039	0.584552683
1015 CI-1	1040 MEK1, MI	EK2 ERK MAPK si COMP	KRASG12C	0.817	0.007	0.184018247
1498 selu	metinib MEK1, MI	EK2 ERK MAPK si COMP	KRASG12C	0.692	0.013	0.299435549
1526 RDI	EA119 MEK1, MI	EK2 ERK MAPK si CSNK2A1	KRASG12C	0.79	0.002	0.061137987
1062 selu	metinib MEK1, MI	EK2 ERK MAPK si CSNK2A1	KRASG12C	0.881	0.004	0.115864071
1498 selu	metinib MEK1, MI	EK2 ERK MAPK si CSNK2A1	KRASG12C	0.741	0.006	0.163150558
1014 RDI	EA119 MEK1, MI	EK2 ERK MAPK si CSNK2A1	KRASG12C	0.782	0.008	0.205302231
1372 Trai	metinib MEK1, MI	EK2 ERK MAPK si CSNK2A1	KRASG12C	0.587	0.045	0.624014981
1060 PD-	-0325901 MEK1, MI	EK2 ERK MAPK si DARS	KRASG12C	0.697	0.025	0.461067753
1014 RDI	EA119 MEK1, MI	EK2 ERK MAPK si DARS	KRASG12C	0.697	0.025	0.461067753
1498 selu	metinib MEK1, MI	EK2 ERK MAPK si EPRS	KRASG12C	0.881	0	0
1526 RDI	EA119 MEK1, MI	EK2 ERK MAPK si EPRS	KRASG12C	0.811	0.001	0.031604522
1014 RDI	EA119 MEK1, MI	EK2 ERK MAPK si EPRS	KRASG12C	0.818	0.004	0.115864071
1372 Trai	metinib MEK1, MI	EK2 ERK MAPK si EPRS	KRASG12C	0.692	0.013	0.299435549
263 FR-	180204 ERK	ERK MAPK si HDAC1	KRASG12C	0.72	0.008	0.205302231
262 VX-	-11e ERK	ERK MAPK si HDAC1	KRASG12C	0.615	0.033	0.533334017
1060 PD-	-0325901 MEK1, MH	EK2 ERK MAPK si IARS2	KRASG12C	0.818	0.004	0.115864071
1014 RDI	EA119 MEK1, MI	EK2 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 RDI	EA119 MEK1, MI	EK2 ERK MAPK si PARS2	KRASG12C	0.72	0.008	0.205302231
1498 selu	metinib MEK1, MI	EK2 ERK MAPK si PARS2	KRASG12C	0.587	0.045	0.624014981
1526 RDI	EA119 MEK1, MI	EK2 ERK MAPK si RPL8	KRASG12C	0.72	0.008	0.205302231
1498 selu	metinib MEK1, MI	EK2 ERK MAPK si RPL8	KRASG12C	0.636	0.026	0.467907815
1372 Trai	metinib MEK1, MI	EK2 ERK MAPK si RPL8	KRASG12C	0.608	0.036	0.56542678
1372 Trai	metinib MEK1, MI	EK2 ERK MAPK si YARS	KRASG12C	0.678	0.015	0.330350852
1498 selu	metinib MEK1, MI	EK2 ERK MAPK si YARS	KRASG12C	0.671	0.017	0.361519728
1014 RDI	EA119 MEK1, MI	EK2 ERK MAPK si YARS	KRASG12C	0.636	0.048	0.637656047

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.10
NCI-H2291	724874_lung	KRAS	(missense:c.35G>T:p.G12V)(missense:c.	(missense:c.46
SHP-77	724872_lung	KRAS	(missense:c.35G>T:p.G12V)	(missense:c.52
COLO-668	910692_lung	KRAS	(missense:c.35G>T:p.G12V)	(missense:c.10)
SW900	724879_lung	KRAS	(missense:c.35G>T:p.G12V)	(nonsense:c.49
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 S4-1. Information of cell lines with KRAS(G12V) mutation in lung cancer

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.492
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)	(ess_splice:c.78
LCLC-97TM1	946361_lung	KRAS	(missense:c.35G>T:p.G12V)	(frameshift:c.10
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

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.29
PANC-03-27	925346_pancreas	KRAS	(missense:c.35G>T:p.G12V)	(ess_splice:c.37
KP-3	1298219_pancreas	KRAS	(missense:c.35G>T:p.G12V)	(frameshift:c.4
MZ1-PC	753595_pancreas	KRAS	(missense:c.35G>T:p.G12V)	(frameshift:c.62
CFPAC-1	906821_pancreas	KRAS	(missense:c.35G>T:p.G12V)	(missense:c.724
PA-TU-8902	1298526_pancreas	KRAS	(missense:c.35G>T:p.G12V)	(missense:c.52)
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.764
PA-TU-8988T	1240201_pancreas	KRAS	(missense:c.35G>T:p.G12V)	(missense:c.844

Table S4-3. Information of cell lines with KRAS(G12V) mutation in pancreatic cancer

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)	(inframe:c.572
PANC-10-05	925348_pancreas	KRAS	(missense:c.35G>A:p.G12D)	(missense:c.764
AsPC-1	910702_pancreas	KRAS	(missense:c.35G>A:p.G12D)	(frameshift:c.40
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. Informa	ion of cell li	nes with BRAF	'mutation in	lung cancer
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Cell line	COSMIC_ID&tissue	Gene	Mutation	TP53
NCI-H1651	910900_lung	BRAF	(missense:c.1125A>T:p.E375D)	(missense:c.52
NCI-H1395	684681_lung	BRAF	(missense:c.1406G>C:p.G469A)	wt
NCI-H2227	688018_lung	BRAF	(missense:c.958G>T:p.A320S)	(ess_splice:c.78
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.1789C>G:p.L597V)	(missense:c.46
NCI-H2405	687821_lung	BRAF	(inframe:c.1454_146916>A:p.L485_P49	(missense:c.81
CAL-12T	753540_lung	BRAF	(missense:c.1397G>T:p.G466V)	(missense:c.404

Cell line	COSMIC_ID&tissue	Gene	Mutation	TP53
NCI-H1355	724866_lung	EGFR	(missense:c.3477G>C:p.Q1159H)	(missense:c.85
NCI-H1975	924244_lung	EGFR	(missense:c.2369C>T:p.T790M)(missen	(missense:c.81
MS-1	753594_lung	EGFR	(missense:c.771A>T:p.E257D)	(missense:c.73
NCI-H1650	687800_lung	EGFR	(inframe:c.2235_2249del15:p.E746_A75	(ess_splice:c.6)
PC-14	753608_lung	EGFR	(inframe:c.2235_2249del15:p.E746_A75	(missense:c.74
HCC-827	1240146_lung	EGFR	(inframe:c.2236_2250del15:p.E746_A75	(inframe:c.652
SHP-77	724872_lung	EGFR	(missense:c.2102A>G:p.Q701R)	(missense:c.52
NCI-H2291	724874_lung	EGFR	(missense:c.1774G>T:p.V592F)	(missense:c.46
H3255	1247873_lung	EGFR	(missense:c.2573T>G:p.L858R)	(ess_splice:c.5
NCI-H1793	908463_lung	EGFR	(missense:c.932G>T:p.C311F)	(missense:c.81
NCI-H1568	1298348_lung	EGFR	(missense:c.3379G>A:p.D1127N)	(missense:c.53
PC-3 [JPC-3]	1240202_lung	EGFR	(inframe:c.2236_2244delGAATTAAGA	(missense:c.84

Table S4-6. Information of cell lines with EGFR mutation in lung cancer

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

Cell line	COSMIC_ID&tissue	Gene	Mutation	TP53
NCI-H1048	687995_lung	NRAS	(frameshift:c.59_60delCA:p.T20fs*11)	(missense:c.81'
COR-L279	910937_lung	NRAS	(missense:c.14A>G:p.K5R)	(ess_splice:c.37
SW1271	1299062_lung	NRAS	(missense:c.182A>G:p.Q61R)	(missense:c.830
NCI-H1299	724831_lung	NRAS	(missense:c.181C>A:p.Q61K)	(ess_splice:c.1_
NCI-H2087	724834_lung	NRAS	(missense:c.181C>A:p.Q61K)	(missense:c.46
NCI-H2135	1298352_lung	NRAS	(missense:c.181C>A:p.Q61K)	(missense:c.830
HCC-15	1240143_lung	NRAS	(missense:c.557G>T:p.C186F)(missense	(missense:c.77)
NCI-H2347	687820_lung	NRAS	(missense:c.182A>G:p.Q61R)	wt