Activation of PI3K from RAS

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ESMO Symposium on Signalling Pathways
Barcelona
Spain
13th March 2015
The major RAS signalling pathways

NF1

RAS

Raf (BRAF)

Raf (BRAF)

MEK

ERK

PI 3-Kinase (PIK3CA)

PTEN

PLCε

Ral-GDS

Ral

Ral-BP1

PLD

Forkhead

PKC Ca²⁺

PLD

Forkhead

Bad

GSK3

Forkhead

Rsk

Elk1

PLA₂

PI(4,5)P₂

PI(3,4,5)P₃

PI(4,5)P₂

PI(3,4,5)P₃

PI(3,4,5)P₃

PDK1

mTOR

p70S6K

Calcium signalling

Proliferation

Transcription

Endocytosis

Proliferation

Transcription

Survival

Proliferation

Translation

Cytoskeleton

Calcium signalling

Proliferation

Transcription

Endocytosis

Proliferation

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Translation

Cytoskeleton
Activation of PI 3-kinase by growth factors involves interaction of PI3K regulatory subunit with receptor tyrosine kinase and PI3K catalytic subunit with RAS.
RAS binds p110\(\alpha\), \(\gamma\) and \(\delta\), RAC binds p110\(\beta\)

Possible ways to selectively target RAS mutant cancer cells

1. Inhibit RAS function directly (target oncogene addiction)

K-Ras(G12C) inhibitors allosterically control GTP affinity and effector interactions

Jonathan M. Ostrem¹*, Ulf Peters¹*, Martin L. Sos¹, James A. Wells² & Kevan M. Shokat¹
Nature 20 Nov 2013
Possible ways to selectively target RAS mutant cancer cells

1. Inhibit RAS function directly (target oncogene addiction)

2. Inhibit RAS controlled downstream signaling enzymes, e.g. RAF, PI3K, mTOR (target oncogene addiction indirectly)
Mice with mutant RAS interaction site in PI3K p110α show massively reduced KRAS induced lung tumour rates

Tony Ramjaun
Surbhi Gupta
Is the interaction of RAS with PI3K\(\alpha\) required for tumour maintenance as well as formation?

- **PIK3CA-RBD/ PIK3CA-loxP Rosa26 Cre-ER / wt K-RasG12D LA2/ wt**
- **PIK3CA-wt/ PIK3CA-loxP Rosa26 Cre-ER / wt K-RasG12D LA2 / wt**
- **PIK3CA-wt/ PIK3CA-wt Rosa26 Cre-ER / wt K-RasG12D LA2 / wt**

**Age to 2-3 months to allow latent KRAS G12D driven lung tumorigenesis**

**Feed with tamoxifen pellets for two weeks to activate Cre deletion of PIK3CA-loxP**

**Assessment of lung tumor burden at age 4-5 months**

Esther Castellano
Clare Sheridan
Effect of inducible p110α-RBD mutant expression on size of established KRAS LA2 lung tumours (CT scans)

E.Castellano, C.Sheridan, ... J.Downward (2013) Cancer Cell, 24, 617

Removal of KRAS – p110α interaction in existing lung tumours leads to long term tumour stasis and partial regression
A genetically encoded platform for the identification of protein-protein interaction inhibitors.

A

B
Split-Intein Circular Ligation of Proteins and Peptides: SICLOPPS
Genetic selection of inhibitors

[Diagram showing genetic selection process with p110α and KRAS proteins, no transcription, and a petri dish labeled 'death']
This screening methodology has yielded an ~15nM inhibitor of heterodimerisation of HIF1α/β.

Ali Tavassoli (2013) JACS 135, 10418
Not all effects of disrupting RAS-p110α interaction are tumour cell autonomous
Host RAS-p110α interaction important in supporting metastasis

B16F10 Melanoma Cells (Tail vein)

Tamoxifen

P110α-Mut
P110α-LoxP
CRE-ER (+/-)

P110α-WT
P110α-LoxP
CRE-ER (+/-)

WT-p110α
MUT-p110α

Number of tumor foci per lung

Average foci area (% of lung area)

Tumor area (% of lung area)

WT-p110α: MUT-p110α

P = 0.001

P = 0.0017

P = 0.0003

181/0 = d
Loss of host RAS-p110α interaction results in reduced blood vessel density and reduced macrophage numbers in tumours.

RAS interaction with PI3K p110α is required for tumor-induced angiogenesis.

Effect of MEK and PI3K inhibitor combination in KRAS LA2 mouse lung tumours

4 weeks treatment micro CT tumour volumes
Combination treatment with MEK and PI3K inhibitors causes marked regression of lung tumours in KRAS LA2 mouse...

... however, this is at the cost of very significant toxicity.

Is it possible to find more selective / less toxic approaches to targeting PI3K pathway in RAS mutant cancers?
Drugs selective for KRAS mutant genotype in Sanger/MGH screen
(The Genomics of Drug Sensitivity in Cancer Project)
www.cancerrxgene.org

Gene IC 50 Effect

p value

20% FDR

Increased sensitivity

Increased resistance
IGF1R inhibitors inhibit PI3K pathway selectively in RAS mutant cells

NVP-AEW541: pAKT/AKT

MUT WT MUT WT

0.00 0.25 0.50 0.75 1.00 1.25 1.50

4 h 24 h

*** ***

pAKT/AKT

NVP-AEW541: Novartis IGF1R kinase inhibitor
KRAS knockdown inhibits PI3K activity and acute RAS activation stimulates PI3K dependent on basal IGF1R activity.
• In KRAS mutant lung cancer, PI3K stimulation downstream of activated RAS is dependent on basal IGF1R activity

• Inhibiting PI3K activity with IGF1R inhibitors may be less damaging to normal cells than the use of PI3K inhibitors

• Combined MEK and IGF1R inhibition may be worth exploring in KRAS mutant lung cancer

• Early stage trials of combinations of MEK and IGF1R inhibitors ongoing

How can major but transient regressions be upgraded to cures?
- Can targeted therapies be combined with immune checkpoint blockade?

**PD-L1 blockade has no anti-tumour activity in** Kras^{LSLG12D/+;Trp53^{F/F}} mice

2 weeks on therapy – volume change

Lung tissue PD-L1 mAb binding

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<th>Treatment</th>
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<td>anti-PD-L1</td>
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n.s
Lack of adaptive immune response to Kras p53 mouse model tumours

2 weeks on therapy, MEKi (GSK1120212/trametinib) plus taxol
GEMMs such as the KP mouse lung cancer model have tumours with <5 coding mutations compared to ~500 non-silent coding mutations in human lung cancer.

Need better mouse models to study the interplay of the immune system and the tumour in the response to therapies, whether chemo, targeted agents or immunotherapies.
Therapy of KRAS mutant cancers

- MEK inhibitors are the only agents with proven, albeit modest, KRAS genotype selectivity in the clinic.
- MEK inhibitors promising as scaffold for combination, e.g. with IGF1R inhibitors or with proteasome inhibitors, also cytotoxics
- Synthetic lethal approach points to combined inhibition of proteasome and ROCK (Steckel 2012, Kumar 2012). However, ROCK inhibitors very early stage in clinic.
- Immune checkpoint therapies may have potential in KRAS mutant cancers, although genotype selectivity unlikely. Current KRAS GEMMs fail to model adequately
Acknowledgements

LRI Pathology
Gordon Stamp
Emma Nye
LRI In Vivo Imaging
Francois Lassailly
May Zaw Thin
LRI High Throughput Screening
Mike Howell
Ming Jian
Becky Saunders
LRI Bioinformatics & Statistics
Gavin Kelly
Phil East
Probir Chakrobarty
Aengus Stewart
LRI Advanced Sequencing
Nik Matthews
Adam Rabinowitz

Southampton
Ali Tavassoli