Functional characterization of KEAP1 mutations in lung squamous cell carcinoma

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KEAP1/NRF2 regulates intracellular redox homeostasis
NRF2 activity modulates survival via redox homeostasis

**NRF2 target genes**
- Heme oxygenase 1 (HMOX1)
- Glutathione synthesis (GCS)
- NADH quinone oxidoreductase 1 (NQO1)
- Multidrug resistance proteins (MRP)

*Mitigate acute spikes in ROS*
*Chemotherapeutic/xenobiotic clearance*
*Control metabolically-derived ROS*

**Diagram:***
- KEAP1 null
- NRF2 null
- Effective Prevention
- Neurodegeneration
- Cancer
- NRF2 mediated transcription
- Risk for Disease
Pathway mutations in KEAP1/NRF2 signaling occur in squamous cell lung carcinoma

- 178 total squamous cell lung carcinomas analyzed
- Mutations in KEAP1 and NRF2 are mutually exclusive
- Primarily in classical subtype
- Collectively KEAP1, NRF2, and CUL3 mutations are altered in 34% of total samples

KEAP1 mutations exhibit differential suppression of NRF2-mediated transcription
KEAP1 mutants differentially bind to interacting proteins

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- **NRF2**
- **KEAP1**
- **ACTIN**
The KEAP1 mutants cluster into four classes:

**Class I: Strong binders of NRF2 but cannot suppress NRF2-mediated transcription**
- NTR 1-60
- BTB 61-179
- IVR 180-314
- KELCH 315-359
- KELCH 361-410
- KELCH 412-457
- KELCH 459-504
- KELCH 506-551
- KELCH 553-598
- CTR 599-624

**Class II: Do not bind NRF2 and cannot suppress NRF2**
- NTR 1-60
- BTB 61-179
- IVR 180-314
- KELCH 315-359
- KELCH 361-410
- KELCH 412-457
- KELCH 459-504
- KELCH 506-551
- KELCH 553-598
- CTR 599-624

**Class III: Weakly bind NRF2 and cannot suppress NRF2**
- NTR 1-60
- BTB 61-179
- IVR 180-314
- KELCH 315-359
- KELCH 361-410
- KELCH 412-457
- KELCH 459-504
- KELCH 506-551
- KELCH 553-598
- CTR 599-624

**Class IV: Behave like wildtype**
- NTR 1-60
- BTB 61-179
- IVR 180-314
- KELCH 315-359
- KELCH 361-410
- KELCH 412-457
- KELCH 459-504
- KELCH 506-551
- KELCH 553-598
- CTR 599-624
KEAP1 mutants differentially bind to interacting proteins

- “Superbinders” only bind more NRF2
- Cannot suppress NRF2-mediated transcription
- Exhibit increased NRF2 half-life
- Have enhanced cell viability in response to chemotherapeutic insult

Mechanism?
"Superbinders": slow cyclers or subpar structures?

- Class I mutants are on "bottom" of KELCH domain, not at KELCH/NRF2 interface.
- Mutants in IVR and linker between IVR and KELCH are also in Class I. Predicted to be near IVR/BTB interface.
- More likely that Class I mutants perturb KEAP1 structure than act as "superbinders".
KEAP1 cysteine residues are stress-specific

- C151 forms adducts with electrophiles
  - H129, K131, R135, K150, and H154
    comprise microenvironment that alters reactivity of C151

- H225/C226 and C613 are reactive to heavy metals

- C288 specific reactivity to alkenals

Is cysteine reactivity in KEAP1 altered in cancer?

McMahon et al, PNAS 2010
Are clustered mutations “pointing” to important regions of KEAP1?

Cys 241, 249, 319, 368, 434, 489 have been shown to react with electrophilic fatty acids as well as sulforaphane.
KEAP1 mutations are hypomorphic and can be further inactivated by interacting proteins

- Overexpression of the ETGE-containing protein DPP3 further activates NRF2 signaling in a KEAP1 mutant background

- DPP3 is overexpressed in tumor verses normal lung squamous cell carcinoma (p=4.6e-14)
Summary

-Mutations in KEAP1 from lung squamous cell carcinoma can be grouped into four phenotypic classes

-The “superbinder” class exhibits enhanced NRF2 activity and stability, and is likely a result of structural changes in the KEAP1 homodimer

-KEAP1 mutations in cancer cluster around cysteines with reactivity to electrophilic compounds

-Overexpression of ETGE-containing proteins can further activate NRF2 activity in a KEAP1 mutant background
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