The landscape of somatic structural rearrangements in RAS pathway genes

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Whole genome sequencing pipeline

- **WGS (6-8X)**
  - ~50 bp reads
  - ~300 insert size

- **Preprocessing**
  - BWA, MarkDuplicates, Realign, Recalib

- **Detection of structural variants**
  - Breakdancer (paired-end mapping)
  - Meerkat (paired-end mapping/split read)

- **QC-Validation**

- **Gene annotation**

- **Filtering**
  - (normal samples)

- **Data interpretation**

- **12 tumor types, 1181 tumor and matched normal (tissue/blood) sample pairs**

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**Breakpoint Diagram**

- **ChrA**
- **Insert**
- **Breakpoint**
- **ChrB**

**Bar Chart**

- **Lungadeno**
- **Bladder**
- **Melanoma**
- **Head&Neck**
- **Prostate**
- **Thyroid**
- **Gastric**
- **Endometrial**
- **Colorectal**
- **Cervical**
- **Low grade glioma**
- **Esophageal**
Incidence of structural rearrangements in 12 tumor types

36845 structural rearrangements in total (12166 gene-gene events)

Validation of gene-gene events:
• *In silico*: rearrangements detected by both DNA callers/by RNAseq
• *In vitro*: PCR amplification of the junction fragment

✓ Validation rate: 80%
Pathways affected: RAS pathway

✓ RAS-MAPK pathway is known to have an important role in cancer.
✓ Mutations in KRAS, NRAS and BRAF have been found to activate RAS/MAPK pathway in many solid tumors.
✓ There is evidence to suggest that there may be alternative mechanisms of activation of the pathway.

• We examined the role of structural aberrations in the activation of the RAS-MAPK pathway in 12 tumor types.
• 199/1181 (17%) TCGA samples have somatic RAS rearrangements.
Rearrangements in RAS pathway
Rearrangements in genes of the RAS pathway are mutually exclusive to most of RAS/RAF somatic aberrations (DNA-level).
Rearrangements affecting 3’UTR in FGFR genes

- The FGFR3 genomic breakpoint lies within its 3’UTR.
- Due to alternative splicing, the fusion transcript FGFR3-ELAV3 is missing exon 18 and the 3’UTR of FGFR3.

The tumorigenic FGFR3-TACC3 gene fusion escapes miR-99a regulation in glioblastoma.

The fusion transcript \textit{FGFR2}–\textit{WDR11} is missing exon 18 and the 3’UTR of \textit{FGFR2}.

The fusion transcript’s 3’UTR is a hybrid of fragments of intron 17 of \textit{FGFR2} and of the 3’UTR of \textit{WDR11}.
Functional validation of driver structural rearrangements

Gateway ORF cDNA clones

cDNA clone pulled from ORF collection

PCR the fusion part of each ORF flanking recombination sites
Incorporated into Entry Clone

Multi-site recombination into destination lentiviral vector

Fusion gene in lentiviral vector

Fusion gene clone in lentiviral vector + Viral packaging helper vectors

Transfection into 293T cells lentivirus production

Virus collection
Infection of Ba/F3 cells with minimal IL-3

IL-3 exhaustion
Transformed Ba/F3 cell selection

Cell Viability Assay

Cell-Titer Glo Assay
The *TRAK1-RAF1* rearrangement is a driver event in melanoma

An inversion in chr3 leads to both *TRAK1-RAF1* fusion, which retains the kinase domain, and its reciprocal partner (*RAF1-TRAK1*).

Overexpression of TRAK1-RAF1 but not of TRAK-RAF1 fusion protein relieves mouse Ba/F3 cells from dependency on IL-3.

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TRAK1-RAF1 activates the RAS pathway

- TRAK1-RAF1 transformed Ba/F3 cells show increased levels of phosphorylated ERK1/2 protein and sensitivity to MEK inhibition.

Kenny Scott, Henry Lu
Summary

- RAS mutations are found at a high frequency in many cancers; structural rearrangements provide an alternative mechanism for activation of the pathway.
- RAS rearrangements are mutual exclusive to most RAS mutations/amplifications.
- Functional studies show the oncogenic potential of RAS rearrangements.

✔ Join me at poster #59!
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