Integrated Genomic Characterization of Pheochromocytoma and Paraganglioma

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On behalf of the Pheochromocytoma TCGA analysis working group
Background on Pheochromocytoma and Paraganglioma

- Rare chromaffin cell neoplasm
  - 1,000 cases per year in the U.S.

- Chromaffin cells: neuroendocrine cells secrete catecholamines (e.g. epinephrine)
  - Named by location

- For brevity, I’ll use “Pheo” in speaking to refer to both Pheochromocytoma and Paraganglioma.

- Up to 30% malignant (Tischler et al, 2014, Endocrine Pathology)
  - For malignant disease, 46% progression-free survival at 1 year (Hescot, 2013)
  - Few markers of metastatic risk
Background on Pheo Genetics and Genomics

• Underlying inherited mutations in ~ 40% (highest of any tumor type)
• 19 susceptibility genes
  – NF1, RET, SDHB, SDHD, SDHA, SDHC, MAX, other less frequent genes.
• Pheo can be familial or sporadic.
• mRNA Expression Clusters
  – Pseudohypoxia and Kinase signaling (Dahia, 2005, Plos Genetics)
  – Potentially up to five clusters (Burnichon)
  – Associate with different susceptibility genes.
The Pheo Cancer Genome Atlas

- Aim is to identify Pheo’s
  - Genomic alterations
  - Integrated classifications
  - Markers of benign vs metastatic disease

- Cohort: 173 patients

- Each case has the following assays, on tumor (T) or germline (G) tissue
  - DNA Whole Exome Sequencing  
  - DNA copy number arrays  
  - mRNA sequencing  
  - miRNA sequencing  
  - DNA methylation arrays  
  - Reverse Phase Protein Arrays (cohort subset)
On a large scale, Pheo has a quiet somatically altered genome.

- Also among lowest by DNA methylation alterations (not shown)
Pheo has diverse mutations

Germline mutations
(classified as pathogenic / likely pathogenic by ACMG guidelines)

- SDHB
- RET
- VHL
- NF1
- SDHD
- MAX
- EGLN1
- TMEM127
- HRAS*
- NF1*
- EPAS1*
- RET*
- ATRX
- CSDE1*
- GPR128*
- SETD2
- VHL
- ARNT
- ARNT
- FGFR1
- BRAF

Somatic Mutations
(* MutSig q<0.1)

> 25% positive

> 40% positive

Ignat Leshchiner
Kate Nathanson
Brandon Wenz
Matt Wilkerson
RET mutation tendencies vary by germline or somatic mutation origin

RET germline mutations

RET somatic mutations

- NF1 and VHL mutations tendencies not different by mutation origin.
**CSDE1** – new driver gene in Pheo

- **Cold Shock Domain Containing E1, RNA-Binding**

- **CSDE1** mutant tumors co-occur with DNA copy number deletion and extreme low expression

- Supports loss of function role for **CSDE1**

- Kobyashi et al. (2013) *Neuroscience*: Knock out of **CSDE1** causes irregular neuronal migration in brain development
CSDE1 Splice Site Mutation causes intron retention

- Tumor mRNA transcripts contain mutation in acceptor splice site, and show intron retention

(Wilkerson et al. 2014 Nucleic Acids Research)
• Deletion regions associated with inherited susceptibility gene

(Brad Murray)
Focal DNA copy number alterations

- Analysis of recurrent somatic copy number alterations (GISTIC)

**Focal copy number deletions**

- CSDE1
- NF1

**Focal copy number amplifications**

- 4q31, 17q21
Novel recurrent *MAML3* fusion gene

- **7 cases with **UBTF-MAML3** fusion**
  - *UBTF* – “upstream binding TF”
  - *MAML3* – “mastermind-like 3”
  - 2 fusion isoforms: UBTF exon 14, UBTF exon 16

- **1 case with **TCF4- MAML3** fusion**
  - TCF4 – “transcription factor 4”

- *MAML3* is highly over-expressed in positive cases

MAML3 mRNA expression

Mapsplice alignments (Wang, Lui, Prins et al. 2010 Nucleic Acids Research)
Novel MAML3 Fusion Gene

- **Exonic Expression Analysis**
  - Supports fusion gene expression pattern
  - Promoter of UBTF or TCF4 driving over-expression of MAML3

Legend:
Intra-gene over-expression under-expression

Stuart Jeffrys (UNC)
Characterization of MAML3 Fusion

- MAML3 Known to be NOTCH co-activator, but fusion gene lacks NOTCH binding site
- Similar to PAX3-MAML3 fusion in sinonasal sarcoma (Wang et al. 2014 Nature Genetics)
- Analysis by platform:
  - mRNA expression
  - miRNA expression
  - DNA Methylation

- MAML3 fusion tumors have activated Wnt signaling

Vonn Walter (UNC)  Gordon Robertson (BCCA)  Ludmila Danilova (JHU)
Pheo classified into 4 expression subtypes

- Detected 4 unsupervised expression subtypes
  - (Wilkerson et al Bioinformatics. 2010 Jun 15;26(12):1572-3)
    - Statistically significant (Sigclust on all pairs $P < 0.05$)
- Major patterns of mRNA expression in Pheo
- Subtype mean profiles correspond to published subtypes
- The subtypes are reproducible molecular classes across cohorts
Differential molecular pathogenesis by mRNA expression subtypes

RNA Subtype
Extra-Adrenal Site

NK1, HRAS, RET, SDHB, VHL, MAX, EPAS1, CSDE1

MAML3 Fusion
BRAF Fusion
NGFR Fusion
NF1 Fusion

Tumor Purity
Leukocyte Fraction
Cortex Cell Presence

Methylation Subtype
miRNA Subtype

Germline Mutation
Somatic Mutation
Gene Fusion

Tumor Purity
Leukocyte Fraction

low
high

Hyper-Methylated
Hypo-Methylated
Normal-Like

Vonn Walter

$P < 0.05$ on each feature with subtype, except singleton fusions
Distinct pathways

Psuedohypoxia

- **SDH**: 10%
- **PHD**: <1%
- **HIF2A**: 5%
- **VHL**: 6%

Highly specific to Pseudohypoxia subtype

PIK3-AKT & MAPK pathways

- **RET**: 8%
- **NGFR**: <1%
- **FGFR1**: 1%
- **NF1**: 13%
- **HRAS**: 10%
- **BRAF**: 1%
- **MAX**: 2%
- **TMEM127**: <1%

Highly specific to Kinase signaling subtype

- **JMJD**
- **TET**
- **α-ketoglutarate**
- **succinate**
- **fumarate**
- **hypermethylation**
- **angiogenesis**
Genomic features of aggressive disease

Aggressive Status
Follow-up
Summary of the new discoveries of the TCGA Pheo Study

1. ~65% cases have a driving germline or somatic mutation.

2. First recurrent fusion gene in Pheo (MAML3)
   - Associates with clinically aggressive disease
   - Found in one expression subtype of sporadic Pheo
   - Overexpresses Wnt signaling pathway

3. First reports of other alterations
   - CSDE1 somatic mutations
   - Fusion genes in (NGFR, BRAF, NF1)
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