Discovery, Validation, and Characterization of a Novel Hereditary Pancreatic Cancer Gene

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Background & Significance: Pancreatic Cancer

- one of the most deadly cancers
  mortality rates ≈ incidence rates

- pathogenesis is poorly understood

- no effective treatment options
  > 90% die in less than 5 years
Basic Science Research in PDAC

What is the genetic basis for PDAC?

Validate the impact of these genetic changes in PDAC?

Mechanism?

Clinical translation?
• early diagnosis
• new therapeutic targets
• tailored-therapy
Background

What is the genetic basis for PDAC?

Germline Mutations → familial PDAC

Common Variants → altered susceptibility to sporadic PDAC

Somatic Mutations → tumorigenesis

Exome/RNA-Seq of Tumors

- kRAS
- TGF-β (SMAD4)
- p53

(Agnieszka Witkiewicz group, UTSW)

(Sean Grimmond group, Melbourne)
What is the genetic basis for PDAC?

**Germline Mutations**
→ familial PDAC

**Common Variants**
→ altered susceptibility to sporadic PDAC
GWAS

**Somatic Mutations**
→ tumorigenesis
Exome/RNA-Seq of Tumors
- kRAS
- TGF-β (SMAD4)
- p53

**NR5A2 (LRH1)**

**National Pancreas Foundation Award, 2012:**

**Nissim S.** Weeks O, Talbot J, Hedgepeth JW, Wucherpfennig J, Schatzman-Bone S, Swinburne I, Cortes M, Alexa K, Megason S, North T, Amacher S, Goessling W.

Iterative use of nuclear receptor Nr5a2 regulates liver and pancreas progenitor formation and differentiation.

Familial Pancreatic Cancer

What is the genetic basis for PDAC?

- **Germline Mutations** → familial PDAC
- **Common Variants** → altered susceptibility to sporadic PDAC
- **Somatic Mutations** → tumorigenesis
  - Exome/RNA-Seq of Tumors
  - *k*RAS
  - TGF-β (SMAD4)
  - p53

Germline genetic variants with high effect size (Klein et al., 2002)
What is the genetic basis for PDAC?

Germline Mutations → familial PDAC

Common Variants → altered susceptibility to sporadic PDAC
GWAS

Somatic Mutations → tumorigenesis
Exome/RNA-Seq of Tumors
krAS
TGF-β (SMAD4)
p53

* variant of uncertain significance (VUS) in known cancer genes

* no variants in known cancer genes → undiscovered genetic etiologies
Candidate Causative Variant

No pathogenic variants were found in known PDAC susceptibility genes including: APC, ATM, BRCA1, BRCA2, CDKN2A, CDK4, CFTR, LKB1, MLH1, MSH2, MSH6, NF1, PALB2, PMS2, PRSS1, SPINK1, TP53

SGTPase = GTPase in Ras superfamily

Filter candidate variants
- protein-altering variants
- heterozygous
- shared in both affecteds
- rare

Assumptions:
- causative variant is rare & has high penetrance
- no phenocopies

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Novel Candidate Allele

SGTPass

• no prior association with cancer
• a small GTPase, function unknown
• mutation SGTPase_TR: early STOP

What is the genetic basis for PDAC?

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novel candidate allele in novel gene

Validate the impact?

Mechanism?

Clinical translation?
Validating New Variants or VUS’s

- **in silico** prediction:
  - requires knowledge of protein structure
  - misses cryptic splice sites

- **in vitro** functional assays:
  - requires in-depth understanding of gene function
  - unclear relationship to cancer *in vivo*

- **in vivo** functional models:
  - mouse: small #'s

- **human epidemiology**:
  - large #'s of cases + controls needed to implicate lower penetrance mutations
  - founder effects: private variants are common

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**Zebrafish:** epidemiologic animal model to query even lower effect size
Zebrafish: *In Vivo* Model

- Zebrafish have a pancreas

  3 months

- Zebrafish develop cancer

  *Pancreatic Cancer*
  
  \textit{ptf1a:Kras-GFP}
  
  3 month-old

  \textit{Li Ex} *

  \textit{mitfa:BRAF(V600E); p53/-}  

  \textit{Coel 2011, Nature}

  *Melanoma*
Zebrafish Population Studies

• Zebrafish advantages over other model systems
  - Rapid *ex utero* development
  - Transparent embryos
  - Amenable to chemical and genetic manipulation
  - Large sibling cohorts

1 adult pair
SGTPase $^{+/\text{TR}}$

x
SGTPase $^{+/+}$

100s of embryos

100s of adult siblings
SGTPase $^{+/\text{TR}}$ vs. SGTPase $^{+/+}$

- Rapid *ex utero* development
- Transparent embryos
- Amenable to chemical and genetic manipulation
- Large sibling cohorts
Co-Segregation of Candidate Variant in Family

<table>
<thead>
<tr>
<th></th>
<th>Cancer</th>
<th>No Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wt</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Mutation</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

p = 0.0476

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SGTPase<sub>TR/+</sub> Promotes Tumor Formation

2 cancer models:

- Spontaneous tumors in <i>tp53</i>/- fish
- Carcinogen-induced cancers

→ validates the causality of this mutation in cancer risk
Novel Candidate Allele

What is the genetic basis for PDAC?

Validate the impact?

Mechanism?

Clinical translation?

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novel candidate allele in novel gene
Unbiased Approaches Reveal RAS pathway Dysregulation in SGTPase mutants

RNASeq:

SGTPase^{+/+} vs. SGTPase^{TR/TR}

3-weeks

RNA-Seq and GSEA consistent with upregulated KRAS activity in mutants

ES = 0.528
NES = 2.073
FDR q = 0.0
Nominal p = 0.001
Unbiased Approaches Reveal RAS pathway Dysregulation in SGTPase mutants

Interacting Proteomics:

SGTPase_TR forms a trimeric complex with RAP1GDS1 and KRAS
Model: SGTPase regulates Ras activity via Rap1gds1

Newly synthesized small GTPases

Kras-4B

Rap1gds1

Transfer to Prenyl transferase
Farnesylation
Geranylgeranylation

SGTPase_TR

Rap1gds1

Transfer to cell membrane
Model:

- Kras
- Rap1gds
- Rap1gds1
- RTK

Accelerated prenylation

Transfer to cell membrane

SGTPase +/TR

SGTPase TR/TR

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Kras

Rap1gds

Rap1gds1

RTK
Progressive Defects in SGTPase \textit{TR/\textit{TR}} Homozygotes

![Images of fish showing progressive defects in SGTPase TR/\textit{TR} homozygotes at different developmental stages (4 dpf, 20 hpf, 7 dpf, 3 wpf, 4 wpf, 8 wpf).]

- \textit{trypsin} at 4 dpf
- \textit{insulin} at 4 dpf
- \textit{fabp10} at 4 dpf
- \textit{cmlc2} at 4 dpf
- \textit{krox20} at 20 hpf
- \textit{myoD} at 20 hpf

- WT at 4 dpf, 20 hpf, 7 dpf, 3 wpf, 4 wpf, 8 wpf
- Het at 4 dpf, 20 hpf, 7 dpf, 3 wpf, 4 wpf, 8 wpf
- Homo at 4 dpf, 20 hpf, 7 dpf, 3 wpf, 4 wpf, 8 wpf

Statistical analysis at different stages:
- 7 dpf: WT, Het, Homo
- 3 wpf: WT, Het, Homo
- 4 wpf: WT, Het, Homo
- 8 wpf: WT, Het, Homo

Graphs showing comparisons between WT, Het, and Homo at different stages with statistical significance markers (n.s., *).
Phenotype of SGTPase $TR/\text{TR}$ Homozygotes

**Cranio-Facial / Bone Defects**

- **Wt / Het**
  - Homozygous SGTPase-$TR^{41}$
  - Lateral
  - Top

- **Homo sibling**

**Abnormal Swimming**

- **Wt / Het**
  - Movie converted to still for distribution.

- **Homo sibling**
  - Movie converted to still for distribution.
Model

Kras

SGTPase_TR

Rap1gds

1

Kras

Accelerated prenylation

SGTPase

+ / TR

SGTPase

TR / TR

Transfer to cell membrane

Rasopathy Phenotype?

Germline defects in Ras pathway → Developmental Abnormalities

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MEK Inhibitor

RTK
Chronic Trametinib Rescues Homozygous Phenotype

Homo

Movie converted to still for distribution.

Homo + Trametinib

Movie converted to still for distribution.
Chronic Trametinib Rescues Homozygous Phenotype

WT

Homo

Control

Trametinib

Vertebral Cortical Thickness (mm)

n.s.

*

## Vehicle

Trametinib
Model:

- **Kras**
- **Rap1gds**
- **Rap1gds1**
- **SGTPase-TR**
- **RTK**

- **Accelerated prenylation**
- **Transfer to cell membrane**

**SGTPase**
- **SGTPase**
  - **SGTPase**
    - **SGTPase**
      - **SGTPase**
        - **SGTPase**
          - **SGTPase**

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**Rasopathy phenotype rescued by MEK-Inhibition**
Model

Accelerated prenylation

Transfer to cell membrane
Novel Candidate Allele

- novel candidate allele in novel gene

Clinical translation:
- evaluate SGTPase mutations in other hereditary cancer syndromes, RASopathies
- drug development: target SGTPase-TR • RAP1GDS1 interaction for prevention or treatment?
Summary

WGS identifies germline mutation in SGTPase

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SGTPase mutants resemble Rasopathy

zebrafish mutants validate causative mutation

unbiased approaches implicate Ras dysregulation

SGTPase mutation accelerates KRAS prenylation
Key Concepts

1) Rare familial cancer syndrome → Broadly relevant disease mechanisms

2) Zebrafish are a powerful tool to functionally validate novel gene variants in cancer risk

- Sporadic 90%
- Familial
- Unsolved Familial PDAC 90%
- Novel candidate genes
- VUS’s in known genes
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