Germline Variation Disrupting Developmental Mechanisms Predisposes to Early Childhood Cancer

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Definitions in this talk

- **Mutation** = a change to your DNA sequence after birth (somatic)

- **Variant/Variation** = a germline (inherited or de novo) change in your DNA sequence compared to the human reference.
Pediatric Tumorigenesis

• Why do children get cancer?

• Pediatric cancer incidence can’t be explained by:

1. High-penetrance “cancer-causing” genes (TP53, APC, etc.)
   • Typically don’t develop cancer until 20's or after

2. Somatic mutation/environmental exposure
   • Requires 2-8 critical mutations for malignant transformation

3. Chromosomal rearrangements
   • Found in healthy people
   • Frequently fail to transform in model systems
Very few mutations in pediatric cancer

- Cancer genomes have an average of 2-8 “driver” mutations.
- Children’s cancers have the fewest somatic mutations.
- Many are immature cell types with defects in developmental mechanisms.

From Vogelstein et al., Science 339; 2013
Bimodal onset of pediatric cancers: pre-school and puberty

Chmielecki et al, Cancer Res 2017; 77(2)
Hypotheses:

1. Pediatric cancer is primarily a developmental defect.

2. Thus, children get cancer partially due to \textit{germline} profiles of rare, damaging variants?
Hypothesis: Children get cancer because of inherited profiles of rare, damaging variants?

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<th>If your full sibling has ALL</th>
<th>Odds Ratio</th>
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<td>If you are <em>not</em> twins</td>
<td>7.07</td>
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<tr>
<td>If you are twins</td>
<td>137.61</td>
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Hemminki & Jiang, *Leukemia* 2002; **16**: 297
Pediatric cancer incidence is increasing.

- Better detection??
- Due to “modern” environmental exposures??

Ages < 20 years

Cancer sites include invasive cases only unless otherwise noted. Rates are per 100,000 and are age-adjusted to the 2000 US Std Population (19 age groups – Census P25-1130). Regression lines are calculated using the Joinpoint Regression Program Version 4.0.3, April 2013, National Cancer Institute. Incidence source: SEER 9 areas (San Francisco, Connecticut, Detroit, Hawaii, Iowa, New Mexico, Seattle, Utah, and Atlanta).
Figure 1 | Feasibility of identifying genetic variants by risk-allele frequency and strength of genetic effect (odds ratio). Reproduced, with permission, from Nature REF. 10 © (2009) Macmillan Publishers Ltd. All rights reserved. GWA, genome-wide association.

Effect size and rare variation

Figure 1 | Feasibility of identifying genetic variants by risk-allele frequency and strength of genetic effect (odds ratio). Reproduced, with permission, from Nature REF. 10 © (2009) Macmillan Publishers Ltd. All rights reserved. GWA, genome-wide association.

8.5% of pediatric cancer patients have germline variants in KNOWN cancer predisposition genes
Cancer predisposition may be more common than we think?

- 29% of >300 pediatric cancer survivors met criteria for a cancer predisposition syndrome.
  - Family history, cancer type, medical history, other conditions
  - Compared to only ~5% for adult cancer survivors

- Including deceased children, ~33% of children with cancer have a predisposition.
Schematic model for genetic and epigenetic variation in cancer as a function of age.
Hypothesis: *Inherited* profiles of rare, damaging variants predispose to IL.

- **Infant leukemia (IL)**
  - >50% of infants (<1 year old) who get leukemia die from disease
  - Survivors often left with lifelong developmental problems
  - ~66% have a translocation in the *MLL* gene
    - H3K4 histone methyltransferase, controls gene transcription
    - >100 identified fusion partners
    - *MLL*-rearrangements rarely induce short latency leukemia *in vitro*
  - No common environmental exposures during pregnancy or infancy identified
Very few somatic mutations in MLL-positive ALL

The landscape of somatic mutations in infant MLL-rearranged acute lymphoblastic leukemias

1.3 non-silent somatic mutations per genome
The largest case-control study of infant leukemia to date.

- Julie Ross, PhD, Logan Spector, PhD & Amy Linabery, PhD;
- Univ of Minnesota

**GERMLINE** DNA from infants that developed IL.

**Exome/Genome Sequencing**

- 114 germline exomes from IL patients
- 20 germline genomes from PCGP IL patients
- 30 germline exomes from healthy children
- 40 germline genomes from 1000 Genomes
- All compared to the ExAC database
Prioritize variants most likely to have a functional impact

1. RARE or NOVEL
   - Found at <1% variant allele frequency in the ExAC database

2. NON-SYNONYMOUS
   - Alter the coding sequence of the resulting protein
Highly significant enrichment

• Used the COSMIC database to identify candidate genes somatically mutated in ALL and AML
  – 126 genes for ALL
  – 655 genes for AML

• Is the observed variation higher in these genes?

http://www.sanger.ac.uk/genetics/CGP/cosmic/
• **MLL1** & **MLL2** are orthologous to drosophila *Trithorax* (*Trx*)
• **MLL3** & **MLL4** are orthologous to *Trithorax-related* (*Trr*)

• COMPASS complexes regulate gene expression during early development
  – H3K4 tri-methylation at promoter sites (on/off)
  – H3K4 mono-methylation at enhancer sites (high/low)
  – Ubiquitination of H2A & H2B – targets and function UNCLEAR!
What are COMPASS complexes?

MLL1 & MLL2 complexes function via:
- retinoic acid receptors at the nuclear membrane
- histone modifications

MLL3 & MLL4 complexes function via:
- retinoic acid receptors at the nuclear membrane
- histone modifications

“Histone crosstalk” refers to a regulatory cascade dependent upon proper modifications of histones.

Adapted from Hu et al 2013
MLL3 and MLL2 are significant pan-cancer genes

Current directions – functional understanding

1. Reprogramming patient fibroblasts/BECs to iPSCs

- Cannot effectively replicate all of this variation in a single animal or cell line
- Acknowledge that variation outside the COMPASS family may be critical for IL etiology
- We have 7 validated clones from 4 patients and 2 clones from one unaffected pediatric control
Pediatric Cancer Predisposition Clinic

- Joint clinic between Heme/Onc, Genetics, GI, Radiology, Endocrinology and Neurology
  [http://www.stlouischildrens.org/our-services/cancer-predisposition-program]

- We offer comprehensive clinical and research services:
  - Genetic counseling
  - Occupational, Physical & Speech Therapy
  - School liaison
  - Research platform:
    - DNA banking
    - Longitudinal phenotype data
    - hiPSC bank
Birth defects and cancer risk:

- Relative Risk increase of 1.4 – 6.0 during childhood depending upon the type of birth defect and cancer.