Deciphering the genetic barcode of cancer susceptibility using mouse models of astrocytoma, MPNST, and NF1

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In any population, why do certain individuals develop cancer?

- Genetic Predisposition
- Diet/Environmental Factors
- Sporadic Events (bad luck!)
Combinatorial effects within a population can give rise to rare cancer

- Genetic Predisposition
- Diet/Environmental Factors
- Sporadic Events (bad luck!)

Understand basic biology of cancer
Apply what we learn to developing therapy
Cancer is a process of accumulating genetic mutations.
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- **Normal Cell**: astrocytes, Schwann cells
- **Cancer**: astrocytoma, MPNST

**modifier genes**

**oncogenes**

**tumor suppressors**

*genes: Nf1, p53*
Cancer is a disease of gene alteration

High-penetrance cancer genes (rare mutations with powerful effects)
e.g. p53, Rb1, APC, Nf1, H-Ras, myc

Low-penetrance modifier genes (common variants with partial effects)
e.g. Pla2g2a, STK15, Ptpj, Sipa1

Imprinted genes/Epigenetic effects (genome modifications inherited from one parent or the other)
Evidence in rhabdomyosarcoma, oligodendroglioma, and paraganglioma

Nf1;p53cis mouse model of Neurofibromatosis type 1
The Genetics of Cancer Susceptibility

Cancer Risk

High-penetrance tumor suppressors
Resistant

Susceptible

Resistant

Susceptible

Reading the Barcode of Cancer Susceptibility

Nf1/p53 mutation

Epigenetic State X

Epigenetic State Y

Modifier GT A

Modifier GT A
Apparent “sporadic” cancers may be the result of more complicated genetic “barcodes”

- These “barcodes” are present before cancer develops, independent of environmental exposures, providing an opportunity for personalized prevention of cancer
Deciphering Susceptibility Codes: Sporadic vs. Familial Cancer vs. Mouse Cancer

- shorter lifespan
- controlled breeding
- controlled diet/environment

- + other mutations
- cancer risk is increased, cancer develops at a younger age

(not to scale)
Neurofibromatosis type 1

- Autosomal dominant, affecting 1 in 3500
- 100% penetrant, but variable expressivity
- Evidence for modifier genes from twin studies
- Characterized by benign lesions in many different organ systems, including:
  - neurofibromas
  - optic nerve gliomas (WHO I)
  - learning disabilities
  - bone fragility
  - changes in brain anatomy (MRI)
- Increased risk for malignancies
  - malignant peripheral nerve sheath tumors
  - astrocytomas/glioblastomas (WHO II-IV)
  - pheochromocytomas
  - rhabdomyosarcomas
  - myeloid leukemia
- p53 is mutated in the transformation of neurofibromas into MPNSTs
Glia cell tumors of the central and peripheral nervous systems are increased in Neurofibromatosis type 1 patients

Astrocytes (normal cell)
- Maintain homeostasis in the central nervous system through interactions with neurons, blood vessels, and basement membranes

Astrocytoma/Glioblastoma Multiforme
- The most common malignant tumor of the CNS
- Incidence of 15 in 100,000 in the general population
- Affects 2% of NF1 patients
- The median survival rate for high-grade astrocytoma (glioblastoma) is 0.4 years
- Only 6% of diagnosed high-grade astrocytoma patients live to 3 years post-diagnosis
- Diffusely infiltrating behavior makes surgical resection difficult to impossible
- Mutation of p53 occurs during astrocytoma initiation

Schwann cells (normal cell)
- Insulate electrical currents of axons in the peripheral nervous system

Malignant Peripheral Nerve Sheath Tumor
- Incidence of 1 in 100,000 in the general population
- Affects up to 10% of NF1 patients
- Tumors can infiltrate along nerve tracks and metastasize
- 5-year survival rate has been estimated between 16-52%, depending on degree of resection of tumors, size, and location
- Mutation of p53 occurs during progression of benign tumors to malignancy
Nf1 and p53 are tumor suppressor genes acting at different points in the control of cell growth and survival.
The Nf1; p53cis, C57BL/6J mouse model of astrocytoma and peripheral nerve sheath tumor

- The average tumor latency is 7 months
- Mutations are maintained by simple Mendelian inheritance
- The p53 pathway is lost and the ras pathway is upregulated at physiologically relevant levels by a single chromosomal loss event
- The genetic background is well defined
The *Nf1;p53cis*, C57BL/6J mouse model of NF1

malignant astrocytomas/glioblastomas
- 73% mutant mice on C57BL/6J
- modified by 129S4/SvJae and CBA/J
- progeny of mutant moms have increased susceptibility
- F1 analysis shows modifiers on chr 11 near *Nf1* and *p53*

malignant peripheral nerve sheath tumors
- 65% mutant mice on C57BL/6J
- modified by A/J and DBA/2J
- progeny of mutant dads have increased susceptibility
- backcross mapping shows linkage to chr 15 and 19

Nf1 and p53 cooperate in tumor suppression
Imprinting on mouse chromosome 11 cooperates with p53 mutation in tumorigenesis.
Mapping modifiers of MPNSTs by backcrossing: Polymorphisms in the A/J strain modify tumorigenesis

<table>
<thead>
<tr>
<th>Progeny</th>
<th>Total</th>
<th>With sarcoma</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPcis, B6, mother cis</td>
<td>47</td>
<td>18</td>
<td>38% (65%)</td>
</tr>
<tr>
<td>NPcis, B6, father cis</td>
<td>91</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>NPcis, B6XA, mother cis</td>
<td>80</td>
<td>20</td>
<td>25% (45%)</td>
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<tr>
<td>NPcis, B6XA, father cis</td>
<td>20</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>NPcis, B6XAXB6, mother cis</td>
<td>97</td>
<td>38</td>
<td>39% (62%)</td>
</tr>
<tr>
<td>NPcis, B6XAXB6, father cis</td>
<td>144</td>
<td>89</td>
<td></td>
</tr>
</tbody>
</table>

- Inheritance of mutant chromosome 11 affects incidence of sarcomas regardless of strain background.
- The A strain confers dominant resistance to sarcomas regardless of inheritance of chromosome 11.
Parents of backcross progeny determine which loci affect susceptibility

Progeny of Nf1;p53cis (B6XA) female X wt B6 male (N=97)
vs. Progeny of wt B6 female X Nf1;p53cis (B6XA) male (N=144)

nerve sheath tumor resistance loci

Karl Broman, John Hopkins University
*nstr1* on mouse chromosome 19 and *nstr2* on mouse chromosome 15 control susceptibility to GEM PNSTs.

**Chromosome 15**

- LOD score vs. Map position (cM)
- **P=0.05**

**Chromosome 19**

- LOD score vs. Map position (cM)
- **P=0.03**
- Blue line: from dad
- Red line: from mom
**nstr1** and **nstr2** are syntenic with chromosomal regions altered in human MPNSTs

Alterations in human MPNSTs (CGAP, N=90)

<table>
<thead>
<tr>
<th>Alteration</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>+8</td>
<td>12 cases</td>
</tr>
<tr>
<td>-8</td>
<td>10 cases</td>
</tr>
<tr>
<td>Addition 8q22</td>
<td>1 case</td>
</tr>
<tr>
<td>Addition 8q24</td>
<td>3 cases</td>
</tr>
<tr>
<td>Translocation 8q22</td>
<td>1 case</td>
</tr>
<tr>
<td>Translocation 8q23</td>
<td>1 case</td>
</tr>
<tr>
<td>+11</td>
<td>2 cases</td>
</tr>
<tr>
<td>-11</td>
<td>17 cases</td>
</tr>
<tr>
<td>11q13 deletion</td>
<td>2 cases</td>
</tr>
<tr>
<td>11q13 addition</td>
<td>1 case</td>
</tr>
<tr>
<td>11q13 translocation</td>
<td>3 cases</td>
</tr>
</tbody>
</table>

Corresponding human chromosomes

- 5p
- 8q22-24
- 22q
- 12q
- 11q13
- 9q
- 9p
- 10q

P=0.05

P=0.03

Using Chromosome Substitution Strains to test mechanisms of resistance

C57BL/6J.Chr 19\textsuperscript{A/J} female  \times  Nf1;p53cis C57BL/6J male  \rightarrow  Nf1;p53cis CSS19 reduced PNSTs?

C57BL/6J.Chr 6\textsuperscript{A/J} female  \times  Nf1;p53cis C57BL/6J male  \rightarrow  Nf1;p53cis CSS6
Chromosomes 19 (nstr1) and 15 (nstr2) modify PNST susceptibility differently.

<table>
<thead>
<tr>
<th>Strain Type</th>
<th>N</th>
<th>% GEM PNST</th>
<th>$\chi^2$ Test P value</th>
<th>Median Age w/ GEM PNST (mo)</th>
<th>Mean Age w/ GEM PNST (mo)</th>
<th>T-test P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPcis\text{pat};CSS-19</td>
<td>30</td>
<td>77%</td>
<td>0.5</td>
<td>5.7</td>
<td>5.9</td>
<td>0.06</td>
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<tr>
<td>NPcis\text{pat};CSS-6</td>
<td>19</td>
<td>84%</td>
<td></td>
<td>4.9</td>
<td>4.9</td>
<td></td>
</tr>
<tr>
<td>NPcis\text{mat};CSS-15</td>
<td>27</td>
<td>37%</td>
<td>0.03</td>
<td>7.3</td>
<td>7.5</td>
<td>0.24</td>
</tr>
<tr>
<td>NPcis\text{mat};CSS-8</td>
<td>20</td>
<td>70%</td>
<td></td>
<td>6.8</td>
<td>6.9</td>
<td></td>
</tr>
</tbody>
</table>

- Nstr2 (chr 15) reduces number of PNSTs, but does not affect latency
- Nstr1 (chr 19) may increase latency of PNSTs, but does not affect penetrance
- Although $NPcis^{\text{mat}};CSS-15$ mice develop significantly fewer GEM PNSTs, they develop significantly more astrocytoma and hematopoietic tumors.
A genetic/epigenetic network for susceptibility to peripheral nerve sheath tumors

Nf1/p53 mutation

Epigenetic State X
- paternal mutant chr 11
  - imprinted gene lost or amp.
  - differential loss of wt p53 (or Nf1)

Epigenetic State Y
- maternal mutant chr 11

 Modifier GT A
- Nstr1 B6/A
  - Resistant (PNST tumor latency incr.)

 Modifier GT B
- Nstr1 B6/B6
  - Susceptible

 Modifier GT A
- Nstr2 B6/A
  - Resistant (PNST tumor incidence decr.)

 Modifier GT B
- Nstr2 B6/B6
  - Susceptible
Using mouse models of cancer to develop new therapies

Candidate targets identified by modifier screens and basic biology studies

Available libraries of therapeutics

“High-throughput” screening of compounds in astrocytoma cells

“High-throughput” screening of compounds in astrocytoma cells

Available libraries of therapeutics

Follow tumor growth and quantify tumor burden using bioluminescence

Nf1-/-;p53-/-+cis;ELUX Tg spontaneous astrocytoma model

treatment studies in subcutaneous or intracranial models (immune-competent and immune-deficient)

toxicity studies in mice

mouse and human tumor cells of different grades and primary astrocytes
14 cell lines of differing tumor grades, sex, and genetic background have been generated thus far by this method.
Using the Nf1-/-;p53-/-+cis mouse model to investigate a potential anti-astrocytoma therapeutic derived from natural compounds

In collaboration with the Molecular Targets Development Program
Schweinfurthin A specifically inhibits mouse and human astrocytoma/GBM cell growth.

Schweinfurthin A shows differential activity in a screen of the NCI60 cell lines: specificity towards brain tumor cell lines.

Mouse and human glioma cells are equally sensitive to Schweinfurthin A.
Summary

• Cancer susceptibility is determined by the interaction of high-penetrance cancer genes, low-penetrance cancer genes, and epigenetic effects

• Epistatic and combinatorial effects can mask the genetic component of cancer susceptibility

• Mouse models of cancer and human familial cancer syndromes are useful to dissect the components of cancer susceptibility

• Mouse tumor cell lines are useful surrogates for human cell lines in preclinical drug testing and allow testing in immune-competent animals

• Brain tumor specific therapeutics may provide a new, more effective approach for the treatment of astrocytoma and glioblastoma.
Acknowledgements

Modifiers of Cancer
Robert Tuskan
Erika Truffer
Kristi Fox
Michelle Perella

Schweinfurthin A
Demir Gürsel

Jessica Hawes
Yvette Connell-Albert
Jessica Walrath
Krishan Kumar

Modifiers of Cancer
C. Dahlem Smith
Karl Broman
Shirley Tsang
John Diehl
David Sun
David Munroe
Dagan Loisel
Jeremy Ledger
Emily Christy
Tyler Jacks
Rod Bronson

Schweinfurthin A
Tommy Turbyville
John Beutler
David Weimer
Jeffrey Neighbors