Genomic Architecture of the KIR and MHC-B and -C Regions in Orangutan

Libby Guethlein
Parham Lab, Stanford University
Natural killer cell function is modulated by families of cell surface receptors

- Receptor variation is germline encoded and not somatically altered
  - Gene content variation
  - Allelic diversity
  - Variegated expression

- Two broad structural groups of receptors
  - Immunoglobulin-like [KIR, LILR, MAIR]
  - Lectin-like [Ly49, CD94:NKG2]

- Both groups have MHC as a ligand

- Studied mammalian species appear to have expanded one group
KIR nomenclature, lineages, and ligands

- **Killer cell Immunoglobulin-like Receptor**
- Naming is done by number of extracellular domains and type of encoded tail
- Lineages were determined by phylogenetic analysis
- Ligands have been determined experimentally

<table>
<thead>
<tr>
<th>Lineage</th>
<th>EC</th>
<th>Ligand</th>
<th>Epitope</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>2D</td>
<td>HLA-G</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>3D</td>
<td>HLA-A and -B</td>
<td>A3/11 or Bw4</td>
</tr>
<tr>
<td>III</td>
<td>2D or 3D</td>
<td>HLA-C</td>
<td>C1(N80) or C2(K80)</td>
</tr>
<tr>
<td>V</td>
<td>3DL3</td>
<td>unknown</td>
<td></td>
</tr>
</tbody>
</table>
MHC nomenclature and background

- **Major Histocompatibility Complex**
- In humans HLA, in other species prefixed by genus/species, i.e. Popy for *Pongo pygmaeus* (orangutan)
- Most KIR ligands are Class I MHC
- Class I molecules have a polymorphic heavy chain complexed with β2m
- Further divided into classical (polymorphic, broad tissue distribution) and non-classical (less polymorphic, restricted tissue distribution, sometimes specialized function)
- Along with their function as a KIR ligand they bind and present peptides to the immune system
- In humans HLA-A, -B, -C, -F, and -G can all act as KIR ligands
- HLA-C has emerged as the dominant ligand in humans and all allotypes are capable to act as a KIR ligand.
Orangutan is the most distant species to express homologs of all known human KIR ligands

<table>
<thead>
<tr>
<th>Primate species</th>
<th>MHC class I genes</th>
<th>KIR</th>
<th>Divergence time from human (million years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E</td>
<td>G</td>
<td>A</td>
</tr>
<tr>
<td>Prosimians</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New World monkeys</td>
<td>green</td>
<td></td>
<td>red</td>
</tr>
<tr>
<td>Old World monkeys</td>
<td>green</td>
<td></td>
<td>red</td>
</tr>
<tr>
<td>Gibbons</td>
<td>red</td>
<td></td>
<td>red</td>
</tr>
<tr>
<td>Orangutans</td>
<td>blue</td>
<td></td>
<td>red</td>
</tr>
<tr>
<td>Gorillas</td>
<td>blue</td>
<td></td>
<td>red</td>
</tr>
<tr>
<td>Chimpanzees</td>
<td>blue</td>
<td></td>
<td>red</td>
</tr>
<tr>
<td>Human</td>
<td>blue</td>
<td></td>
<td>red</td>
</tr>
<tr>
<td>Cognate receptor in human</td>
<td>CD94: NKG2</td>
<td>lineage I KIR</td>
<td>lineage II KIR</td>
</tr>
</tbody>
</table>
KIR genes are organized head-to-tail with gene lengths of approx. 15kb and 2 kb between loci.
KIR haplotypes vary in gene content
Initial orangutan haplotype determined by sequencing overlapping cosmids

Cosmids were sequenced by generating two subclone libraries [BamHI and EcoRI] that were then used to determine the complete sequence. Strategy chosen to ensure proper assembly of closely related genes.
Subsequent sequencing by the Oragutan Genome Project revealed a second haplotype.

Popy-H2 was the result of complete sequencing of a single BAC. Analysis of the read archives has identified Popy-H3 as the other haplotype present in the individual sequenced.
A third haplotype structure identified through sequencing of two overlapping BACs
A third haplotype structure identified through sequencing of two overlapping BACs

- Initial sequencing with 454
  - de novo assembly in MIRA with each BAC individually
  - manual editing
  - assessed gene content
  - construction of reference from two known haplotypes
  - hybrid reference/de novo assembly in MIRA with combined BACs
  - manual editing
  - finishing and check of assembly

- Confirmatory sequencing with PacBio
  - de novo assembly confirmed the 454 assembly and finished areas that had not been completed
Sequenced KIR haplotypes reveal expansion of lineage III KIR and diversification through tail swap
Unlike KIR genomic architecture in the MHC is more conserved
All Popy-C alleles encode C1 epitopes and Popy-B alleles can be broadly divided into two subgroups one of which contains alleles encoding Bw4 or C1 epitopes.
Initial typing results and BACs are consistent with a model of a single Popy-C locus and two Popy-B loci.
Proposed haplotype structure based primarily on the typing results with some sequence overlap

- Initial sequencing with 454
  - de novo assembly in MIRA with each BAC individually
  - manual editing
  - Many hybrid contigs with two alleles mixed in
  - Phasing was not clear for much of the polymorphism

- Sequencing with PacBio
  - de novo assembly

Hap 1 (BAC 3)
- C1
- B
- Bw4

Hap 2 (BAC 1 & 2)
- C1
- C
- B
- B
Orangutans have a minimum of two Popy-B loci and one ‘hybrid’ Popy-B/C locus.
Additional BACs identified by searching BAC ends

13 BACs found by in silico probing the BAC end library with full length BAC sequence

10 overlapped the existing clones

Prepped and sequenced with PacBio
The additional BACs have extended both haplotypes and 'anchored' the ends.

Chimpanzee

Human

H1

H2

HCG22
HCG27
HLA-C
HLA-B
MICA
MICB
TNF
The additional BACs have extended both haplotypes and 'anchored' the ends.
Summary

- Orangutan KIR haplotypes have diversified by duplication and recombination
  - Many of the events involve swapping of tail encoding exons
- Diversification of Popy-KIR has occurred after speciation
- Popy-B can be divided into two broad groups only one of which encodes potential KIR ligands and these appear to occupy separate loci
- Popy-C and Popy-B*03:02 occupy the same locus
Acknowledgements

Stanford
Peter Parham
Arnav Moudgil
Paul Norman
Parham Lab

Biomedical Primate Research Centre
Ronald Bontrop
Natasja de Groot
Corinne Heijmans

Stanford Genome Technology Center
Farbod Babrzadeh

Pacific Biosciences
Swati Ranade
Brett Bowman
Nicole Rapicavoli

Yerkes Regional Primate Center

NIH
AI31168/AI24258