Knockout mice created by TALEN-mediated gene targeting
Young Hoon Sung, In-Jeoung Baek, Duk Hyoung Kim, Jisun Jeon, Jaehoon Lee, Kyunghee Lee, Daewon Jeong, Jin-Soo Kim, and Han-Woong Lee

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Gene-specific knockout mice

- Knocked out gene – existing gene is replaced or disrupted
- Causes change in mouse phenotype
- One of the most powerful ways to study gene function in living animals
Review on TALENs

Overview of how to generate knockout mice with TALENs

1. Design specific TALENs
2. Inject TALEN mRNA into mice embryo
3. Generate mutant mice

Inject TALEN mRNA into mice embryo

Generate mutant mice

F₀

F₁
Step 1: Design and synthesize highly active TALENs

- HA Tag – for antibody tagging
- NLS – nuclear localization signal
- TALEN module – target either Pibf1 or Sepw1
- FokI nuclease domain – creates non-specific cleavage
Step 2: Identify mutant mice

Pibf1-TALEN:

<table>
<thead>
<tr>
<th>Founders</th>
<th>331 bp</th>
<th>190 bp</th>
<th>Amino acid sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
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</table>

* Wild-type sequence
* Δ21 bp + aa change
* Δ21 bp + aa change
* Δ3 bp
* Δ5 bp (frameshift)
* Δ1 bp (frameshift)
* WT
* Δ5 bp (frameshift)
* WT
* Δ12 bp
* Δ21 bp
* Δ24 bp
* Δ11 bp (frameshift)
* Δ1 bp (frameshift)
* Δ27 bp + aa change
* Δ2 bp (frameshift)
* Δ24 bp
Pibf1 shows no off-target cleavage
Step 3: Investigate Dose dependency for Pibf1-TALEN

Table 1: TALEN-mediated Pibf1 gene targeting in C57BL/6J mice

<table>
<thead>
<tr>
<th>Dose of TALEN-Pibf1 mRNA (ng/μl)</th>
<th>Number of injected zygotes</th>
<th>Number of surviving zygotes</th>
<th>Two-cell embryos</th>
<th>Transferred embryos</th>
<th>Newborns</th>
<th>Founders*</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>276</td>
<td>263 (95.3%)</td>
<td>262 (99.6%)</td>
<td>243</td>
<td>13 (5.3%)†</td>
<td>10 (76.9%)††</td>
</tr>
<tr>
<td>20</td>
<td>183</td>
<td>176 (96.2%)</td>
<td>176 (100%)</td>
<td>176</td>
<td>39 (22.2%)†</td>
<td>19 (48.7%)</td>
</tr>
</tbody>
</table>

- Mutation rate approximately proportional to injection dose of Pibf1-TALEN mRNA
- More frequent bi-allelic mutations in high dose
- Relatively large deletions more frequent with high-dose
- Higher number of mutant mice produced by low-dose injections
Step 4: Produce $F_1$ offsprings and determine genotypes

- **Founder #1 ($\Delta 21 \text{ bp}/\Delta 21 \text{ bp}$)**
  - WT
  - $\Delta 21$

- **Founder #6 ($\Delta 12 \text{ bp}/\Delta 21 \text{ bp}$)**
  - WT
  - $\Delta 12$
  - $\Delta 21$

- **Founder #8 ($\Delta 11 \text{ bp}/\Delta 24 \text{ bp}$)**
  - WT
  - $\Delta 11$
  - $\Delta 24$
Conclusion

- TALEN activity in one-cell embryos is sufficient to induce mutations.
- TALEN activity not likely to be maintained after the first cleavage of one-cell embryos.
- TALEN-mediated gene targeting is an efficient method for creating heritable null mutations in a specific locus of the mouse genome.
Limitations

• 15% gene knockouts are developmentally lethal, limiting ability to study development into adult mice
• Same gene has different functions in humans
• Limited control over base pair deletion
• Off-target binding
• Dose-dependent activity
Significance and Future Developments

- Target specific sequences in the genome
- Easy to manipulate TALENs to target any sequence
- Can target other genes
- Find a more specific method for cleavage
Discussion Questions

• Is there a way to make the DNA cleavage more predictable?
• Is this method really more efficient than current methods for creating knockout mice?
• What other methods may this be combined with to create more control?