An Estrogen Receptor alpha Functional Mutant is Protective in Murine Lupus

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Lupus and Sex Bias

Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder that affects skin, joints, kidneys, brain and other organs.

- Patient diagnosed with SLE at age 20 still has a 1 in 6 chance of dying by 35 years of age.

Lupus is a disease that primarily affects women and minorities.

- 90% of those diagnosed are women, the vast majority between the ages of 15 and 45 years old.

- In pre-menarchal and post-menopausal females the ratio is 2:1.

Key unanswered question in lupus:

- What is the mechanism underlying the 9:1 female predominance in this disease?

Contreras et al. Clin Nephrol 2002
Rahman & Isenberg, NEJM, 2008
ERαKO/NZM mice

Females, but not males, had significantly prolonged survival compared to WT.

- Reduced proteinuria
- Lower renal pathology scores
- Lower urea nitrogen
- Higher serum anti-dsDNA levels

Svenson et al., Clinical Immunol, 2008
Toll like receptor (TLR)-induced production of inflammatory cytokines is reduced in ERαKO mice

- In multiple immune cell types, such as DCs, as well as kidney mesangial cells.

Lox = TLR7 agonist  
CpG = TLR9 agonist

Cunningham et al., Clin Immunol, 2012
Svenson et al, AJMS, 2014
Hormone levels in ERαKO Mice

- The hormonal profile of ERαKO females is abnormal
- ERαKO females have hypergonadism and partial endocrine sex reversal
  - LH, estradiol and testosterone are all elevated in KOs
ERαKO mice are a functional, not deletional, knockout of ERα

<table>
<thead>
<tr>
<th>Strain Name</th>
<th>Isoform Structures</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wild type</td>
<td>ERα66</td>
</tr>
<tr>
<td></td>
<td>ERα46</td>
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</tbody>
</table>

[Diagram of ERα isoform structures]
1. To what extent are hormone levels (E2 and T2) impacting the phenotype?

2. Is there a difference between the original *functional* ERα KO and a *deletional* ERα KO?

Ovariectomize mice and administer E2 (and thus correct hypergonadism) to determine the phenotypes of the 2 strains.
NZM Survival

Log rank test p=0.052
Female NZM2410 ERαKO mice were protected from lupus disease expression (effect required E2).

ERα-/- were not protected.
Dipstick albumin at death mg/dL

NZM WT

NZM ERαKO

NZM ERαKO - OVX

NZM ERαKO - OVX + E2

NZM Ex3a - OVX + E2

Proteinuria
Multiple ER\(\alpha\) Splice Variants

ER\(\alpha\) (66kDa) and its N-terminally truncated isoform (46kDa) are both predominantly nuclear transcription factors. Data suggest that the isoforms have different activities:

- Liganded ER\(\alpha\)66 prepares the promoter to respond to ligand through sequentially targeting chromatin remodeling complexes and general transcription factors.
- Liganded ER\(\alpha\)46 recruits co-repressors.

Metivier, et al., EMBO journal, 2004
ERα46 is devoid of AF-1

Similar to the ERα in ERαKO mice we would expect that transactivation would be ineffective in a cell context predominately mediated by AF-1 but activation would still be effective in a cell context sensitive to AF-2.
Bone marrow-derived dendritic cell (BM-DC) numbers are reduced in NZM/ERαKO vs. NZM/Ex3a mice.
Kidney DCs are reduced in ERαKO mice.
Kidney DCs are reduced in ERαKO mice

**Percent Positive cells**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Percent CD11c+/CD11b+/MHCII+</th>
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<tbody>
<tr>
<td>WT OVX + E2</td>
<td></td>
</tr>
<tr>
<td>ERαKO OVX + E2</td>
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</tr>
<tr>
<td>Ex3a OVX + E2</td>
<td></td>
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**Absolute # CD11c+/CD11b+/MHCII+**

<table>
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<tr>
<th>Condition</th>
<th>Positive cells x 10^4</th>
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Gene expression and pathway differences between NZM WT and NZM ERαKO DCs by RNAseq

**Genes**
- IL-6 signaling
- TLR/MyD88 signaling
- Interferon signaling

**Pathways**
- NFkB pathway
- PI3K/mTOR pathway
- Androgen Receptor signaling
- NOD signaling
- NOTCH signaling
- Cell cycle and apoptosis pathways
Summary

• Female lupus prone ERαKO mice have significantly decreased renal disease and prolonged survival if:
  • They are either unmanipulated (previous study) or
  • They are both OVX’d and estrogen-repleted. Thus, estrogen is required for the protective effect. The phenotype suggests activity of the mutant ERα protein.
  • ERα deficiency (Ex3a) is not protective in the setting of OVX and/or E2 repletion.

• Preliminary flow data revealed that dendritic cell numbers from BM, kidneys, and spleen are reduced in NZM/ERαKO but not NZM/Ex3a animals, which again suggests an inhibitory mechanism of the mutant ERα, rather than requirement of ERα.
Summary

• Given that the mutant ERα is nearly structurally identical to the endogenous ERα46 splice variant, we hypothesize that this small isoform is immune modulatory.
  
  • Selectively targeting this isoform (with a SERM?) may be protective in lupus
  
  • Looking for ideas as to how we can exploit this as a therapeutic intervention.

• We are also looking for new ways to look at ERα at the level of the transcriptome – perhaps with ATAC-seq?
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