The Power of Biospecimens in Understanding Disease Progression in ALS

Peter H. King, M.D.
Chief of Neurology, Birmingham VA Medical Center
Professor and Vice Chair of Neurology, UAB
Objectives

• General concepts of disease marker discovery
• Sharing the UAB/BVAMC experience in using ALS tissue

  • **Example 1:** Discovery of muscle biomarkers and new directions for understanding disease mechanisms
  • **Example 2:** Validation of HuR as a new regulator of central neuroinflammation in ALS
  • **Example 3:** Validation of peripheral neuroinflammation in ALS and support for a novel treatment direction
Importance of Biomarker discovery by “omics”

- Markers for clinical assessment
- Reveals novel molecular pathways
- Provides direction for mechanistic studies and novel therapies
- Her2
- BCR-ABL kinase

Swinnen, Nature Rev, 2014
Post Mortem Biospecimens in ALS

Before

Normal

After

ALS

King and Mitsumoto, 1996

F5 tornado

Aerial bombing
ALS Tissue: Discovery and Validation

- IPSC

- ALS patient derived
- Sporadic or familial
- Rapid testing
- Mechanistic
- Not an organism
- No aging effect

- "Gold" standard
- Sporadic form
- Microenvironment
- End-stage
- QC RNA, protein

- Temporal and spatial evolution
- Microenvironment
- Mechanistic
- Genetic manipulation
- Genetic based
- Incomplete recapitulation of pathology
### Example 1: Biomarker Discovery

#### Muscle samples

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>ALS</th>
<th>Myopathy</th>
<th>Neuropathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>22</td>
<td>39</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td>Age Range</td>
<td>32–74</td>
<td>27–82</td>
<td>38–74</td>
<td>33–88</td>
</tr>
<tr>
<td>Mean Age (y)</td>
<td>54 ± 11</td>
<td>59 ± 12</td>
<td>56 ± 14</td>
<td>60 ± 11</td>
</tr>
<tr>
<td>Gender (M:F)</td>
<td>2.6:1</td>
<td>1.2:1</td>
<td>1.1:1</td>
<td>3.3:1</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>–</td>
<td>Spinal (77%)</td>
<td>Inflammatory Axonal neuropathy</td>
<td>Non-specific</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bulbar (23%)</td>
<td>Mitochondrial Plexopathy</td>
<td>GBS (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Necrotizing</td>
<td>GBS</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a* Patient samples were used for either qPCR or western blot analysis. CIDP, Chronic inflammatory demyelinating polyradiculoneuropathy; GBS, Guillain Barre syndrome.

---

Si et al., Ann Clin Transl Neurol. 2014
**Smad 8 Validation**

**qPCR**

Smad 8

<table>
<thead>
<tr>
<th>RQ</th>
<th>Normal</th>
<th>ALS</th>
<th>Myo</th>
<th>Neuro</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10</td>
<td>20</td>
<td>30</td>
<td>40</td>
</tr>
</tbody>
</table>

Hoechst

WGA

p-Smad

Merge

Si et al., Ann Clin Transl Neurol. 2014
Smad8 in the G93A SOD1 mouse

ALS mouse

- Rotarod score
- Weight

qPCR

- WT
- G93A

IHC

- p-Smad
- WGA
- Hoechst
- Merge

Si et al., Ann Clin Transl Neurol. 2014
CYP27B1 and ALS Disease progression: a prospective study

Study patients

<table>
<thead>
<tr>
<th>Sex</th>
<th>Age</th>
<th>Onset</th>
<th>Duration (m)</th>
<th>Study duration (m)</th>
<th>ALSFRS-R</th>
<th>ΔFRS</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>59</td>
<td>Bulbar</td>
<td>7</td>
<td>6</td>
<td>28</td>
<td>13</td>
<td>2.5</td>
</tr>
<tr>
<td>M</td>
<td>64</td>
<td>Bulbar</td>
<td>8</td>
<td>6</td>
<td>36</td>
<td>21</td>
<td>2.5</td>
</tr>
<tr>
<td>M</td>
<td>41</td>
<td>Spinal</td>
<td>15</td>
<td>12</td>
<td>24</td>
<td>12</td>
<td>1.0</td>
</tr>
<tr>
<td>M</td>
<td>44</td>
<td>Spinal</td>
<td>17</td>
<td>12</td>
<td>30</td>
<td>19</td>
<td>0.9</td>
</tr>
<tr>
<td>M</td>
<td>54</td>
<td>Spinal</td>
<td>47</td>
<td>12</td>
<td>34</td>
<td>27</td>
<td>0.6</td>
</tr>
<tr>
<td>M</td>
<td>66</td>
<td>Spinal</td>
<td>17</td>
<td>12</td>
<td>44</td>
<td>42</td>
<td>0.2</td>
</tr>
<tr>
<td>F</td>
<td>53</td>
<td>Spinal</td>
<td>26</td>
<td>12</td>
<td>43</td>
<td>40</td>
<td>0.3</td>
</tr>
<tr>
<td>M</td>
<td>62</td>
<td>Spinal</td>
<td>16</td>
<td>12</td>
<td>29</td>
<td>27</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Si et al., J Steroid Bioch, 2020
Lessons Learned

• Coordinated molecular program initiated in skeletal muscle in ALS at early pre-symptomatic stages
• Diversity of novel disease-associated pathways
  • Smads in muscle denervation/reinnervation and miRNA regulation
  • Role of local Vitamin D in denervated skeletal muscle
  • TGF-β: role in muscle fibrosis and inflammation; link to Smads
• Potential markers for tracking disease progression
Example 2  Central neuroinflammation: validation

- HuR is an RNA binding protein in the ELAV family
- Binds to AUUUA sequences and stabilizes mRNA and increases translation
- Translocates to cytoplasm when activated
- Expressed in microglia/macrophages
HuR and Neuroinflammation

RNA Sequencing of microglia

Validation of targets

Matsye et al., Glia, 2017
HuR and ALS

[Image of Western blot analysis showing HuR and Gapdh bands with WT1, MT1, WT2, and MT2 lanes labeled.]

[Image of immunofluorescence staining showing HuR, IBA1, and HuR/IBA1 in MT, WT, ALS1, ALS2, and Control conditions.]
Lessons Learned

• HuR is a major regulator of inflammatory cytokine production through posttranscriptional pathways
• Human tissue validation of ALS mouse findings: HuR is activated and upregulated in microglia.
• HuR may be a therapeutic target for slowing disease progression in ALS
Example 3  Peripheral inflammation in ALS: validation

**Neutrophils**

G93A Rat ALS muscle

- αBT
- Elastase
- Myelin

**Mast cells**

G93A Rat ALS Sciatic nerve

- Tryptase
- DAPI

Trias et al. JCI Insight, 2018
Validation in Human ALS

NMJ/muscle

αBT  Elastase   DAPI  αBT  Elastase Chymase   DAPI

JCI Insight, 2018
CD68+, CSF-1R+ Macrophages in ALS Nerve Roots

Trias et al., 2019
Schwann cells orchestrate peripheral nerve inflammation through the expression of CSF1, IL-34, and SCF in amyotrophic lateral sclerosis

Trias et al., Glia, 2019
Lessons learned

- Peripheral neuromuscular inflammation in Rat ALS model is validated in human ALS tissue.
- Rat model indicates an evolution of peripheral inflammation with disease progression.
- Provide rationale for masitinib.
Conclusions

• Post mortem ALS tissue is essential for discovery of new pathways and validation of pathways discovered in non-human ALS models
• The importance of animal models for assessing temporal evolution of biomarkers
• The importance of normal controls and disease controls
• Discovery in ALS will not move forward without the cooperation and courage of our patients
...We are also grateful to our patients who donated their spinal cord tissues postmortem for ALS research.
Collaborative Teams

Laboratory

BVAMC ALS Clinic

Support

National Institute of Neurological Disorders and Stroke

U.S. Department of Veterans Affairs

The University of Alabama at Birmingham

Knowledge that will change your world