Structure Determination of a Bioengineered Human/Porcine Factor VIII for Hemophilia A Treatment,

and

Improvements to the Human Factor VIII Model

IAN SMITH
SPIEGEL LAB
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This talk will:

• Introduce:
  • The bleeding disorder Hemophilia A
  • The Blood Coagulation Cascade
  • Blood Coagulation Factor VIII (FVIII) protein

• 3-D molecular structure of novel human/porcine chimeric FVIII for Hemophilia A therapy

• Implications for future research
Central Dogma of Biology

DNA synthesis (replication) → DNA

RNA synthesis (transcription) → RNA

Protein synthesis (translation) → PROTEIN

Levels of protein organization:
- Primary protein structure is sequence of a chain of amino acids.
- Secondary protein structure occurs when the sequence of amino acids are linked by hydrogen bonds.
- Tertiary protein structure occurs when certain attractions are present between alpha helices and pleated sheets.
- Quaternary protein structure is a protein consisting of more than one amino acid chain.

Amino Acids → Pleated sheet → Alpha helix

Pleated sheet → Alpha helix

Figure 1-4 Molecular Biology of the Cell, Fifth Edition (© Garland Science 2008)
Blood Coagulation Cascade

FVIII upregulates formation of FXa by 200,000 times.


Hemophilia A and Factor VIII (FVIII)

- X-linked disease that affects 1 in 5000 males worldwide

Hemophilia Degrees of Severity (% FVIII or FIX)

- Normal: 50-150%
- Mild: 5-40%
- Moderate: 1-5%
- Severe: <1%

- Hemophilia A replacement therapy with recombinant FVIII

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Shen et al, Blood, 2008, 111, 1240-1257
• ~30% patients receiving FVIII concentrate replacement therapy acquire an inhibitory alloantibody. 

Bray et al, Blood, 1994, 83 (9), 2428-2435
Immune Tolerance Induction (ITI)

Table 1
Predictors of success following immune tolerance induction (ITI): data from the International Immune Tolerance Registry (IITR) and North American Immune Tolerance Registry (NAITR) [5,11].

<table>
<thead>
<tr>
<th>Variable</th>
<th>Success rate (%)</th>
<th>Cutoff for ‘good risk’</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 20</td>
<td>70–78</td>
<td>&lt; 20 years</td>
</tr>
<tr>
<td>&gt; 20</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Peak titer (BU/mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 20</td>
<td>85–89</td>
<td>&lt; 20 BU/mL</td>
</tr>
<tr>
<td>&gt; 20</td>
<td>44–58</td>
<td></td>
</tr>
<tr>
<td>Pre-ITI titer (BU/mL)</td>
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<td></td>
</tr>
<tr>
<td>&lt; 10</td>
<td>78–79</td>
<td>83</td>
</tr>
<tr>
<td>&gt; 10</td>
<td>41–65</td>
<td>40</td>
</tr>
<tr>
<td>Dose (IU/kg/day)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 200</td>
<td>48–66</td>
<td>72–83</td>
</tr>
<tr>
<td>&gt; 200</td>
<td>86</td>
<td>41</td>
</tr>
</tbody>
</table>

BU, Bethesda unit.
Human / Porcine Chimeric FVIII (HP47)

- hFVIII expresses poorly. [Serum] = ~0.5nM
- Recombinant porcine FVIII displays increased cellular secretion
- Human/Porcine Chimera as high output protein therapeutic
- HP47 demonstrates higher expression and comparable activity to hFVIII
Protein Crystallography

Building the FVIII molecular model

- Assess model agreement to electron density with R factors
  - $R_{\text{work}}$ and $R_{\text{free}}$
- Scale of 0.0 (perfect fit) - 0.6 (random/poor fit)

FVIII amino acids (red) Electron density (blue wire mesh)

T. Splettstoesser
HP47 Crystal Structure

- At 3.2Å, highest resolution FVIII model to date
  - More confidently rebuild sections

HP47 Model Overview

- ~85% of the 1467aa HP47 sequence built into model

Quality of the current model

- \( R_{\text{work}} \): 0.1972
- \( R_{\text{free}} \): 0.2863
- Ramachandran outliers: 10.40%
  - Space Group: \( P2_1 \)
Improvements to the Human Model FVIII

2008 FVIII Structure (PDB: 2R7E)

New human/porcine FVIII structure

Newly refined FVIII Structure
Improvements to the Human Model FVIII

- Enhance basic understanding of FVIII’s function in coagulation
- Develop more effective Hemophilia A therapeutics

New human/porcine FVIII structure

Newly refined FVIII Structure
Summary

- Hemophilia A is a X-linked disease caused by a lack of blood coagulation factor VIII (FVIII) protein.

- Patients who develop an immune response to FVIII rely on large quantities of improved therapeutics.

- Determined crystal structure of novel Human/Porcine Chimeric therapeutic. Improved human FVIII protein model.
Questions?