Mechanisms of Daptomycin Resistance and the Seesaw Effect in Multi-Drug Resistant Enterococci

NAME OF STUDENT

Candidacy Exam

August 25, 2017
• Major nosocomial pathogen
• Endocarditis, bacteremia, UTIs, meningitis
• High intrinsic resistance to antibiotics (aminoglycosides, cephalosporins, beta-lactams)
• High genetic plasticity

http://www.cdc.gov/drugresistance/biggest_threats.html
Daptomycin

- Lipopeptide antibiotic
- Used as a “last resort” for MDR-enterococcal infections (Breakpoint MIC = 4µg/ml)
- Observed clinical resistance in VRE
- Disrupts cell membrane integrity
The LiaFSR system regulates DAP-R in enterococci
\textit{liaXYZ} are effectors of the LiaFSR stress response.
DAP-R leads to redistribution of anionic phospholipids

_E. faecalis_ Diversion

Phospholipid microdomain

DAP

NAO Staining = Visualization of enriched anionic PL microdomains (Cardiolipin)

The Seesaw Effect - *Efs, Efm, MRSA*

- β-lactam Resensitization
  - PBP5
- Daptomycin Resistance
  - LiaFSR
The Seesaw Effect- *Efs, Efm, MRSA*

Exploited in combination therapy with DAP + β-lactam for severe MDR infections

- **β-lactam Resensitization**
- **Daptomycin Resistance**

Mechanism? Fulcrum?
Overall Goal: Characterize LiaX and determine its role in antibiotic resistance

- 533 AA
- Surface exposed
- Mutations present in DAP-R clinical strains
- Evolutionary adaptation of DAP-S clinical strain—Ct truncation of liaX (fs AA 289) sufficient for high level resistance
Overall hypothesis

LiaX is a multifunctional protein that
→ Regulates daptomycin resistance through negative inhibition of liaYZ
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→Activates the liaFSR system in the presence of extracellular stress
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LiaX is a multifunctional protein that
→ Regulates daptomycin resistance through negative inhibition of liaYZ
→ Activates the liaFSR system in the presence of extracellular stress
→ Modulates the seesaw effect through interactions with PBP5
Aim 1: Characterize the localization of LiaX as it pertains to the CE stress response to AMPs
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1. Evaluate LiaX protein levels and localization under DAP stress and upon the development of resistance
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2. Determine the role of LiaX in resistance to AMPs \textit{in vitro} and \textit{in vivo}
Aim 1: Characterize the localization of LiaX as it pertains to the CE stress response to AMPs

1. Evaluate LiaX protein levels and localization under DAP stress and upon the development of resistance
2. Determine the role of LiaX in resistance to AMPs in vitro and in vivo
3. Assess if extracellular LiaX can protect DAP-S strains from antibiotic attack by activating the liaFSR stress response
Aim 1 Preliminary Data

LiaX (with the Ct alone) negatively regulates DAP-R and CM remodeling.
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LiaX (with the Ct alone) negatively regulates DAP-R and CM remodeling.
Extracellular LiaX in DAP-R strains

Scale bar 0.5 μM. Secondary antibody conjugated to 18 nM gold particles.
DAP-R spent media protects DAP-S strain

LiaX binds DAP (Kd = 0.05uM)
Localization hypothesis

LiaX in CW

DAP-S
S613
Localization hypothesis

LiaX in CW

S613

Activation of stress response through liaFSR
High liaXYZ transcription
Localization hypothesis

- LiaX in CW
- Activation of stress response through liaFSR
- High secretion
- High surface exposure
- High liaXYZ transcription
- Oligomerization

S613 -> R712
Localization hypothesis

DAP-S OG

LiaX in CW
Activation of stress response through mutation in *liaX* Change in LiaX protein conformation like a Ct truncation
Localization hypothesis

Activation of stress response through mutation in liaX

Change in LiaX protein conformation like a Ct truncation

OG

OG-liaX^{NT}

Nt of LiaX more surface exposed and secreted

LiaX in CW
AMP resistance hypothesis

DAP-R
ΔLiaR
MIC 8

Susceptible
DAP
LL37
Nisin
HBD 3
Broad spectrum

AMP resistance hypothesis

- DAP-R
- MIC 8
- ΔLiaR

Susceptible DAP
LL37
Nisin
HBD 3
Broad spectrum


Graph showing C. elegans survival over time.
AMP resistance hypothesis

DAP-S  \( \Delta \text{LiaX} \)  \( \Delta \text{LiaX-Ct} \)  Resistant DAP LL37 Nisin HBD3 Broad Spectrum MIC 2
AMP resistance hypothesis

Resistant
DAP
LL37
Nisin
HBD3
Broad Spectrum

DAP-S $\Delta$LiaX $\Delta$LiaX-Ct

OG $\Delta liaX$
OG$\Delta liaX$: pAT392::liaX

C. elegans survival, %

Time, d
DAP attack on a DAP-S strain
DAP insertion
Oligomerization
Damage begins

LiaS

LiaF

Membrane damage

LiaR
Time to cell death < Time to mount a response

Membrane damage happens first and continues

LiaFSR response is temporally delayed in DAP-S strains
Extracellular protection hypothesis
LiaX-DAP complex activates stress response before cell death

DAP-R

R712

LiaX

DAP

LiaS

LiaF

Ca2+

DAP-S strain

DAP-R

LiaR

P

Secondary site

Consensus site
Aim 2: Dissect the role of LiaX in regulating DAP-R through protein interactions
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1. Characterize the liaX interactome in DAP-R and DAP-S strains
2. Study the liaX and liaYZ interaction as mechanism of regulation of DAP-R
Aim 2 Preliminary Data

LiaX regulates DAP-R by inhibiting liaYZ
Aim 2 hypothesis - LiaX and LiaYZ interaction

Full length LiaX in DAP-S strains
Aim 2 hypothesis - LiaX and LiaYZ interaction

Full length LiaX in DAP-S strains

DAP-R strain with Ct truncation of LiaX
Aim 2 hypothesis - LiaX and LiaYZ interaction

Full length LiaX in DAP-S strains

Remodel CM likely through cardiolipin synthase
Aim 3: Elucidate the role of LiaX in mediating the seesaw effect through interaction with PBP5
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1. Study PBP5-liaX colocalization in DAP-S strains and PBP5 mislocalization in DAP-R strains
2. Assess PBP5 protein levels and β-lactam binding to PBPs in DAP-R strains
### Aim 3 Preliminary Data

<table>
<thead>
<tr>
<th>Strain</th>
<th>DAP MIC (ug/ml)</th>
<th>Ceftriaxone MIC</th>
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<tbody>
<tr>
<td>OG</td>
<td>2</td>
<td>32</td>
</tr>
<tr>
<td>OGΔliaX</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>OG-liaX&lt;sup&gt;NT&lt;/sup&gt;</td>
<td>12</td>
<td>6</td>
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<tr>
<td>Complements</td>
<td>4</td>
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</tbody>
</table>

β-lactam Resensitization vs. Daptomycin Resistance

LiaX
LiaX-Pbp5 pull down

Used LiaX or Nt-LiaX as bait and PBP5 as prey
Controls: no bait, GFP used as bait/prey
Pull-down and Bacterial 2hybrid show interaction

Bacterial 2 hybrid system
Tags are on the Ct end of both LiaX and PBP5

LiaX, Nt-LiaX, Pbp5, LiaX+, Pbp5, Nt-LiaX+
Pbp5

- control + control

LiaX-T25 and PBP5-T18
PG synthesis mislocalized

NADA Staining of nascent PG synthesis

A

B

C

S613 Equatorial rings

R712 Aberrant staining and side wall synthesis
Aim 3 hypothesis

LiaX- PBP5 interaction

Nt LiaX
Ct LiaX
PBP5
LiaY LiaZ

Full length LiaX in DAP-S strains
LiaX- PBP5 interaction

Full length LiaX in DAP-S strains

Ct truncation in DAP-R strains

Increased β-lactam access
Model of the LiaFSR and LiaX mediated stress response
Absence of stress ("OFF")

Basal transcription

liaX liaY liaZ
“ON” state via LiaFSR

Membrane damage

LiaS

LiaF

LiaX

LiaY LiaZ

PBP5

B-lactam resensitization

CM Remodeling by recruitment of cardiolipin synthase

High transcription

liaX liaY liaZ
“ON” state $\rightarrow$ via LiaX

B-lactam resensitization

CM Remodeling by recruitment of cardiolipin synthase
This project aims to

1. Dissect the mechanism by which LiaX regulates the CE stress response
2. Identify the mechanism for the LiaX modulation of the see-saw effect in enterococci
3. Study the DAP “resistome” --> expose many new therapeutic targets