Heat Shock Protein 90
Cellular Chaperone and Target for Potentiating Antifungal Agents

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Heat Shock Response and Heat Shock Proteins

- Heat Stress:
  - Formation of actin stress fibers
  - Fragmentation of golgi and ER
  - Fewer functional mitochondria and lysosomes
  - Protein aggregation
  - Loss of membrane integrity
  - Manifests in **cell cycle arrest**
- Cells respond quickly and transiently to emergence of unfolded proteins by turning on expression of heat shock proteins (Hsps).
- Hsps act as molecular chaperones

Image of Ferruccio Ritossa viewing chromosomal puffs in the early 1960s.

Heat Shock Proteins

• Constitutively expressed
• 50-200 genes up-regulated in response to stressors
  • Molecular chaperones
  • Proteolytic system
  • DNA repair
  • Metabolic enzymes
  • Transcription factors or kinases
  • Maintenance of cytoskeleton
  • Membrane stability
• Powered by ATP hydrolysis

“Quis custodiet ipsos custodes?”
“Who will guard the guardians themselves?”

Attributed to the Roman poet and ethicist Juvenal
First century AD

Profile of Up-Regulated Proteins in S. cerevisiae
Cells experienced a temperature shift from 25 to 35°C over the course of 10 minutes.

Heat Shock Protein 90 (Hsp90) are Highly Conserved

- Not found in archaea.
- Found 103 protein encoding genes for HSP90 family members across 32 genomes studied.

Heat Shock Protein 90

- Comprises 1-2% of cellular proteins
- Exists as a homodimer in the cytoplasm
- Acts on client proteins (signal transducers):
  - Growth
  - Cell survival
  - Development
  - Signal transduction
  - Protein trafficking
  - Receptor maturation
  - Adaptive and innate immunity

The Mode of Action of Heat Shock Protein 90

- Act on substrates in an iterative and cyclical nature through ATP hydrolysis
- Unknown conformational change from ATP hydrolysis

Co-Chaperones and Post-Translational Modifications Regulate Hsp90 Function

- Facilitate interactions with other chaperone systems
- Trigger or inhibit ATPase activity
- Recruitment of client proteins
- PTMs:
  - Acetylation
  - Phosphorylation
  - Nitrosylation

Inhibitors of Hsp90 Function

<table>
<thead>
<tr>
<th>Binding site</th>
<th>Chemical class</th>
<th>Selected examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-terminal ATP-binding pocket</td>
<td>Benzoquinone ansamycin</td>
<td>GA, 17AAG, 17DMAG</td>
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<tr>
<td>N-terminal ATP-binding pocket</td>
<td>Macrolide</td>
<td>Radicicol and related oxime derivatives, β-zearalenol</td>
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<td>N-terminal ATP-binding pocket</td>
<td>Purine scaffold</td>
<td>PU24FC1</td>
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<td>N-terminal ATP-binding pocket</td>
<td>Pyrazole</td>
<td>CCT018169</td>
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<td>N-terminal ATP-binding pocket</td>
<td>Hybrid</td>
<td>Radamycin, GA dimer, GA-testosterone, GA-oestrogen</td>
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<td>C-terminus</td>
<td>Noviosylocoumarin crosslinker</td>
<td>Novobiocin, coumermycin, cisplatin</td>
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<tr>
<td>Unknown</td>
<td>Histone deacetylase inhibitor</td>
<td>Dapsipeptide, SAHA</td>
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</tbody>
</table>

The Role of Hsp90 in Cancer

- Over expression of Hsp90:
  - Breast cancer
  - Pancreatic carcinoma
  - Leukemia
- Cytoprotective stress response
- Tolerate mutations that cause imbalanced signaling and avoid apoptosis
- Surface expression enhances cell invasion

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Clients</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td>Uncontrolled proliferation</td>
<td>Receptor tyrosine kinases, serine/threonine kinases, steroid hormone receptors</td>
<td>110,142,143,108</td>
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<td>Immortalization</td>
<td>Telomerase</td>
<td>144</td>
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<tr>
<td>Impaired apoptosis</td>
<td>AKT</td>
<td>49</td>
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<tr>
<td>Angiogenesis</td>
<td>HIF1α</td>
<td>145</td>
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<tr>
<td>Invasion/metastasis</td>
<td>MMP2</td>
<td>63</td>
</tr>
</tbody>
</table>

HIF1α, hypoxia-inducible factor 1α; Hsp90, heat-shock protein of 90 kDa; MMP2, matrix metalloproteinase 2.

Armamentarium of Antifungal Agents: Mechanisms of Action

Azoles:
- Inhibit Erg11 (lanosterol 14α-demethylase)
- Build-up of toxic sterol intermediate
- Cell membrane stress
- Abrogates production of ergosterol

5-Flucytosine:
- Blocks DNA and RNA synthesis

Polyenes:
- Bind and extract ergosterol

Armamentarium of Antifungal Agents: Mechanisms of Action

Echinocandins:
• Block the activity of β-(1,3)-D-glucan synthase
• Compromise cell-wall integrity

Canonical Mechanisms of Antifungal Resistance

- Increased expression of multidrug transporters
  - *Candida* and *Aspergillus*
- Overexpression or mutation of Erg11
  - *Candida* and *Aspergillus*
- Mutation of Erg3
- Mutation of Fks1

Hsp90 Enables the Emergence of Fungal Drug Resistance

- Phosphotase calcineurin is involved in modulating cellular signaling.

Inhibition of Hsp90 Function Mitigates Antifungal Resistance

Hypothesis: If Hsp90 facilitates antifungal resistance, then inhibiting the function of Hsp90 would prevent the progression of drug resistance and improve the activity of current antifungals.
HSP90 Inhibitors Synergize with Fluconazole to Drive Antifungal Activity against *C. albicans*

- Utilized fluconazole (FL) resistant *C. albicans* clinical isolate Caci-2.
- Azoles are fungistatic.
- Co-treatment of Hsp90 inhibitors with fluconazole is fungicidal and clears fungal infection.
HSP90 Inhibitors Synergize with Fluconazole to Drive Antifungal Activity against *C. albicans* in an Invertebrate Model

- Gave lethal injections of fluconazole resistant *C. albicans* in greater wax moth.
- Co-treatment with fluconazole and Hsp90 inhibitors abolished fungal infection.
Co-treatment with Hsp90 Inhibitors Impedes the Emergence of Caspofungin Resistance in *A. fumigatus*

- Utilized caspofungin (CS) resistant *A. fumigatus* clinical isolate.
- Echinocandins are **fungistatic**.
- Co-treatment of Hsp90 inhibitors with caspofungin improved its ability to clear the fungal infection.
Genetic Inhibition of HSP90 Expression Increases the Efficacy of Fluconazole in a Murine Model

- Mouse studies showed the need for fungal specific HSP90 inhibitors.
- Generated a strain of *C. albicans* with a tetracycline repressible promoter (*tetO*).
- WT HSP90 functionally complements *tetO-HSP90/hsp90Δ*
- Further studies showed tetracycline-mediated inhibition of HSP90 expression eradicated fungal infection.
Summary

Results:
• Pharmacological inhibition of Hsp90 improves efficacy of both fluconazole and caspofungin in resistant *C. albicans* and *A. fumigatus* respectively.
• Observed higher survival rates after co-treatment in invertebrate model.
• Genetic inhibition of *HSP90* expression improves effectiveness of fluconazole in a murine model.

Challenges:
• Hsp90 is highly conserved and plays a central role in the cell:
  • Cellular signaling
  • Host stress and immune responses
• During a preliminary mouse study, observed toxicity from Hsp90 inhibition in infected mice.
• Need for fungal selective inhibitors
Thank you! Any questions?