UNIVERSITI PUTRA MALAYSIA

COMPARISON OF CANDIDA HSP90 PROTEIN CONTENT AND GENE EXPRESSION IN IRANIAN AND MALAYSIAN CANDIDA INFECTED PATIENTS

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By
VAJIHOZAMAN KHALILI

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Abstract of thesis presented to the senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Doctor of Philosophy

COMPARISON OF CANDIDA HSP90 PROTEIN CONTENT AND GENE EXPRESSION IN IRANIAN AND MALAYSIAN CANDIDA INFECTED PATIENTS

By

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January 2013

Chairman: Abdah Binti Md Akim, PhD
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Introduction: Hsp90 is one of the most abundant and conserved proteins in eukaryotes such as fungal pathogens. Hsp90 is involved in the stability and biosynthesis of proteins required for making the cell wall and is involved in determining cell wall thickness, especially at high temperature. Its interaction with some specific proteins is necessary in order to increase Candida resistance against lytic enzymes and high temperature for continual viability and adaptation to stress conditions.
General Objective:

To compare the concentration and gene expression of HSP90 in Candida species from Malaysian and Iranian populations in in vitro and in vivo and the role of this protein in the pathogenesis of Candida species.

Specific Objectives

1) To determine the amount of HSP90 and its gene expression in different Candida species isolated from Iranian and Malaysian patients.
2) To isolate HSP90 from different Candida species via chromatography techniques.
3) To evaluate HSP90 gene expression in different Candida species via Real-time PCR.
4) To determine differences in the amount and gene expression levels of HSP90 in Candida spp i) isolated from human patients, ii) isolated from mice kidneys and iii) under the shock conditions (25°C and 42°C).
5) To investigate possible correlations between the HSP90 levels and gene expression with Candida spp virulence during infection.
6) To establish systemic and non-systemic candidiasis in a mouse model and to evaluate the amounts and gene expression levels of HSP90.
Methodology: In this thesis, Hsp90 concentrations and gene expression levels of Candida species in sixteen Malaysian and sixteen Iranian patients were investigated in response to temperature changes. Following purification and measurement of Candida Hsp90, evaluation of HSP90 gene expression levels was performed in vitro and in vivo conditions in Candida species including C. krusie, C. parapsilosis, C. albicans and C. tropicalis. In both systemic and non-systemic infections the gene expression and amount of HSP90 were evaluated at three situations: i) Candida Hsp90 obtained from Malaysian and Iranian patients , ii) Hsp90 isolated from mouse kidneys infected with Candida cells and iii) Candida Hsp90 after treatment with temperatures 25˚C (low temperature) and 42˚C (thermal shock). Hsp90 purification was performed by two kinds of ion exchange and affinity chromatography using DEAE-Cellulose and hydroxyapatite respectively. Real-time PCR was used in order to evaluate the gene expression of Hsp90.

Result: The results showed that Hsp90 concentrations and gene expression levels in isolates obtained from both human patients and kidneys of mouse infected with Candida cells were higher in C. albicans compared to non-albicans Candida in both Malaysian and Iranian populations (p<0.05). A significant increase was observed in the amount and gene expression of Hsp90 isolated from Malaysian patients in comparison with Iranian patients in both systemic and non-systemic infections (p<0.05). In both populations, the highest gene expression and concentration of Hsp90 was observed in Candida cells after at thermal shock (42˚C) treatment, followed by Candida isolated from mice kidneys. An increase
in the amount and gene expression level of Hsp90 was seen in mice body because when *Candida* cells entered mouse’s body, they encounter different stress factors such as heat and a powerful immune system in this case Hsp90 increased as a defensive response to protect *Candida* cells in order to survive and maintain viability leading to cell proliferation and more infection in the body. An increase in gene expression was observed in the Malaysian isolates compared to the Iranian samples at different temperatures, but there was a significant difference only in *Candida* cells isolated from patients (before injection) (p<0.05).

**Conclusion:** Despite the existence of homologies in Hsp90, there are differences at Hsp90 concentration and gene expression in the two populations at different conditions. The amount of Hsp90 and gene expression increase in *Candida* cells entering the host body (mice) in order to battle with the strong immune system and other stress factors for the survival and viability of *Candida* cells leading to more infection in the mice.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PERBANDINGAN KANDUNGAN PROTIN DAN EKSPRESI GEN HSP90 CANDIDA DALAM PESAKIT-PESAKIT IRAN DAN MALAYSIA YANG DIJANGKITI CANDIDA

Oleh

VAJIHOZAMAN KHALILI

Januari 2013

Pengerusi: Abdah Binti Md Akim, PhD

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Pengenalan: Hsp90 adalah salah satu protin yang paling banyak dan terpelihara dalam organisma eukaryote seperti pathogen fungi. Hsp90 terlibat dalam penstabilan dan biosintesis protin yang diperlukan oleh dinding sel serta ketebalannya, terutamanya pada suhu tinggi. Interaksi protin ini dengan beberapa protin spesifik diperlukan untuk meningkatkan ketahanan sel-sel Candida terhadap enzim lisis dan suhu tinggi, untuk kebolehidupan dan adaptasi kepada keadaan tekanan.
Objektif Keseluruhan:

Perbandingan konsentrasi dan ekspresi gen HSP90 dalam spesis Candida daripada populasi Malaysia dan Iran in vitro dan in vivo, serta peranan protin ini dalam patogenesis spesis Candida.

Objektif Spesifik :

1) Untuk menentukan amaun HSP90 dan ekspresi gen dalam pelbagai spesis Candida yang diperolehi daripada pesakit-pesakit Iran dan Malaysia.
2) Untuk mengasingkan HSP90 daripada pelbagai spesis Candida melalui teknik-teknik kromatografi.
3) Untuk menaksir ekspresi gen HSP90 dalam pelbagai spesis Candida melalui “Real-Time PCR”.
4) Untuk menentukan perbezaan amaun dan ekspresi gen HSP90 dalam Candida spp yang: (i) diasingkan daripada pesakit manusia, (ii) diasingkan daripada ginjal tikus, dan (iii) di bawah keadaan terkejut (25ºC and 42ºC).
5) Untuk menyelidik korelasi berkemungkinan di antara paras dan ekspresi gen HSP90 dengan virulens Candida spp dalam jangkitan.
6) Untuk menghasilkan model tikus (i) sistemik dan (ii) bukan-sistemik; serta menaksir amaun dan paras ekspresi gen HSP90.

Keputusan: Keputusan menunjukkan bahawa konsentrasi serta ekspresi gen Hsp90 lebih tinggi dalam isolat-isolat C. albicans yang diperolehi daripada kedua-dua pesakit manusia dan ginjal tikus yang dijangkiti Candida berbanding Candida bukan-albicans dalam kedua-dua populasi Malaysia dan Iran (p<0.05). Amaun dan ekspresi gen Hsp90 yang diasinkan daripada jangkitan sistemik dan bukan-sistemik para pesakit Malaysia lebih tinggi berbanding dengan para pesakit Iran (p<0.05). Akan tetapi, perbezaan ini tidak dilihat dalam isolate-isolat yang diperolehi daripada tikus yang dijangkiti Candida. Dalam kedua-dua populasi, ekspresi gen dan konsentrasi Hsp90 yang
paling tinggi dilihat dalam keadaan kejutan haba (42˚C), diikuti oleh Candida yang diasengkan daripada ginjal tikus. Peningkatan dalam amaun dan ekspresi gen Hsp90 dilihat dalam badan tikus kerana ketika sel-sel Candida memasuki badan tikus, sel-sel ini telah menemui fakto-faktor tekanan yang berlainan, seperti haba dan system imun yang kuat. Dalam kes ini, Hsp90 meningkat sebagai respons defensif untuk melindungi kebolehidupan sel-sel Candida lalu membolehkan proliferasi, mengakibatkan lebih banyak infeksi dalam badan. Peningkatan dalam ekspresi gen telah dilihat dalam isolat-isolat Malaysia berbanding sampel Iran pada suhu berlainan, tetapi terdapat perbezaan signifikan hanya dalam sel Candida yang diasengkan daripada para pesakit sebelum suntikan (p<0.05).

Kesimpulan: Walaupun terdapat homologi tinggi dalam Hsp90, terdapat perbezaan dalam konsentrasa dan ekspresi gen Hsp90 antara dua populasi dari keadaan yang berlainan. Dalam Candida yang memasuki badan hos (tikus), amaun dan ekspresi gen Hsp90 meningkat untuk melawan system imun yang kuat serta factor-faktor tekanan lain, untuk membolehkan kebolehidupan Candida yang mengakibatkan paras infecksi yang lebih tinggi dalam tikus.
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I certify that a Thesis Examination Committee has met on 14 January 2013 to conduct the final examination of Vajihozaman Khalili on her thesis entitled "Comparison of Candida HSP90 Protein Content and Gene Expression in Iranian and Malaysian Candida Infected Patients" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The Committee recommends that the student be awarded the Doctor of Philosophy.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or other institutions.

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VAJIHOZAMAN KHALILI
Date: 14 January 2013
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>iii</td>
</tr>
<tr>
<td>ABSTRAK</td>
<td>vii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>xi</td>
</tr>
<tr>
<td>APPROVAL</td>
<td>xiii</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>xv</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xxi</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xxiv</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>xxxi</td>
</tr>
</tbody>
</table>

## CHAPTER

### 1 INTRODUCTION

1.1 Background                                                           | 1    |
1.2 Hypothesis                                                          | 4    |
1.3 Research questions                                                  | 4    |
1.4 Objectives of this study                                           | 5    |
   1.4.1 General Objective                                              | 5    |
   1.4.2 Specific Objective                                             | 5    |

### 2 LITERATURE REVIEW

2.1 Fungi                                                               | 7    |
2.2 *Candida* species                                                  | 7    |
   2.2.1 *Candida albicans*                                            | 9    |
2.3 Non-*albicans* species                                             | 10   |
   2.3.1 *Candida parapsilosis*                                        | 10   |
   2.3.2 *Candida tropicalis*                                          | 11   |
   2.3.3 *Candida krusei*                                              | 11   |
2.4 *Candida* infections                                               | 12   |
   2.4.1 Virulence factors                                             | 13   |
2.5 Diversity of disease                                               | 13   |
   2.5.1 Mucocutaneous Candidiasis                                     | 14   |
   2.5.2 Systemic Candidiasis                                          | 15   |
2.6 Mortality/Morbidity                                                | 15   |
2.7 Predisposing factors for Candidiasis                               | 16   |
   2.7.1 Immune Deficiency                                             | 16   |
   2.7.2 Broad-Spectrum Antibiotics                                    | 17   |
2.8 Chaperones and Heat Shock Proteins                                 | 17   |
   2.8.1 History related to molecular Chaperones                       | 18   |
2.9 Different kinds of Heat shock protein                              | 19   |
   2.9.1 Protein folding and its importance, both protein              | 19   |

xvi
folding and Heat Shock Proteins are required for protein function

2.10 The essential regions in Hsp90 20
2.11 Multi complexes of Hsp90 and co-chaperones 20
  2.11.1 What are Co-chaperones? 21
2.12 Genetic studies on the Candida HSP90 molecule 21
  2.12.1 The effect of point mutations on the HSP90 gene 21
  2.12.2 The regulation of Hsp90 22
2.13 Properties of Hsp90 22
  2.13.1 Molecular weight 22
  2.13.2 Hsp90 concentration 23
  2.13.3 Candida Hsp90 breakage by cytoplasmic protease enzyme (ycaB) 24
  2.13.4 Hsp90 and its fragments appear as immunodominant antigens 24
  2.13.5 The importance of the 47KD fragment 25
  2.13.6 Toxicity of Candida Hsp90 25
  2.13.7 The role of Hsp90 in the virulence and pathogenesis of Candida albicans 26
2.14 Hsp90 and temperature alterations 27
  2.14.1 Relationship between virulence and Hsp90 at high temperatures 29
  2.14.2 Relationship between Hsp90 and temperature in morphogenetic transitions 30
2.15 Hsp90 in other Candida 32
2.16 The relationship between biological functions in fungi and Hsp90 32
  2.16.1 Comparison of Hsp90 function in some important biological Processes in normal circumstances and under stress conditions Such as high temperature 33
2.17 Mitoen-activated protein- kinase (MAP-K) network 35
2.18 Hsp90 differences between eukaryotes and prokaryotes 36

3 MATERIALS AND METHODS
3.1 Candida species 37
3.2 Categorization of Candida species samples 38
  3.2.1 The source of two clinical isolates and controls 38
    Candida species
  3.2.2 Definition of clinical Candida species Systemic and non-Systemic 40
3.3 Specific identification of Candida species 41
  3.3.1 Culture conditions and storage for Candida species 42
3.4 Evaluating the effects of temperature changes 43
3.5 Disrupting yeast cells in order to get cell extract 44
3.6 Isolation of Candida Hsp90 45
3.6.1 Ion Exchange Chromatography (Anion Exchange Chromatography) 45

3.6.2 Affinity Chromatography 47

3.7 Identification and detection of the presence of Hsp90 by Polyacrylamide Gel Electrophoresis Method

3.7.1 SDS-PAGE Laemmli 49

3.7.2 SDS-PAGE Mini-protean 3 Method 51

3.8 Immunoblotting 52

3.9 Determination of the amount of Hsp90

3.9.1 Bradford Assay 55

3.10 Real-Time Reactions

3.10.1 Specification and determination of the amount of first material 56

3.10.2 Purification of total RNA from Candida cells (RNA Extraction) 57

3.11 Quantification of RNA 60

3.12 The qualification analysis of RNA

3.12.1 Ribosomal RNA band integrity 60

3.12.2 Purity of RNA 61

3.13 Reverse Transcription (cDNA synthesis) 61

3.14 Polymerase Chain Reactions (PCR)

3.14.1 Nucleotide sequences of primers C. albicans HSP90 gene and 18S rRNA 66

3.15 Cycle threshold (CT) 67

3.16 Standard Curve and qPCR amplification efficiency 67

3.17 No Template Control (NTC) 68

3.18 The Melting Curve Analysis (Dissociation Curve) 68

3.19 Data Analysis and ∆∆CT Method 69

3.20 Evaluation change of Candida Hsp90 in vivo 70

4 RESULTS

4.1 Maintenance of Candida Cell Growth and Stock cultures 73

4.2 The presumptive identification of Candida species 74

4.3 The cellular extraction of Candida cells (crude extraction) 74

4.3.1 SDS-PAGE Gel Electrophoresis 75

4.4 Purification of Hsp90 77

4.4.1 Ion Exchange Chromatography (Anion Exchange) 78

4.4.2 Affinity chromatography 83

4.5 Immunoblotting 88

4.6 Determination of Hsp90 Concentration (Bradford Method) 90

4.7 Real Time –Polymerase Chain Reaction

4.7.1 RNA purification 91

4.7.2 cDNA synthesis 92

4.7.3 Polymerase Chain Reactions (PCR) 92

4.8 The Melting Curve (dissociation curve) 92

4.9 The Amplification Efficiency 93
4.10 No Template Control (NTC)
4.11 Control Samples
  4.11.1 Investigation amount of Hsp90 in Crude extract of *Candida spp* obtained from Malaysian and Iranian controls
  4.11.2 Investigation amount of Hsp90 in Anion exchange extract of *Candida spp* Obtained from Malaysian and Iranian Controls
  4.11.3 Comparison and investigation amount of Hsp90 in extract obtained from affinity Chromatography in *Candida spp* isolated from Malaysian and Iranian controls
4.12 Investigation of Hsp90 concentration in clinical Candida species obtained from Malaysian and Iranian patients
  4.12.1 Investigation amount of Hsp90 in Crude extract of isolate *Candida spp* obtained from Malaysian and Iranian patients
  4.12.2 Investigation amount of Hsp90 in Anion exchange extract of *Candida spp* obtained from Malaysian and Iranian Patients
  4.12.3 Investigation protein of Hsp90 in affinity of isolate *Candida spp* obtained from Malaysian and Iranian Patients
4.13 Evaluation and comparison of Hsp90 concentration in clinical isolates obtained from Malaysian and Iranian patients following changes in temperature
  4.13.1 Evaluation of Hsp90 concentration in clinical isolates (*Candida spp*) obtained from Malaysian and Iranian patients at 25°C
  4.13.2 Investigation of Hsp90 concentration in clinical isolates *Candida spp* obtained from Malaysian and Iranian patients under treatment high temperature (42°C)
4.14 Evaluation of gene expression of HSP90 in *Candida spp* obtained from Malaysia and Iranian patients before and after changes in temperature
  4.14.1 Evaluation the gene expression of HSP90 in *Candida spp* obtained from Malaysian and Iranian patients before treatment with temperature
  4.14.2 Investigation of gene expression of Hsp90 in *Candida spp* obtained from Malaysian and Iranian Patients at 25 °C.
  4.14.3 Investigation of gene expression of Hsp90 in *Candida spp* obtained from Malaysian and Iranian Patients at 42 °C.
4.15 Animal Model
4.15.1 Investigation of colony produced in different mice tissues by Candida spp obtained Malaysian and Iranian Patients into mice body

4.15.2 Evaluation of Hsp90 concentration in Candida spp obtained from mice Kidney infected by Candida isolated from Malaysian and Iranian patients

4.15.3 Evaluation and comparison of HSP90 gene expression among Candida spp in cells obtained from mice kidneys infected by Candida cells isolated from Malaysian and Iranian patients

4.16 Comparison and evaluation of Hsp90 concentration in Affinity chromatography extract clinical isolates Candida spp obtained from Malaysian and Iranian patients (37°Cb), Mice kidneys (37°Ca), and shock conditions (42°C and 25°C)

4.17 Comparison and evaluation the gene expression of HSP90 in clinical isolates Candida spp obtained from Malaysian and Iranian patients (37°C b), Mice kidneys (37°C a), and shock conditions (42°Cand 25°C)

5 DISCUSSION
5.1 Candida Hsp90 purification
5.2 The effect of temperature changes on Hsp90
5.3 The effect of temperature changes on gene expression of HSP90 in Candida cells
5.4 HSP 90 in Candida Species
5.5 Stress and HSP 90
5.6 Partial Degradation of Hsp90
5.7 The optimum temperature for induction of Heat shock proteins
5.8 Hsp90 and Virulence
5.9 The Evaluation Hsp90 concentration and its gene expression in vivoconditions (Animal Model)
5.10 Evaluation and comparison Hsp90 concentration and its gene expression between Candida species with regard to pathogenicity of Hsp90

6 CONCLUSION

FUTURE RECOMMENDATIONS

BIBILOGRAPHY

APPENDICES

BIODATA OF STUDENT