UNIVERSITI PUTRA MALAYSIA

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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

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January 2013

Chairman: **Abdah Binti Md Akim, PhD**
Faculty: **Medicine and Health Sciences**

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 (25ºC and 42ºC).
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.

Metodologi: Tesis ini telah menyiasat perubahan suhu tehadap Hsp90 dari segi perbandingan konsentrasi dan paras ekspresi gen Hsp90 yang diperolehi daripada spesis Candida masing-masing dalam enam belas pesakit Malaysia dan Iran. Selepas penulenan dan pengukuran Hsp90 Candida, penaksiran paras ekspresi gen HSP90 telah dilakukan secara in vitro dan in vivo dalam Candida spp, termasuk C. krusei, C. parapsilosis, C. albicans and C. tropicalisis. Dalam kedua-dua infeksi sistemik dan bukan-sistemik, ekspresi gen HSP90 serta amaunnya telah ditaksir dalam tiga keadaan: i) Hsp90 Candida yang diperolehi daripada pesakit-pesakit Malaysia dan Iran, ii) Hsp90 yang diasingkan daripada ginjal tikus yang dijangkiti sel-sel Candida, dan iii) Hsp90 Candida selepas rawatan suhu rendah (25˚C) dan kejutan haba (42˚C). Penulenan Hsp90 telah dilakukan menggunakan dua jenis kromatografi penukaran ion dan afiniti menggnakan DEAE-selulosa serta hidroksiapatit. “Real-time PCR” telah digunakan untuk menaksir 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 diasingkan 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 diasingkan 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 diasingkan daripada para pesakit sebelum suntikan ($p<0.05$).

Kesimpulan: Walaupun terdapat homologi tinggi dalam Hsp90, terdapat perbezaan dalam konsentrasi 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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This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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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.

VAJIHOZAMAN KHALILI

Date: 14 January 2013

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