

**THE EFFECTS OF ENDOGENOUS HEAT
SHOCK PROTEIN HSP70 ON
MACROPHAGES ACTIVITIES**

AHMAD ISMAIL KHALED ABDO

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**THE EFFECTS OF ENDOGENOUS HEAT SHOCK PROTEIN HSP70 ON
MACROPHAGES ACTIVITIES**

Ahmad Ismail Khaled Abdo

March 2017

Main Supervisor: Professor Dr. Mohd Effendy Abd.Wahid, Ph.D.

Co- Supervisor: Associate Professor Dr. Yeong Yik Sung, Ph.D.

School: Institute of Marine Biotechnology

Macrophages are key players in the immune system. They play major roles in pathogen clearance, antigen presentation and wound healing, with primary function in phagocytosis and cytokines secretion. Upon infection when phagocytosis and cytokines production are manifold, heat shock protein 70 (HSP70), a molecular chaperone are upregulated robustly. The role of endogenous HSP70 in regards with macrophages activities was explored herein. For analyzing the protein expression pattern, macrophages were challenged with different zymosan concentrations at 1, 3 and 6 hours. HSP70 was knocked out using RNAi technique for examining its effects on macrophage functions, specifically, phagocytosis and nitric oxide secretion, which were measured by fluorescent microscopy and spectrophotometric method respectively. HSP70 inhibition affected microtubules that were detected using immunostaining. HSP70 was increased in response to exposure time but not

zymosan concentration. On other aspect, knockdown of HSP70 with a siRNA inhibited nitric oxide production by 28% after 18 hr of zymosan activation when compared to control, perhaps to the inhibition of inducible nitric oxide synthase (iNOS) by disruption of nuclear factor kappa-B (NF-kB) pathway. Phagocytosis was also affected by HSP70 knockdown, the number of engulfed particles was greatly reduced and macrophages were unable to phagocytose large particles. However, siRNA transfected cells formed pseudopods during zymosan challenge, but the internalization of particles was impaired. Microscopic examination revealed that siRNA transfected cells had similar morphology with the control, but at the same time possessed slower growth. HSP70 was reported to bind and regulate microtubules, the latter are crucial for mitosis and phagocytosis. Further investigations revealed that microtubules were remarkably elevated in the absence of HSP70 in both the resting and the activation state. These results hypothesized the importance of HSP70 for nitric oxide production by affecting NF-kB pathway, and phagocytosis through microtubules regulation.

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Kesan Protein Renjatan Haba HSP70 Dalam Ke atas Aktiviti Makrofaj

Ahmad Ismail Khaled Abdo

March 2017

penyelia Utama : Professor . Dr. Mohd Effendy Abd.Wahid, Ph.D.

penyelia Bersama: Profesor Madya Dr. Yeong Yik Sung, Ph.D.

Sekolah: Institut Bioteknologi Marin

Makrofaj merupakan pemain penting dalam sistem imuniti tubuh. Mereka memainkan peranan yang besar dalam penyingkiran patogen, penunjukan antigen dan penyembuhan luka, dengan fungsi utama dalam perembesan sitokin. Sewaktu jangkitan di mana fagositosis dan penghasilan sitokin digandakan, protein renjatan haba 70 (HSP70), yang merupakan protein pengiring diupregulasi secara kukuh. Peranan HSP70 dalaman yang berkaitan dengan aktiviti makrofaj diterokai dalam waktu tersebut. Untuk penganalisaan corak ekspresi protein, makrofaj telah dicabar dengan kepekatan zymogen pada masa yang berbeza, 1, 3 dan 6 jam. HSP70 disingkirkan dengan menggunakan teknik RNAi untuk melihat kesannya terhadap fungsi makrofaj, khususnya fagositosis dan rembesan nitrik oksida, yang diukur melalui mikroskopi fluoresen dan juga kaedah spektrofotometrik. Perencatan HSP70- memberi kesan terhadap mikrotubul yang dapat dikesan menggunakan pewarnaan-

immuno. HSP70 juga meningkat dalam tindakbalas terhadap masa dedahan tetapi bukannya terhadap kepekatan zymosan. Pada aspek lain, penyingkiran HSP70 melalui siRNA merencat penghasilan nitrik oksida sebanyak 28% selepas 18 jam pengaktifan zymosan apabila dibandingkan dengan kawalan, mungkin terhadap perencatan yang diaruhkan oleh nitrik oksida sintase (iNOS) melalui gangguan terhadap faktor nuklear laluan kappa-B (NF-kB). Fagositosis juga terkesan dengan penyingkiran HSP70, bilangan partikel yang ditelan juga berkurangan dan makrofaj tidak dapat memfagosit partikel besar. Walau bagaimana pun, sel-sel transfeksi siRNA membentuk poda-palsu sewaktu dicabar dengan zymosan, tetapi internalisasi partikel yang telah terjejas. Pemeriksaan mikroskopik menunjukkan yang sel transfeksi siRNA mempunyai morfologi yang sama dengan kawalan, tetapi dalam masa yang sama mempunyai pembesaran yang lebih perlahan. HSP70 dilaporkan bercantum dan mengawalatur mikrotubul, yang penting untuk mitosis dan fagositosis. Pemeriksaan seterusnya mendapati bahawa mikrotubul meningkat secara luar biasa sewaktu ketiadaan HSP70 dalam keadaan rehat dan aktif. Keputusan ini memberi hipotesis tentang kepentingan HSP70 untuk penghasilan nitrik oksida dengan memberi kesan kepada laluan NF-kB, dan fagositosis melalui regulasi mikrotubul.