Hsp72
translocation and secretion
in in vivo and in vitro models

Thesis submitted in accordance with the requirements
of the University of Liverpool
for the degree of Doctor of Philosophy by

Francesca Leoni

March 2009
University of Liverpool
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Part 2 of 2
Declaration

The work presented in this thesis is original and has not been submitted previously in support of any qualification or course.

Signed

Date
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Abstract

Evidence suggesting that Hsp72 is actively participating in cellular signalling as well interacting with immune system dynamics has been increasing. This is true in healthy, stressed and diseased cells but to different degrees. Modulation of the plasma membrane association and secretion in the extracellular environment by different types of stressors is the key event that leads to different degrees of immune system activation. Hence a better understanding of the mechanisms of Hsp72 secretion and association with plasma membrane is crucial.

This thesis investigated the tissue source and mechanism of Hsp72 surface presentation to plasma membrane structures and release in relation with different cellular and physiological stressors. *In vivo* models confirmed that different tissue types determine specific Hsp72 responses following the same stress and increase serum Hsp72 dependant on intensity and duration of the stress. Diseases models confirm that Hsp72 responses in specific cell populations is related to disease progression, while *in vitro* models clearly showed that there are multiple mechanisms of secretion and surface presentation, dependent on the nature of the stressor as well as the intensity and duration.

This observations clearly change the view of extracellular Hsp72 as a danger signal and lead to a revision of the original danger model. It also suggests that manipulation of Hsp72 translocation through the different pathways involved may prove effective therapeutically.
Publications


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Abbreviation List

ABC = ATP Binding Cassette transporter
AIF = Apoptosis Inducing Factor
ALP= Alkyl lysophospholipids
AMI =Acute Myeloid Leukaemia
APC = Antigen Presenting Cell
BA = Benzyl Alcohol
BF = Biceps Femoris
CK = Creatinine Kinase
Cer = Ceramide
Cyt C = Cytochrome C
DC = Dendritic Cells
DIDS = Diisothiocyanatostilbene-2, 2′-disulfonate
DoCer = Dodecasaccharide Ceramide
DRM = Detergent Resistant Microdomain
E = Epinephrine
ER = Endoplasmic Reticulum
GalCer = Galactosyl Ceramide
Gb3 = Globotraosyl Ceramide
GFR = Growth Factor Receptor
GM-1 = Ganglioside M-1
GPRC = G protein coupled receptor
GM-CSF
HSF-1 = Heat Shock Factor-1
HSE = Heat shock element
HSP = Heat Shock Protein
Hsp70 = Heat Shock Protein 70
Hsp72 = Heat Shock Protein 72
Hsp90 = Heat Shock Protein 90
IFN-γ = Interferon –γ
KNK437 = N-formyl-3, 4-methylenedioxy-benzylidene-y-butyro lactam
IL-1β = Interleukin-1β
IL-6 = Interleukin-6
IL-10 = Interleukin-10
IL-12 = Interleukin-12
IM54 = 2- (1H-indol-3-yl-)-3-penthylamino-malemide
LacCer = Lactosyl Ceramide
LD = Longissimus Dorsi
LPS = Lipopolysaccharide
MDR = Multi Drug Resistance
MEF = Murine Embryonic Fibroblast
MFI = Mean of Fluorescence Intensity
MHC = Major Hystocompatibility Complex
MVB = Multi Vesicular Bodies
MRP = Multidrug Resistance Protein
NK = Natural Killer Cells
PBL = Peripheral Blood Lymphocytes
PBMC = Peripheral Blood Mononuclear Cells
PDT = Photo Dynamic Therapy
PgP = P-Glycoprotein
PhA = Phenetyl Alcohol
PS = Phosphatidylserine
RMFI = Relative mean of fluorescence intensity
ROS = Reactive Oxygen Species
SC = Scavenger Receptors
SLE = Systemic Lupus Eritematosus
TLR = Toll Like Receptor
TNF-α = Tumour Necrosis Factor -α
UPS = Ubiquitin Proteosome System