Localisation of Heat Shock Proteins in Haematological Malignancies

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

Nina Claire Dempsey

August 2009
Declaration

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

Signed:

Date:
Acknowledgments

I would like to thank Prof. John Williams for giving me the opportunity to carry out this work and for his excellent supervision. I would also like to thank Dr Christine Hoyle, not only for providing the clinical samples but for her supervision throughout my PhD. Thank you to Elyse Ireland and Rob Coleman for their invaluable technical support and thank you to everyone in the research lab; Ola Altaie, Francesca Leoni, Helen Williams, Ian Hurley and Neil Pickles for making my PhD an enjoyable experience! A special thanks goes to my Mum and Dad, and Ian for their enormous support at home.
Abstract

Although a number of HSPs have been shown to be up-regulated in a wide range of human cancers, the full significance of this remains to be determined. The localisation of HSPs seems to be critical in determining their role in cancer cell survival; High intracellular levels (iHsp) appear to be advantageous to the tumour cell, inhibiting key steps in apoptosis, while in some circumstances, surface expression (sHsp) appears to be detrimental to the cell, aiding immune recognition by various effector cells. Consequently, clarifying the importance of HSP cellular location in the cancer setting may lead to the development of novel therapies based upon manipulation of HSP localisation. This thesis had two major aims; (1) to investigate the cellular localisation of HSPs in leukocytes from patients with both myelocytic and lymphocytic malignancies in order to establish relationships between apoptosis and stage of disease (2) to study the synergistic effect of four chemotherapeutic drugs with membrane fluidising agents, compounds which have the potential to modulate HSP localisation.

Hsp90 and Hsp27 expression was shown to be restricted to the inside of peripheral blood leukocytes, while Hsp72 was localised both intracellularly and on the cell surface. In CLL, iHsp90 and iHsp27 levels were found to be significantly higher than in control subjects, while surface and intracellular Hsp72 was shown to be expressed either at very high levels or at very low levels. Furthermore, iHsp90 levels were found to be associated with stage of disease, while iHsp27 levels were shown to negatively correlate with levels of apoptosis. CLL patients with stable disease were found to express higher levels of iHsp72 than patients with progressive disease.

However, in AML and MDS, levels of all HSPs in peripheral blood were found to be similar to those seen in control subjects, but disease patients showed a much wider range of expression. In AML, levels of sHsp72 positively correlated in all cell types, an observation not made in MDS patients or control subjects. HSP localisation was shown to be affected by membrane fluidising agents, with a movement of Hsp72 and Hsp60 to the cell surface. This effect was not due to proteotoxicity and supports data implicating the cell membrane in the regulation of HSP responses. This manipulation of HSP localisation and the increase in membrane fluidity resulted in increased sensitivity of CLL cells to three chemotherapeutic agents and points to the possibility that manipulation of membrane fluidity, may have significant value in the development of new treatment regimes.
Table of Contents

Chapter 1. Introduction

1.1 Cancer
1.1.1 Essential Steps in Cancer Development 1
1.1.2 The Immune System and Cancer 3
1.1.3 Cancer Treatment 6

1.2 Cell Death
1.2.1 Apoptosis 12
1.2.2 Necrosis 14
1.2.3 Autophagy 14
1.2.4 Anoikis 14

1.3 Heat Shock Proteins
1.3.1 HSP Nomenclature 17
1.3.2 HSP Regulation 18
1.3.3 Hsp27 19
1.3.4 Hsp60 21
1.3.5 Hsp72 22
1.3.6 Hsp90 25

1.4 Mechanisms of HSP Release
1.4.1 Classical Release Pathway 26
1.4.2 Lipid-Raft Associated Release 27
1.4.3 Exosome Associated Release 28
1.4.4 Lysosome Pathway 28
1.4.5 Secretory-like Granules 29
1.4.6 ABC Transporters 29

1.5 Haematological Malignancies 30
1.6 Aims of the Thesis 31

Chapter 2. Materials & Methods

2.1 Materials
2.1.1 Consumables 33
2.1.2 Blood Processing 34
2.1.3 Cell Culture 34
2.1.4 Cell Viability Assays 34
2.1.5 Membrane Fluidising Agents 35
2.1.6 Chemotherapeutic Agents 35
2.1.7 Flow Cytometry 36
2.1.8 Western Blotting 38
2.1.9 Hsp72 ELISA 41
2.1.10 Fluorescence Microscopy 43
2.1.11 Real-Time PCR 43
2.1.12 Equipment 44

### 2.2 Methods

2.2.1 Blood Collection and Processing 45
  - 2.2.1.1 Total Leukocyte Isolation 45
  - 2.2.1.2 PBMC Purification 45

2.2.2 Cell Culture 46

2.2.3 Flow Cytometry 46
  - 2.2.3.1 Identification of Leukocyte Populations 46
  - 2.2.3.2 Caspase-3 Analysis 47
  - 2.2.3.3 Annexin V/Propidium Iodide 47
  - 2.2.3.4 Surface Hsp72, Hsp27 and Hsp90 Analysis 47
  - 2.2.3.5 Intracellular Hsp72, Hsp27 and Hsp90 Analysis 48
  - 2.2.3.6 ZAP-70 Analysis 49
  - 2.2.3.7 T Regulatory Cell Detection 49
  - 2.2.3.8 DR4/DR5 Analysis 50

2.2.4 Western Blot Analysis 50
  - 2.2.4.1 Protein Assay 50
  - 2.2.4.2 Gel Electrophoresis & Western Blotting 50
  - 2.2.4.3 Heat Shock Protein Detection 51

2.2.5 Hsp72 ELISA 51
  - 2.2.5.1 Hsp72 ELISA of serum 51
  - 2.2.5.2 Hsp72 ELISA of Jurkat Cell Extracts 52
Chapter 3. Heat Shock Protein Localisation in Chronic Lymphocytic Leukaemia

3.1 Introduction

3.1.1 Chronic Lymphocytic Leukaemia 59
3.1.2 Prognostic Markers in CLL 60
3.1.3 Treatment of CLL 61
3.1.4 CLL and Autoimmune Haemolytic Anaemia 63
3.1.5 Immunodeficiency in CLL 63
3.1.6 Heat Shock Protein Expression in CLL 64
3.1.7 Aims 65

3.2 Methods

3.2.1 Total Leukocyte Isolation 66
3.2.2 PBMC Purification 66
3.2.3 Caspase-3 Analysis 66
3.2.4 Surface HSP Analysis 66
3.2.5 Intracellular HSP Analysis 66
3.2.6 ZAP-70 Analysis 67
3.2.7 T Regulatory Cell Analysis 67
3.2.8 Western Blot Analysis 67
3.2.9 Hsp72 ELISA 67
Chapter 4. Heat Shock Protein Localisation in Myeloid Malignancies

4.1 Introduction  
4.1.1 Acute Myeloid Leukaemia  
4.1.2 Prognostic Markers in AML  
4.1.3 Treatment of AML  
4.1.4 Myelodysplastic Syndrome  
4.1.5 Prognostic Markers in MDS  
4.1.6 Treatment of MDS  
4.1.7 HSP Expression in AML and MDS  
4.1.8 Aims  

4.2 Methods  
4.2.1 Total Leukocyte Isolation  
4.2.2 Caspase-3 Analysis  
4.2.3 Surface HSP Analysis  
4.2.4 Intracellular HSP Analysis  
4.2.5 Hsp72 ELISA  
4.2.6 Statistical Analysis  

4.3 Results  
4.3.1 Active Caspase-3 Activity in AML and MDS  
4.3.2 Hsp72 Localisation in AML and MDS  
4.3.3 Release of Hsp72 into Serum
4.3.4 Hsp90 and Hsp27 Localisation in AML and MDS

4.4 Discussion
4.4.1 Active Caspase-3 Levels in AML and MDS
4.4.2 Surface Hsp72, Hsp90 and Hsp27 in AML and MDS
4.4.3 Intracellular Hsp72, Hsp90 and Hsp27 in AML and MDS
4.4.4 Extracellular Hsp72 in AML and MDS

4.5 Summary

Chapter 5. Synergistic Action of Chemotherapeutic Drugs and Membrane Fluidisers

5.1 Introduction
5.1.1 Cell Membrane Fluidity
5.1.2 Combination Therapy
5.1.3 Aims

5.2 Methods
5.2.1 Cell Culture
5.2.2 Drug Treatment
5.2.3 Caspase-3 Plate Based Assay
5.2.4 Propidium Iodide Assay
5.2.5 Annexin V/Propidium Iodide Assay
5.2.6 Flow Cytometry
5.2.6.1 Surface and Intracellular HSP Analysis
5.2.6.2 DR4/DR5 Analysis
5.2.7 Fluorescence Microscopy
5.2.7.1 DR4/DR5 Staining
5.2.7.2 Hoescht Staining
5.2.8 Statistical Analysis

5.3 Results
5.3.1 Determination of Sub-Lethal Doses of Membrane Fluidising Treatments
5.3.2 Determination of Sub-Lethal Doses of Chemotherapeutic Agents
5.3.3 Combination Treatment of Jurkat Cells 152
5.3.4 Combination Treatment of Primary CLL Cells 161
5.3.5 Localisation of HSPs Following Membrane Fluidising Treatments 165
5.3.6 Manipulation of HSP Localisation Prior to Combination Treatments 168

5.4 Discussion 171
5.5 Summary 174

Chapter 6. Membrane Regulation of the HSP Response

6.1 Introduction 6.1.1 Membrane Fluidisation and HSP Response 175
6.1.2 Aims 176

6.2 Methods 6.2.1 Cell Culture 177
6.2.2 Drug Treatment 177
6.2.3 Surface and Intracellular HSP Analysis by Flow Cytometry 177
6.2.4 Hsp72 ELISA 177
6.2.5 RT-PCR 177
6.2.6 Statistical Analysis 178

6.3 Results 6.3.1 Time Course Analysis of HSP Localisation in Response to Fluidisers 179
6.3.2 Hsp72 ELISA of Fluidiser-Treated Cell Extracts and Supernatant 185
6.3.3 Real-Time PCR Analysis of Fluidiser Treated Cells 186

6.4 Discussion 191
6.5 Summary 193
Chapter 7. Discussion and Conclusion

7.1 HSP Localisation in Haematological Malignancies 194
   7.1.1 HSPs and Apoptosis in Haematological Malignancies 195
   7.1.2 Hsp27 and Hsp90 in Haematological Malignancies 196
   7.1.3 Localisation of Hsp72 in Haematological Malignancies 197
   7.1.4 Extracellular Hsp72 in Haematological Malignancies 199

7.2 Synergistic Action of Chemotherapeutic Agents and Membrane Fluidisers: Dependence upon HSP Localisation? 200

7.3 Implications of the Results 203
7.4 Future Work 204
7.5 Conclusion 205

Chapter 8: References 207
List of Figures

Chapter 1. Introduction

Figure 1.1.1 Immune Responses to cancer 4
Figure 1.1.2 Regulation of Apoptosis by HSPs 16

Chapter 3. Heat Shock Protein localisation in Chronic Lymphocytic Leukaemia

Figure 3.3.1 Active Caspase-3 expression in CLL patients and control subjects 69
Figure 3.3.2 Active Caspase-3 expression in CLL patients grouped according to Binet stage 69
Figure 3.3.3 Comparison of surface Hsp72 localisation analysed using the cmHsp70.1 and the Stressgen antibody 71
Figure 3.3.4 Identification of malignant CLL cells 75
Figure 3.3.5 Surface and Intracellular Hsp72 expression on the malignant cells from CLL patients 75
Figure 3.3.6 Surface and Intracellular Hsp72 localisation in representative CLL patients 76
Figure 3.3.7 Comparison of sHsp72 in high- and low-expressing patients 77
Figure 3.3.8 Comparison of iHsp72 in high- and low-expressing patients 78
Figure 3.3.9 Western Blot of intracellular Hsp72 expression in high- and low-expressing CLL patients 78
Figure 3.3.10 Surface and Intracellular Hsp72 in CLL patients grouped according to Binet stage 79
Figure 3.3.11 Comparison of intracellular Hsp72 expression in CLL patients with stable and progressive disease 80
Figure 3.3.12 Extracellular Hsp72 in serum from CLL and control subjects 82
Figure 3.3.13  Comparison of Extracellular Hsp72 in CLL patients treated with or not treated with corticosteroids
Figure 3.3.14  Effect of corticosteroid treatment on extracellular Hsp72 levels in CLL patients.
Figure 3.3.15  Intracellular Hsp90 expression in Lymphocytes from CLL patients and control subjects
Figure 3.3.16  Intracellular Hsp90 expression in Lymphocytes from CLL patients grouped according to Binet stage
Figure 3.3.17  Intracellular Hsp27 expression in Lymphocytes from CLL patients and control subjects
Figure 3.3.18  Intracellular Hsp27 expression in Lymphocytes from CLL patients grouped according to Binet stage
Figure 3.3.19  Correlation analysis between intracellular Hsp27 and levels of active caspase-3 in total lymphocytes
Figure 3.3.20  Expression of ZAP-70 in CD5+/CD19+ cells
Figure 3.3.21  Intracellular Hsp90 in ZAP-70+ and ZAP-70- CLL patients
Figure 3.3.22  Numbers of T-regulatory cells in PBMCs from CLL and control subjects.

Chapter 4. Heat Shock Protein Localisation in Myeloid Malignancies

Figure 4.1.1  Schematic representation of Haematopoiesis
Figure 4.3.1  Gating of the neutrophil, monocyte, lymphocyte and stem cell precursor cell populations in control subjects, AML patients and MDS patients.
Figure 4.3.2  Active Caspase-3 in leukocytes from control subjects and patients in complete remission
Figure 4.3.3  Active Caspase-3 in leukocytes from AML patients, MDS patients and control subjects
Figure 4.3.4  Hsp72 localisation in leukocytes from control subjects and patients in complete remission
Figure 4.3.5  Surface localisation of Hsp72 in leukocytes from AML patients, MDS patients and control subjects 118
Figure 4.3.6  Intracellular localisation of Hsp72 in leukocytes from AML patients, MDS patients and control subjects 119
Figure 4.3.7  Correlation analysis of surface Hsp72 on different cell types from AML patients. 120
Figure 4.3.8  Extracellular Hsp72 in serum from AML patients, MDS patients, control subjects and patients in complete remission 122
Figure 4.3.9  Hsp90 and Hsp27 localisation in leukocytes from control subjects and patients in complete remission 124
Figure 4.3.10  Intracellular Localisation of Hsp90 in leukocytes from AML patients, MDS patients and control subjects 125
Figure 4.3.11  Intracellular localisation of Hsp27 in Leukocytes from AML patients, MDS patients and control subjects 126

**Chapter 5. Synergistic Action of Chemotherapeutic Drugs and Membrane Fluidisers**

Figure 5.3.1  Analysis of apoptosis and necrosis in BA-treated Jurkat cells. 142
Figure 5.3.2  Analysis of apoptosis and necrosis in Ethanol-treated Jurkat cells 142
Figure 5.3.3  Analysis of apoptosis and necrosis in PhA-treated Jurkat cells 143
Figure 5.3.4  Analysis of apoptosis and necrosis in Bupivacaine-treated Jurkat cells 143
Figure 5.3.5  Analysis of apoptosis and necrosis in heat-treated Jurkat cells 144
Figure 5.3.6  Cell viability analysis in fluidiser-treated Jurkat cells 145
Figure 5.3.7  Analysis of apoptosis and necrosis in Doxorubicin-treated Jurkat cells 147
Figure 5.3.8 Analysis of apoptosis and necrosis in Cyclophosphamide-treated Jurkat cells.

Figure 5.3.9 Analysis of apoptosis and necrosis in Lovastatin-treated Jurkat cells.

Figure 5.3.10 Expression of DR4 and DR5 on Jurkat cells.

Figure 5.3.11 Analysis of apoptosis and necrosis in TRAIL-treated Jurkat cells.

Figure 5.3.12 Cell viability analysis in drug-treated Jurkat cells.

Figure 5.3.13 Apoptosis in Jurkats treated with TRAIL combination therapy.

Figure 5.3.14 Apoptosis in Jurkats treated with Doxorubicin combination therapy.

Figure 5.3.15 Apoptosis in Jurkats treated with Cyclophosphamide combination therapy.

Figure 5.3.16 Apoptosis in Jurkats treated with Lovastatin combination therapy.

Figure 5.3.17 Cell viability analysis in Jurkat cells treated with Doxorubicin combination therapy.

Figure 5.3.18 Cell viability analysis in Jurkat cells treated with TRAIL combination therapy.

Figure 5.3.19 Cell viability analysis in Jurkat cells treated with Cyclophosphamide combination therapy.

Figure 5.3.20 Staining of nuclear material in Jurkat cells treated with Doxorubicin combination therapy.

Figure 5.3.21 Apoptosis in Primary CLL cells treated with TRAIL combination therapy.

Figure 5.3.22 Apoptosis in Primary CLL cells treated with Doxorubicin combination therapy.

Figure 5.3.23 Apoptosis in Primary CLL cells treated with Cyclophosphamide combination therapy.

Figure 5.3.24 Intracellular HSP analysis in fluidiser-treated Jurkat cells.

Figure 5.3.25 Surface HSP analysis in fluidiser-treated Jurkat cells.
Figure 5.3.26  Effect of heat shock pre-treatment on the cytotoxic effect of Doxorubicin combination treatment  
169

Figure 5.3.27  Effect of heat shock pre-treatment or heat shock combination treatment on Doxorubicin-induced apoptosis  
169

Figure 5.3.28  Effect of methyl-β-cyclodextrin pre-treatment on the synergistic action of Doxorubicin and Ethanol  
170

Figure 5.3.29  Effect of methyl-β-cyclodextrin pre-treatment on intracellular Hsp72 levels in Ethanol-treated Jurkat cells  
170

Chapter 6. Membrane Regulation of the HSP Response

Figure 6.1.1  Induction of an HSP response via alteration in membrane fluidity  
176

Figure 6.3.1  Surface and Intracellular Hsp72 analysis in fluidier-treated Jurkat cells  
181

Figure 6.3.2  Surface and Intracellular Hsp60 analysis in fluidier-treated Jurkat cells  
182

Figure 6.3.3  Surface and Intracellular Hsp90 analysis in fluidier-treated Jurkat cells  
183

Figure 6.3.4  Surface and Intracellular Hsp27 analysis in fluidier-treated Jurkat cells  
184

Figure 6.3.5  Effect of fluidiser treatments on Hsp72 levels in Jurkat cell extracts  
187

Figure 6.3.6  Effect of alcohols on Hsp72 detection by ELISA  
188

Figure 6.3.7  Effect of fluidisers on Hsp72 release from Jurkat cells  
189

Figure 6.3.8  Effect of fluidisers on Hsp72 mRNA levels in Jurkat cells  
190
List of Tables

Chapter 1. Introduction

Table 1.1 New nomenclature for HSPs referred to in this thesis 17

Chapter 3. Heat Shock Protein localisation in Chronic Lymphocytic Leukaemia

Table 3.3.1 The subtypes of CLL according to the Binet classification 59
Table 3.3.2 Genetic defects found in CLL cells and their associated prognoses 61
Table 3.3.3 Characteristics of the CLL patients and control subjects involved in this section of the study 68
Table 3.3.4 Effect of sodium chloride treatment on sHsp72 expression analysed using the Stressgen antibody 72
Table 3.3.5 Comparison between the levels of HSPs, caspase-3 and Treg numbers in CLL patients and control subjects. 99

Chapter 4. Heat Shock Protein Localisation in Myeloid Malignancies

Table 4.1.1 The subtypes of AML according to the FAB classification 102
Table 4.1.2 The subtypes of MDS according to the FAB classification 105
Table 4.3.1 Characteristics of the AML, MDS, CR patients and control subjects involved in this section of the study. 110
Table 4.3.2 Comparison between the levels of HSPs and caspase-3 in AML patients, MDS patients, control subjects and CR patients. 132
Chapter 5. Synergistic Action of Chemotherapeutic Drugs and Membrane Fluidisers

Table 5.3.1  Percentage of Jurkat cells positive for DR4 and DR5 as determined by flow cytometry  149

Chapter 7. Discussion

Table 7.1.1  Association of HSP over-expression with tumour grade and prognosis  194
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>17-AAG</td>
<td>17-allylaminogeldanamycin</td>
</tr>
<tr>
<td>17-DMAG</td>
<td>17-dimethylaminoethyldamino-17-demethoxygeldanamycin</td>
</tr>
<tr>
<td>ABC</td>
<td>ATP-binding cassette</td>
</tr>
<tr>
<td>ADCC</td>
<td>Antibody-dependent cellular cytotoxicity</td>
</tr>
<tr>
<td>AIHA</td>
<td>Autoimmune Haemolytic Anaemia</td>
</tr>
<tr>
<td>ALDH</td>
<td>Aldehyde dehydrogenase</td>
</tr>
<tr>
<td>ALL</td>
<td>Acute Lymphoblastic Leukaemia</td>
</tr>
<tr>
<td>AML</td>
<td>Acute Myeloid Leukaemia</td>
</tr>
<tr>
<td>APAF-1</td>
<td>Apoptotic protease-activating factor 1</td>
</tr>
<tr>
<td>APCs</td>
<td>Antigen Presenting Cells</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine Triphosphate</td>
</tr>
<tr>
<td>BA</td>
<td>Benzyl Alcohol</td>
</tr>
<tr>
<td>Bag-</td>
<td>Bcl-2–associated athanogene</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary DNA</td>
</tr>
<tr>
<td>CLL</td>
<td>Chronic Lymphocytic Leukaemia</td>
</tr>
<tr>
<td>CML</td>
<td>Chronic Myeloid Leukaemia</td>
</tr>
<tr>
<td>CR</td>
<td>Complete Remssion</td>
</tr>
<tr>
<td>CTLA-</td>
<td>Cytotoxic T Lymphocyte Antigen</td>
</tr>
<tr>
<td>CTLs</td>
<td>Cytotoxic T-Lymphocytes</td>
</tr>
<tr>
<td>Cyclo</td>
<td>Cyclophosphamide</td>
</tr>
<tr>
<td>Daxx</td>
<td>Death Associated protein 6</td>
</tr>
<tr>
<td>DCs</td>
<td>Dendritic Cells</td>
</tr>
<tr>
<td>DD</td>
<td>Death Domain</td>
</tr>
<tr>
<td>DED</td>
<td>Death Effector Domain</td>
</tr>
<tr>
<td>DISC</td>
<td>Death-Inducing Signaling Complex</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>Dox</td>
<td>Doxorubicin</td>
</tr>
<tr>
<td>DRM</td>
<td>Detergent-Resistant Microdomain</td>
</tr>
<tr>
<td>EBRT</td>
<td>External Beam Radiotherapy</td>
</tr>
<tr>
<td>EGFR</td>
<td>Epidermal Growth Factor Receptor</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-Linked Immunosorbent Assay</td>
</tr>
<tr>
<td>ER</td>
<td>Endoplasmic Reticulum</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Name</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>ErbB2</td>
<td>Human Epidermal growth factor Receptor 2</td>
</tr>
<tr>
<td>FADD</td>
<td>Fas-Associated Death Domain</td>
</tr>
<tr>
<td>FLT3</td>
<td>fms-like tyrosine kinase receptor-3</td>
</tr>
<tr>
<td>FoxP3</td>
<td>Forkhead Box P3</td>
</tr>
<tr>
<td>GA</td>
<td>Geldenamycin</td>
</tr>
<tr>
<td>GITR</td>
<td>Glucocorticoid-Induced Tumour Necrosis Factor Receptor Family-Related Gene</td>
</tr>
<tr>
<td>GR</td>
<td>Glucocorticoid Receptor</td>
</tr>
<tr>
<td>HMGB1</td>
<td>High Mobility Group Box 1</td>
</tr>
<tr>
<td>HMG-CoA</td>
<td>3-hydroxy-3-methyl-glutaryl-CoA</td>
</tr>
<tr>
<td>HSEs</td>
<td>Heat Shock Elements</td>
</tr>
<tr>
<td>HSF</td>
<td>Heat Shock Factor</td>
</tr>
<tr>
<td>HSP</td>
<td>Heat Shock Protein</td>
</tr>
<tr>
<td>IAPs</td>
<td>Inhibitor of Apoptosis Proteins</td>
</tr>
<tr>
<td>IFN-</td>
<td>Interferon</td>
</tr>
<tr>
<td>iHSP</td>
<td>Intracellular HSP</td>
</tr>
<tr>
<td>IL-</td>
<td>Interleukin</td>
</tr>
<tr>
<td>IgVH</td>
<td>Immunoglobulin variable heavy chain gene</td>
</tr>
<tr>
<td>LAMP1</td>
<td>Lysosomal-associated membrane protein 1</td>
</tr>
<tr>
<td>mβcd</td>
<td>methyl-β-cyclodextrin</td>
</tr>
<tr>
<td>MDS</td>
<td>Myelodysplastic Syndrome</td>
</tr>
<tr>
<td>MHC</td>
<td>Major Histocompatability Complex</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger RNA</td>
</tr>
<tr>
<td>N-CAM</td>
<td>Neural Cell Adhesion Molecule</td>
</tr>
<tr>
<td>NF-κB</td>
<td>Nuclear Factor Kappa B</td>
</tr>
<tr>
<td>NK cell</td>
<td>Natural Killer Cell</td>
</tr>
<tr>
<td>OPG</td>
<td>Osteoprotegerin</td>
</tr>
<tr>
<td>PBMC</td>
<td>Peripheral Blood Mononuclear Cells</td>
</tr>
<tr>
<td>PDGF</td>
<td>Platelet Derived Growth Factor</td>
</tr>
<tr>
<td>PhA</td>
<td>Phenethyl Alcohol</td>
</tr>
<tr>
<td>PS</td>
<td>Phosphatidyl Serine</td>
</tr>
<tr>
<td>RT-PCR</td>
<td>Real-Time Polymerase Chain Reaction</td>
</tr>
<tr>
<td>sHSP</td>
<td>Surface HSP</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>siRNA</td>
<td>Small Interfering RNA</td>
</tr>
<tr>
<td>Smac/DIABLO</td>
<td>Second Mitochondrial Activator of Caspases/Direct IAP-binding Protein of Low Isoelectric Point</td>
</tr>
<tr>
<td>TAAs</td>
<td>Tumour Associated Antigens</td>
</tr>
<tr>
<td>TCR</td>
<td>T-Cell Receptor</td>
</tr>
<tr>
<td>TGF</td>
<td>Transforming Growth Factor</td>
</tr>
<tr>
<td>Th</td>
<td>T-helper Cell</td>
</tr>
<tr>
<td>TNF-</td>
<td>Tumour Necrosis Factor</td>
</tr>
<tr>
<td>TNFR</td>
<td>TNF Receptor</td>
</tr>
<tr>
<td>TRAIL</td>
<td>Tumour Necrosis Factor-Related Apoptosis Inducing Ligand</td>
</tr>
<tr>
<td>TRAIL-R</td>
<td>TRAIL Receptor</td>
</tr>
<tr>
<td>Tregs</td>
<td>T-Regulatory Cells</td>
</tr>
<tr>
<td>TSAs</td>
<td>Tumour Specific Antigens</td>
</tr>
<tr>
<td>TSP-1</td>
<td>Thrombospondin-1</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular Endothelial Growth Factor</td>
</tr>
<tr>
<td>ZAP-70</td>
<td>Zeta-Associated Protein-70</td>
</tr>
</tbody>
</table>