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Heat Shock Proteins in Chronic Lymphocytic Leukaemia

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1. Introduction

Over the last decade, research has implicated a group of molecular chaperones termed Heat Shock Proteins (HSPs) as major contributors to cancer progression and the development of chemo-resistance. HSPs were initially discovered as a group of proteins that were strongly induced in response to heat shock and other cellular stresses. Under non-stress conditions, they have a wide variety of roles within many sub-cellular compartments where they facilitate protein folding, prevent protein unfolding and assist protein transport across membranes. Due to their roles in maintaining protein conformation, HSPs are vital when other cellular proteins start to unfold due to cellular stress such as high temperature, exposure to cytotoxic chemicals or oxidative stress and are up-regulated rapidly in these situations (Ciocca and Calderwood, 2005). Under these stressful conditions, HSPs prevent protein aggregation, stabilise cell membranes and inhibit many of the key steps in cell death pathways (Beere & Green, 2001), thereby enabling cell survival in conditions that would otherwise be lethal. Importantly, in normal cells, once the stress has passed, HSP levels return to baseline and these proteins return to their regular house-keeping duties. However, a growing body of research shows that in tumour cells, including Chronic Lymphocytic Leukaemia (CLL) cells, HSPs remain elevated and may contribute to prolonged tumour cell survival via several mechanisms that remain to be fully revealed.

Human HSPs were originally identified as stress-induced proteins and were traditionally split into five families based on their molecular weight. These five families were the HSP27, HSP60, HSP90, HSP70 and HSP110 families. However, the sequencing of the human genome led to the identification of additional members of well established HSP families. Furthermore, the expansion of these HSP families resulted in many HSPs being referred to by several different names. Therefore, attempts to draw comparisons between studies examining specific HSPs has proved challenging. Consequently, Kampinga et al. (2009) recently introduced guidelines for the nomenclature of human HSPs which ensures that the genes and proteins are named in a consistent manner (Table 1). Readers are referred to Kampinga et al. (2009) for a complete listing of human HSP nomenclature. This chapter will focus on the involvement of the major HSPs, HSPB1 (Hsp27) HSPA1A (Hsp72) and HSPC1 (Hsp90) in the development and progression of CLL.

Gene Name	New Protein Name	Old Names
HSPB1	HSPB1	Hsp27, Hsp28; Hsp25
DNAJB1	DNAJB1	Hsp40
HSPD1	HSPD1	Hsp60
HSPA8	HSPA8	HSC70, Hsp71, Hsp73
HSPA9	HSPA9	Mortalin, mtHsp70, GRP75
HSPA1A	HSPA1A	Hsp72, Hsp70-1, HSPA1
HSPC1	HSPC1	Hsp90, HSP90A, Hsp89, HSP90AA
HSPH2	HSPH2	Hsp110

(Adapted from Kampinga et al. 2009)

Table 1. New nomenclature for human HSPs referred to in this chapter

The up-regulation of a number of HSPs in response to stress stimuli is regulated by the transcription factor Heat Shock Factor-1 (HSF1) (Wu, 1995). Under non-stress conditions, members of the DNAJ and HSPA families along with HSPC1 are bound to monomeric HSF1 within the cytosol. However, under stress conditions these HSPs dissociate from HSF1 and bind misfolded proteins, suggesting that these HSPs have a higher affinity for misfolded proteins compared to HSF1. As a consequence HSF1 trimerises and migrates to the nucleus where, in this state, it has a high binding affinity for cis-acting DNA sequence elements known as Heat Shock Elements (HSEs) in the promoter region of the HSP genes resulting in transcription of HSP genes (Sorger , 1991). Once the stress is discontinued, the trimeric forms of HSF1 dissociate from the HSEs and are converted back to HSF1 monomers with the inability to bind DNA. It is believed that the increase in HSPs within the cell following the heat shock response are themselves negative regulators of HSF, binding to HSF and preventing further trimerisation (Wu, 1995). HSPC1 has been shown to be a negative regulator of HSF1, as immunodepletion of HSPC1 results in enhanced HSF1 activity (Zou et al. 1998).

2. HSPs in cancer

The potential importance of HSPs in tumour biology is clear when considering their activities in light of the Hanahan & Weinberg concept of the hallmarks of cancer (Hanahan & Weinberg 2000; Hanahan & Weinberg 2011), where the development of a malignant phenotype requires six modifications:

- self-sufficiency with respect to growth signals
- limitless replicative potential
- insensitivity to anti-growth signals
- sustained angiogenesis
- capacity for tissue invasion and metastasis
- evasion of apoptosis

HSPs have the potential to contribute to each of these modifications, and three HSPs (HSPB1, HSPA1A and HSPC1) can be shown to influence at least two of these modifications. Not surprisingly it is these three HSPs that feature most in tumour studies.

HSPB1 belongs to the family of small heat shock proteins. Besides its role as an ATP-independent molecular chaperone, it is involved in the regulation of cell differentiation, the acquisition of thermotolerance and the inhibition of apoptosis and cell senescence. HSPB1 is up-regulated in response to a wide variety of stress stimuli such as oxidative stress, exposure to anti-cancer agents and radiation (Lanneau et al. 2008). As a molecular chaperone, HSPB1 prevents protein aggregation and stabilises partially denatured proteins ensuring refolding by other chaperones such as HSPA1A and HSPC1.

HSPA1A is the main inducible member of the HSPA family and is not normally present in significant concentrations in non-stressed normal cells. HSPA1A mainly exists as a dimer, and similarly to HSPB1 is up-regulated in response to a wide variety of stress stimuli and promotes cell survival. HSPA1A has a high affinity for hydrophobic amino acids and will rapidly bind to partially denatured proteins in preparation for refolding or disposal.

HSPC1 is the most abundant member of the HSPC family, and is constitutively expressed in all eukaryotic cells where it binds a specific set of 'client proteins' including steroid receptors, non-receptor tyrosine kinases and cyclin-dependent kinases (McLaughlin et al. 2002). HSPC1 operates as part of a multimeric chaperone complex which includes members of the HSPA and DNAJ families, along with many other 'co-chaperones' and immunophillins (Holzbeierlein et al. 2010). It exists as a dimer, consisting of three major domains per monomer; A C-terminal homodimerisation domain, a middle ATP-hydrolysis-regulating domain, and an N-terminal, ATP binding domain (Krukenberg et al. 2011).

At one time HSPs were thought to be solely intracellular proteins (iHSPs). However certain HSPs have been found to be released from viable cells into the extracellular milieu (eHSPs) (Hightower & Guidon, 1989; Hunter-Lavin et al. 2004) and have also been found expressed on the tumour cell surface (sHSPs). The exact mechanism involved in the transport of these HSPs from the cytosol to the cell surface and extracellular environment have not been fully determined, but evidence suggests that HSPs may employ a number of methods including lipid-raft transport (Hunter-Lavin et al. 2004; Gastpar et al 2005; Vega et al. 2008), exosomal transport (Clayton et al. 2005) and ABC transporters (Mambula & Calderwood 2006). Regardless of their route of exit, sHSPs and eHSPs have critical roles in a variety of processes ranging from cell invasion and metastasis to immunomodulation.

3. Intracellular HSPs

3.1 Intracellular HSPs and prognosis

The up-regulation of HSPs inside cancer cells compared to normal cells has been well documented and has led to numerous studies investigating their prognostic and therapeutic potential (Calderwood et al. 2006; Ciocca et al. 2005; Sherman & Multhoff. 2007). In many cases high levels of HSPs have been found to be advantageous to the cancer cell, which is hardly surprising when considering that HSPB1, HSPA1A and HSPC1 are all anti-apoptotic factors, and would suggest that these proteins should have prognostic potential. However, although elevated HSPA1A, HSPB1, and HSPC1 are associated with poor prognosis in leukaemia, this pattern is not repeated in other cancers (Table 2) (Jaattela, 1999; Ciocca & Calderwood, 2005). In a minority of cancer types, the over-expression of HSPs appears to correlate with a positive prognosis for the patient (Kawanishi et al. 1999; Sagol et al. 2002; Santarosa et al. 1997) and at present it is still unclear as to how HSP expression may influence cancer progression.

Increased levels of HSPB1 have been documented in various cancers including breast, liver and prostate (Cornford et al. 2000; Romani et al. 2007; Vargas-Roig et al. 1997) and high levels have been found to correlate with poor prognosis (Duval et al. 2006; Thomas et al. 2005). However, there are contradictions within the literature; In breast cancer, for example, increased levels of HSPB1 have been associated with prolonged survival in oestrogen receptor-negative cases (Love & King, 1994). Low levels of HSPB1 in ovarian cancer have also been found to correlate with decreased survival (Geisler et al. 2004). However, these contradictions may be attributed to HSPB1, (synonymous with p24 and p29), being an oestrogen-regulated protein (Adams & McGuire, 1985; Ciocca & Luque, 1991).

Increases in HSPA1A have been observed in breast cancer (Tauchi et al. 1991), colorectal cancer (Milicevic et al. 2007; Shotar, 2005), kidney cancer (Ramp et al. 2007) and leukaemia (Chant et al. 1995; Thomas et al. 2005). Additionally, elevated HSPA1A has been associated with resistance to cancer therapy and/or poor prognosis for the patient (Thomas et al. 2005; Vargas-Roig et al. 1998). However, the relationship between the presence of HSPs and prognosis cannot be extrapolated to all cancers, as high intracellular HSPA1A expression has been correlated with a positive prognosis in oesophageal (Kawanishi et al. 1999), pancreatic (Sagol et al. 2002) and renal cancer (Santarosa et al. 1997).

HSPC1 levels are elevated in a number of cancer types including oesophageal squamous cell carcinoma (Wu et al. 2009), invasive breast carcinoma (Zagouri et al. 2010) and leukaemia (Thomas et al. 2005). Furthermore, in some cases, elevated HSPC1 levels have been associated with decreased survival and adverse karyotypes (Pick et al. 2007; Thomas et al. 2005).

Cancer type	HSPA1A	HSPB1	HSPC1
Breast	Poor	Variable	n/d
Endometrial	Poor	n/d	Good
Kidney	Good	n/d	n/d
Leukaemia	Poor	Poor	Poor
Lung	n/d	n/d	n/d
Osteosarcoma	Good	Poor	Variable
Ovary	n/d	Poor	n/d

(Adapted from Jaattela, (1999) and Ciocca & Calderwood, (2005))

Table 2. Association of HSP over-expression with prognosis

It is clear that different HSPs have distinct roles in normal cells and therefore it is not inconceivable that they may also have a variety of roles in tumour development. Indeed, the over-expression of one particular HSP within a tumour cell does not necessarily signify elevated levels of other HSPs within the same patient; In CLL particularly, HSPB1, HSPA1A and HSPC1 increases have been observed amongst patients but not necessarily simultaneously (Dempsey et al. 2010a). Similarly there is little correlation between the expression of HSPB1, HSPA1A and HSPC1 in prostate cancer (Cornford et al. 2000) or AML (Thomas et al. 2005). It is therefore imperative to understand the individual roles of these proteins in cancer development by studying them together within a specific sample but also considering them as distinct entities.

3.2 Intracellular HSPs and resistance to apoptosis

Mechanistic investigations have shown that HSPB1 interferes with the apoptotic pathway in several ways including the prevention of cytochrome c and Smac/DIABLO release from the mitochondria (Chauhan et al. 2003a; Paul et al. 2002) and direct interaction with cytochrome c (Garrido et al. 2001; LeBlanc, 2003; Samali et al. 2001) and Daxx (Charrette et al. 2000) thereby inhibiting their function. There is clear evidence that HSPB1 levels correlate with chemo-resistance (Schepers et al. 2005; Vargas-Roig et al. 1998). Studies designed to explore the role of HSPB1 in the development of chemo-resistance have utilised various techniques including siRNA and gene transfection. Depletion of HSPB1 has been shown to overcome resistance to drug-induced apoptosis (Chauhan et al. 2003b) while transfection of full-length HSPB1 into cancer cells confers resistance to Cisplatin and Doxorubicin (Richards et al. 1996).

HSPA1A is involved in carcinogenesis at many different stages from the enhancement of activity of many different oncogenes and inhibition of tumour suppressor genes such as p53 (Yaglom et al. 2007), to promoting the survival of tumour cells through inhibition of apoptosis (Khaleque et al. 2005; Nylandsted et al. 2000). Similarly to HSPB1, HSPA1A interacts with several key proteins in cell death pathways (Beere & Green, 2001). Direct interaction with APAF-1 and mutated p53 (Iwaya et al. 1995; Lehman et al. 1991) has been documented as well as direct inhibition of procaspases. Several studies have shown that depletion of HSPA1A results in increased sensitivity to chemotherapeutic drugs (Zhao & Shen, 2005) and ultimately apoptosis of various cell lines including breast, colon and prostate (Nylandsted et al. 2000), highlighting the importance of this protein for cell survival.

A wide variety of client proteins are held by HSPC1 in both tumour and non-tumour cells, and as a result it is involved in several vital signalling pathways including PI3K/Akt, Erk1/2, JNK and NFκB pathways. It is the chaperoning of a variety of oncoproteins such as mutant B-Raf (Grbovic et al. 2006), Bcr/Abl (An et al. 2000) and mutant p53 (Lin et al. 2008), as well as the signalling pathway components, that has sparked interest into HSPC1's potential role in tumour development and progression. HSPC1 also cooperates with HSPA1A in inhibiting apoptosome formation. Furthermore, HSPC1 interferes with death receptor mediated apoptosis by stabilising the anti-apoptotic protein Receptor Interacting Protein (RIP) thereby promoting cell survival.

Clearly HSPB1, HSPA1A and HSPC1 interfere with apoptotic signalling at multiple points (Figure 1).

4. Extracellular HSPs

4.1 Extracellular HSPs in immunomodulation

As a consequence of their molecular chaperoning role, HSPs exit the cell bound to a wide variety of antigenic peptides. These extracellular HSP-peptide complexes bind to surface receptors on Antigen Presenting Cells (APCs) in a process termed cross-presentation (Bolhassani & Rafati, 2008). During the process of necrosis, when cell stress is highest and the cellular components leak out of the cell, these HSP-peptide complexes are abundant and can signal stress to local APCs, which in-turn can induce a Cytotoxic T-Lymphocyte (CTL)

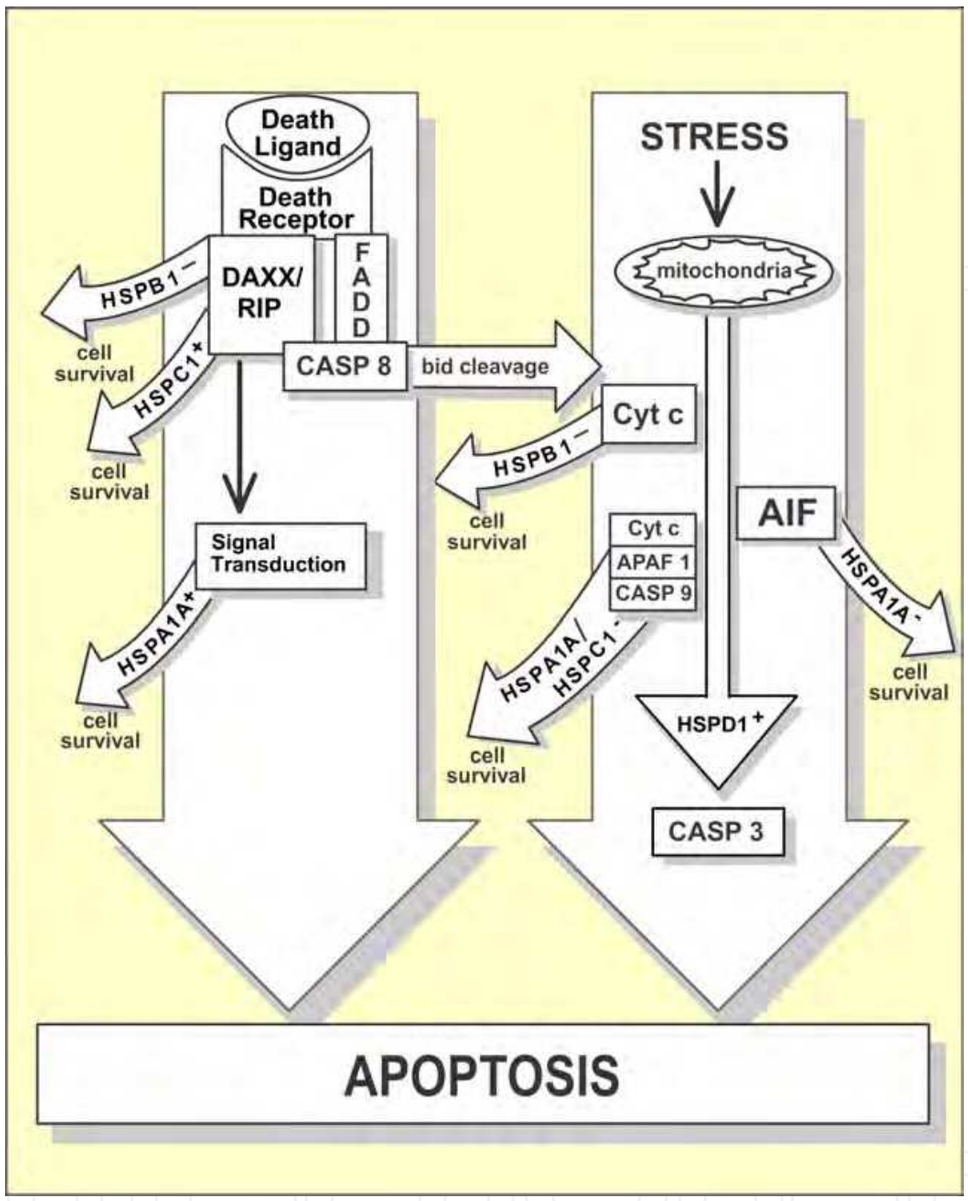


Fig. 1. Regulation of the Death Receptor and Mitochondrial Apoptosis Pathways by HSPs (Taken from Dempsey, 2009). Two main arrows represent the Death Receptor-mediated- and mitochondrial- apoptosis pathways. HSPB1 inhibits apoptosis by directly interacting with Daxx, and sequestering cytochrome c released from the mitochondria. HSPD1 has been shown to directly promote the proteolytic maturation of caspase-3, thereby displaying a pro-apoptotic role. HSPA1A inhibits apoptosis by inhibiting the activation of protein kinases involved in signal transduction pathways (e.g. JNK), binding to AIF thereby neutralising its effects and binding to Apaf-1 resulting in inhibition of apoptosome formation. HSPC1 has been shown to stabilise the anti-apoptotic protein, RIP and has also been shown to work synergistically with HSPA1A to inhibit apoptosome formation. Apoptosis induced by Death Receptors also results in activation of the mitochondrial pathway via cleavage of the pro-apoptotic protein Bid. A minus sign indicates negative regulation while a positive sign indicates positive regulation.

response. Similarly, viable tumour cells with high HSP levels may also actively release HSP-peptide complexes into the surrounding environment. Research investigating the HSP-APC interactions has revealed a number of APC receptors capable of binding HSPs; Scavenger receptors (SRs), Toll-like receptors (TLRs), CD40, CD14 and CD91 (Calderwood et al. 2007; Basu et al. 2001) allow HSP-peptide complexes to interact with a number of different immune cell types including Dendritic cells, Macrophages, Monocytes and NK-cells (Murshid et al. 2008).

The binding of HSPA1A-peptide complexes to the SR LOX-1 on Dendritic cells, for example, results in the internalisation of the HSP-peptide complex (Arnold-Schild et al. 1999) via the phagocytic pathway, and re-presentation of the antigenic peptide on MHC-I on the Dendritic cell surface. Work by Kurotaki et al. (2007) has revealed the significance of HSPs in this antigen cross presentation process; Dendritic cells were found to bind and internalise HSPC1-peptide complexes and generate a T-cell response, while unconjugated peptide was unable to stimulate this same response. In order for APCs to effectively activate a T-cell response, they must undergo maturation to express appropriate co-stimulatory molecules. Indeed, HSPA1A has been shown to stimulate the expression of CD40, CD83 and CD86 on Dendritic cells (Bausero et al. 2005; Kuppner et al. 2001). Additionally, the expression and release of pro-inflammatory cytokines such as IL-1β, IL-6, IL-12 and TNF-α is stimulated by HSPs (Asea et al. 2000; Baretto et al. 2003; Todryk et al. 1999). There appears to be a positive feedback system in which APC-released pro-inflammatory cytokines augment transcription and release of HSPA1A from tumour cells (Baretto et al. 2003). The immunomodulatory properties of extracellular HSPs have identified them as a potential 'danger signal', released by cells in order to signal stress to the immune system (Todryk et al. 2000; Williams & Ireland 2008). Furthermore, as HSP-peptide complexes can induce both innate and adaptive immunity, they have become attractive candidates for immunotherapy protocols. Clinical trials have investigated the use of HSP-based vaccines on solid tumours and lymphomas but results have been inconclusive (Murshid et al. 2008). The technique involves isolating and purifying HSP from the patient's tumour cells and re-applying this as a vaccine in order to stimulate an immune response. A critical advantage of using such treatments is that, by exploiting the chaperoning ability of HSPs, a broad range of tumour-specific peptides, unique to that specific tumour will be targeted.

4.2 Distinct roles of membrane bound HSPs

A number of HSPs are also present on the tumour cell surface, and have distinct roles dependent on the specific HSP. HSPA1A in particular has been found embedded in the cell membrane of a variety of tumour cell types (Kleinjung et al. 2003; Pfister et al. 2007) and work from our lab has shown sHSPA1A on CD5+/CD19+ cells from CLL patients (Dempsey et al. 2010a). To date, it appears that the membrane localisation of HSPA1A is specific to tumour cells and has attracted much attention as a potential therapeutic target (Gehrmann et al. 2008; Krause et al. 2004), the implications of which will be discussed later in this chapter.

sHSPA1A acts a recognition structure for activated NK-cells (Gehrmann et al. 2003; Gross et al. 2003; Multhoff et al. 1999) and is therefore detrimental for the tumour cell. The region of

the HSPA1A molecule exposed to the extracellular milieu of tumours has been identified as the 14-mer peptide TKDNNLLGRFELSG (TKD peptide) (Multhoff et al. 2001). The stimulation of NK cells using the TKD peptide and low dose IL-2 results in up-regulation of CD94/CD56 on NK cells and initiates NK-cell killing of sHSPA1A+ tumour cells (Gross et al. 2003; Gross et al. 2008; Multhoff et al. 1999). Interestingly, purified (perforin-free) granzyme B from NK-cells was shown to bind to HSPA1A and result in granzyme B internalisation into sHSPA1A+ tumour cells (Gross et al. 2003). Stimulation of NK-cells was also observed following incubation with sHSPA1A+ tumour-cell derived exosomes (Gastpar et al. 2005). Wei et al. (1996) demonstrated that sHSPA1A is also a target for δγT-cells. Furthermore, the induced *in-vivo* expression of sHSPA1A on tumour cells increases their immunogenicity, resulting in both a CTL-mediated and NK-cell mediated response and rejection of the tumour (Chen et al. 2002a). The expression of sHSPA1A, therefore, overcomes many of the characteristics that tumour cells develop to evade immune recognition, including down-regulation of MHC molecules, and down-regulation of co-stimulatory and adhesion molecules (Bausero et al, 2005; Chen et al. 2002a). The literature to date, suggests that the cellular location of HSPA1A is crucial in determining disease outcome as iHSPA1A is advantageous to the tumour, while sHSPA1A appears to be detrimental.

In contrast to this theory, the small number of studies to date, investigating the presence of sHSPA1A in primary cells from leukaemic patients suggest that its presence may in fact be detrimental for the patient. The study by Gehrmann et al. (2003) demonstrated that although sHSPA1A acted as a recognition structure for NK cells, sHSPA1A expression could be correlated with unfavourable or intermediate cytogenetics. Similarly, sHSPA1A levels were found to be higher in AML patients with treatment-refractory disease and active disease than patients in complete remission, suggesting a correlation with poor prognosis (Steiner et al. 2006). Furthermore, AML patients continuing to express moderately high levels of sHSPA1A after achieving remission were shown to have a shorter relapse-free survival time than remission patients with lower levels of sHSPA1A (Steiner et al. 2006). No correlation between sHSPA1A expression and stage of AML or resistance to chemotherapy could be determined by Steiner et al. (2006), suggesting that surface localisation of HSPA1A cannot be investigated in isolation. Schilling et al. (2007) demonstrated co-expression of HSPA1A with phosphatidylserine (PS) on the surface of hypoxic tumour cell lines. Additionally, exogenously added HSPA1A was shown to bind to PS on the cell surface and enhance the response to radiation. The results indicate that the radiotherapy-resistance observed in many hypoxic tumours may be overcome by prior treatment with HSPA1A.

In addition to HSPA1A, cell surface expression of HSPC1 and HSPB1 on tumour cells has been reported (Becker et al. 2004; Brameshuber et al. 2010; Ferrarini et al. 1992; Shin et al. 2003). sHSPC1 has been shown to be involved in tissue invasion and metastasis (Eustace et al. 2004), while the role of sHSPB1 is still unclear. Indeed, inhibiting cell surface expression of HSPC1, both *in-vitro* and *in-vivo*, reduces cell motility and invasiveness (Eustace et al. 2004; Tsutsumi et al. 2008). Work by Sidera et al. (2008) proposes that in some cancer cell types, sHSPC1 interacts with and activates HER-2, resulting in homodimerisation with ErbB-3, signal transduction pathway activation and ultimately actin rearrangement. Secretion of HSPC1 in exosomes has also been discovered, and has been shown to contribute to cancer cell invasiveness (McCready et al. 2010).

It is clear that extracellular HSPs have critical roles in tumour development and immune responses and we therefore suggest that mechanistic investigations of tumour biology should include analysis of extracellular, as well as intracellular, HSPs.

5. HSPs in leukaemia

Although a large amount of data exists regarding the expression of HSPs in cancer, the majority of studies have focused on solid tumours. Cells within a solid tumour mass are subjected to high levels of stress; the tumour microenvironment is often hypoxic and nutrient deficient and there may be infiltration by large numbers of immune cells. Therefore it is not surprising that HSP levels are elevated in many solid tumour types. Leukaemia cells however, are not subjected to these same stresses and as a result may not necessarily show elevated levels of HSPs.

Of the limited number of studies investigating HSP expression in leukaemia, a number have focused on correlating HSP expression with prognosis and clinical outcome, while others have directed research towards HSP expression and susceptibility to apoptosis. One study by Thomas et al. (2005) explored the expression of intracellular HSPB1, HSPD1, HSPA1A, HSPC1 and HSPH2 in both peripheral blood and bone marrow from patients with Acute Myeloid Leukaemia (AML). It was noted that complete remission rates were higher in patients with lower HSP expression and that overall survival was also significantly longer in these patients. Similarly, overall survival was found to be significantly greater in Myelodysplastic Syndrome (MDS) patients with lower HSPB1, HSPD1, HSPC1 and HSPH2 expression (Duval et al. 2006). A study exploring the expression of surface HSPA1A (sHSPA1A) in bone marrow aspirates from AML patients demonstrated that patients in complete remission express significantly lower levels of sHSPA1A when compared to patients with active AML (Steiner et al. 2006). Taken together these results suggest that higher levels of HSP expression both internally and on the surface of the leukaemic cell are advantageous to the cancer cell and therefore an adverse factor for the patient. This is in contrast to studies that have demonstrated the involvement of sHSPA1A in immune recognition and tumour regression (Chen et al. 2002; Multhoff et al. 1999; Gross et al. 2003; Gross et al. 2008).

HSP expression in AML and MDS has also been found to correlate with expression of the myeloblast surface antigen CD34, a well established poor prognostic factor (Duval et al. 2006; Thomas et al. 2005). Moreover, AML and MDS patients with intermediate or unfavourable karyotypes display a higher level of HSP expression than patients with favourable karyotypes. In contrast, Steiner et al. (2006) did not establish a correlation between sHSPA1A expression and cytogenetic risk group or FAB subtype. A study into expression of HSPD1, HSPA1A, HSPA8 (constitutive and heat-inducible forms) and HSPC1 in AML cells showed a heterogeneous expression of all three HSPs and no correlation was found between this extensive range of HSP expression and clinical outcome (Chant et al. 1995). However, a more recent study by the same group demonstrated that although AML cells show a broad range of HSPA1A expression, this expression correlates with susceptibility to apoptosis (Chant et al. 1996). Surprisingly, the correlation between HSPA1A expression and apoptosis was positive, indicating that cells with higher levels of the protein are more susceptible to apoptosis. This finding is contradictory to the theory that HSPA1A

protects cells from apoptosis. In spite of this correlation between HSPA1A and apoptosis, none was found between expression of HSPC1 and susceptibility to apoptosis. In contrast, levels of HSPA1A in MDS did not correlate with either pro- or anti-apoptotic protein levels (Duval et al. 2006). However, levels of HSPB1, HSPD1, HSPC1 and HSPH2 negatively correlated with the expression of the pro-apoptotic proteins Bad and Bak, while correlating positively with the anti-apoptotic proteins Bcl-2 and Bcl-Xl.

Research into acute lymphoblastic leukaemia (ALL) has shown decreased expression of HSPA1A and HSPB1 in bone marrow aspirates from patients who achieved complete remission when compared to those patients who did not achieve complete remission (Campos et al. 1999). Additionally, ALL cells displaying the Bcr/Abl fusion protein contained high levels of HSPA1A (Nimmanapalli et al. 2002). Further studies have demonstrated that HSPA1A contributes to the Bcr-Abl–mediated resistance to apoptosis by chemotherapeutic agents such as etoposide. Moreover, down-regulation of HSPA1A can sensitise these Bcr/Abl ALL cells to cytotoxic drugs (Guo et al. 2005). The Bcr/Abl fusion protein was shown to be a client protein of HSPC1. Furthermore, inhibition of HSPC1, but not HSPA1A, in myeloid cells was found to result in degradation of Bcr/Abl (Peng et al. 2007).

6. HSPs in chronic lymphocytic leukaemia

An extensive study by our group analysed the levels of HSPB1, HSPA1A and HSPC1 in CD5+/CD19+ and CD5-/CD19+ cells from CLL patients and normal lymphocytes from control subjects (Dempsey et al. 2010a). At first glance, it would appear that levels of both iHSPC1 and iHSPB1 are significantly higher in CLL patients overall than normal age-matched control subjects. An initial analysis of our data would also support the hypothesis that elevated intracellular HSP leads to tumour cells being resistant to apoptosis (Khaleque et al. 2005; Nylandsted et al. 2000; Thomas et al. 2005; Vargas-Roig et al. 1998); Caspase-3, a marker of apoptosis, was found to be lower in CLL patients compared to age-matched control subjects, while levels of iHSPB1 and iHSPC1 were higher in CLL patients. We also observed a negative correlation between levels of caspase-3 and iHSPB1. An observed difference in caspase-3 levels between CLL and control subjects is not surprising since the underlying basis of CLL is an inability for B-lymphocytes to commit to apoptosis. There was, however, no difference in caspase-3 levels between patients at different stages of the disease which suggests that the progression of CLL is more likely to be a result of an increased cellular clonal replication rather than an increased resistance to apoptosis from the same cells. A more detailed analysis of the data has demonstrated that CLL patients in different Binet stages express distinct levels of iHSPC1; Levels of iHSPC1 in Binet stage A patients are significantly higher than levels observed in Binet stage B and C patients. This is surprising, as the anti-apoptotic nature of these proteins would suggest they may be elevated in patients with a more advanced disease. However, the elevated levels of HSPC1 in patients with a less severe disease could be interpreted as a stress response in the early stages of the disease, which aids CLL cells in surviving immune destruction. As the disease progresses and CLL cells replicate uncontrollably, these very high HSPC1 levels are no longer required for cell survival and therefore begin to decrease.

Our data has also revealed that CLL patients can be divided into two groups based on their expression of iHSPA1A in CD5+/CD19+ cells; one group of patients presents very low levels of iHSPA1A while the other group expresses levels up to 1000-fold higher. This is also the case for sHSPA1A, although the difference is less pronounced. It should be noted that patients expressing very low levels of iHSPA1A are not necessarily the same patients that express low levels of sHSPA1A as no correlation between sHSPA1A and iHSPA1A could be found. Interestingly these CLL patients classified into the 'low sHSPA1A or iHSPA1A expressing' groups display HSPA1A levels similar to levels in non-malignant CD5-/CD19+ cells from the same patients and also lymphocytes from control subjects. In spite of this differential expression of HSPA1A amongst CLL patients, neither sHSPA1A nor iHSPA1A can be correlated with stage of disease or cytogenetic abnormality. Our data supports the work by Chant et al. (1995) who have demonstrated a wide range of HSP expression amongst AML patients. We have also determined that CLL patients with stable disease (not requiring treatment) possess significantly higher levels of iHSPA1A than patients with progressive disease (requiring treatment), which is in line with the decrease in HSPC1 seen as the disease progresses.

There is also considerable variation in the levels of extracellular HSPA1A (eHSPA1A) present in the serum of CLL patients. When considered as a group, levels of eHSPA1A in CLL patients are not significantly different from levels in control subjects. However, a more detailed analysis of the data reveals a correlation between the levels of extracellular and intracellular HSPA1A in CLL patients. Interestingly patients receiving corticosteroid treatment display significantly lower levels of HSPA1A in serum when compared to patients not receiving corticosteroid treatment, with some steroid-treated patients showing near-undetectable levels of eHSPA1A. This suggests that steroid treatment may totally inhibit the secretion of HSPA1A, although this remains to be confirmed. However, in addition to their negative effects on lymphocyte proliferation, T-cell activation and NK-cell function, corticosteroids have been shown to regulate transcription factors. As eHSPA1A and iHSPA1A levels correlate and eHSPA1A levels are lower in steroid-treated patients, it could be deduced that the transcription of HSPA1A has been inhibited. This conclusion is supported by the decrease in HSPA1A release observed following commencement of glucocorticoid treatment in CLL patients.

CLL patients often display resistance to corticosteroid therapy which is attributed to the imbalanced expression of glucocorticoid receptor (GR) isoforms. Indeed, higher expression of the transcriptionally inactive glucocorticoid receptor-beta (GR-beta) in relation to the hormone-activated transcription factor GR-alpha has been observed in CLL cells (Shahidi et al. 1999). However, as the GR is a client protein of HSPC1, variation in HSPC1 concentration may result in defective ligand binding, which may also contribute to resistance (Bailey et al. 2001). Indeed it has been demonstrated that the ratio of HSPC1 to GR expression is significantly higher in steroid-resistant compared to steroid-sensitive multiple sclerosis patients (Matysiak et al. 2008). This suggests that CLL patients in Binet stage A, whose HSPC1 expression is high, may have a reduced sensitivity for the steroid.

In summary although HSPs appear to be elevated in CLL patients compared to control subjects, closer examination of the data reveals that this is not the case in all CLL patients and in fact, there is a large variability in HSPB1, HSPA1A and HSPC1 amongst patients

which can be associated with disease stage and also treatment regime. The results indicate that the concept of HSPs being over-expressed in cancer may in fact be oversimplified. Indeed, it is now becoming apparent that there is also great variability in HSP expression in solid tumours such as breast cancer (Zagouri et al. 2010). These findings are crucial in helping to determine whether future treatments targeting HSPs maybe considered as a universal option.

7. Heat shock proteins as therapeutic targets

Many of the most recently developed anti-cancer treatments are targeted towards a single specific oncoprotein. Although in many cases, this has been largely successful (Flaherty et al. 2010; Kantarjian et al. 2003) cancer cells may acquire resistance to these treatments via a number of mechanisms including secondary mutations in the target binding domain and activation of alternative signalling pathways (Nazarian et al. 2010; Shah et al. 2002). Indeed, by their very nature, cancers accumulate oncogenic mutations as they progress and therefore a treatment strategy that involves the selective targeting of a specific protein kinase, for example, is unlikely to show continued success. As stated earlier, HSPs contribute to each of the six hallmarks of cancer and as a result have multiple molecular targets. In particular, HSPC1 appears to play a fundamental role in the development and maintenance of several tumour characteristics (Neckers, 2007). HSPC1 binds and stabilises a broad array of mutated oncoproteins (An et al. 2000; Minami et al. 2002; Pashtan et al. 2008), so the targeting of this single chaperone, should in theory, destabilise and degrade a wide variety of signalling kinases, therefore simultaneously targeting a number of cell signalling pathways. Furthermore, by selectively inhibiting HSPC1, all six 'hallmarks of cancer' (Hanahan & Weinberg, 2001; Hanahan & Weinberg, 2011) can be targeted in parallel, thereby dramatically reducing the probability that a resistant clone will develop. Since the identification of the first HSPC1 inhibitors 25 years ago, interest into these novel therapies has expanded considerably and the development of newer inhibitors has focussed on increasing efficacy and reducing side effects. Indeed a large body of data now exists documenting their anti-cancer effects on a wide variety of tumour cell types (Kim et al. 2009) both in isolation and in combination with common chemotherapeutic agents. HSPC1 inhibitors are currently grouped according to their site of action; N-terminal inhibitors and C-terminal inhibitors.

7.1 N-terminal HSPC1 inhibitors

The original N-terminal inhibitors, geldanamycin (GA) and radicicol were naturally occurring antibacterial products discovered in *Streptomyces hygroscopicus*. Initial experiments using these compounds revealed binding to HSPC1 and consequent degradation of the HSPC1 client protein v-src (Whitesell et al. 1994). Although subsequent experiments proved that these natural HSPC1 inhibitors were able to cause the degradation of a wide range of client proteins, the non-specific toxicity of these compounds prevented their clinical use. However, these experiments led to the development of the first clinically applicable HSPC1 inhibitor, 17-AAG (17-allylamino-17-demethoxygeldanamycin, also known as tanespimycin) (Holzbeierlein et al. 2010). As a derivative of GA, 17-AAG resulted in the degradation of a variety of oncogenic proteins in a number of tumour cell types, while displaying reduced toxicity. This compound was entered into clinical trials and tested on a broad array of

cancers including a large variety of haematological malignancies such as AML, ALL, CML and CLL. Detailed reviews of clinical trial data can be found in Kim et al. (2009), Taldone et al. (2008) and Holzbeierlein et al. (2010). In addition to showing initial promise as a novel therapy in isolation, 17-AAG also demonstrated synergism with a number of chemotherapeutic agents such as Bortezomib (Richardson et al. 2010), Trastuzumab (Modi et al. 2007) and Paclitaxel (Ramalingam et al. 2008). An alternative derivative of GA, 17-DMAG (17-dimethylaminoethylamino-17-demethoxygeldanamycin, also known as alvespimycin) was subsequently produced, with greater solubility and showed similar initial success in phase I and II clinical trials. Based on the results using 17-AAG and 17-DMAG, interest into the development of alternative HSPC1 inhibitors has increased greatly and has produced a number of N-terminal inhibitors including IPI-504 (Retaspimycin hydrochloride), AUY922, BIIB021 and SNX-2112 (Eccles et al. 2009; Hanson & Vesole, 2009; Lundgren et al. 2009; Okawa et al. 2008). In spite of these promising results and continuous research and development, clinical trials involving N-terminal inhibitors have not been without limitations. Hepatotoxicity and poor solubility of the drugs have been frequently reported. Furthermore, despite the idea of targeting the six 'hallmarks of cancer' through the inhibition of HSPC1, drug resistance has become an issue. This has been attributed to the increase in HSPB1 and HSPA1A following HSPC1 inhibition (Dakappagari et al. 2010; Ravagnan et al. 2001) due to enhanced activity of HSF-1, thereby increasing cellular resistance to apoptosis. This increase in HSPB1 and HSPA1A appears to be a complication related to the use of all N-terminal inhibitors (Taldone et al. 2008). A review by Holzbeierlein et al. (2010) highlights three significant clinical trials involving 17-AAG in which no anti-cancer response was observed and recruitment into the trials was terminated prematurely. Similarly, unexpected problems have been encountered using 17-DMAG. Another issue with HSPC1 inhibition is that, in addition to chaperoning many oncogenic proteins, HSPC1 also chaperones many other proteins with anti-tumour activities. The tumour suppressor proteins LKB1 (Boudeau et al. 2003), LATS1, LATS2 (Huntoon et al. 2010) and wild-type p53 (Walerych et al. 2004) are all down-regulated following treatment with HSPC1 inhibitors. These results highlight the potential disadvantages of HSPC1 inhibitor therapies.

7.2 C-terminal HSPC1 inhibitors

Research into HSPC1 inhibitors has primarily focused on N-terminal inhibition, however a small number of C-terminal inhibitors have been identified. Novobiocin was the first C-terminal HSPC1 inhibitor to be identified, and similarly to GA, is a naturally occurring antibiotic produced in *Streptomyces niveu*. However, novobiocin was found to have a weak affinity for its target and as a result was not therapeutically applicable. Subsequently, research has focused on the synthesis of novobiocin analogues with a stronger affinity for HSPC1. According to a review by Holzbeierlein et al. (2010), over 300 analogues have been screened resulting in the identification of compounds with improved activity and selectivity of tumour over non-tumour cells. It has also been noted that the anti-cancer agent cisplatin and the polyphenolic flavanoid Epigallocatechin-3-gallate (EGCG) can inhibit HSPC1 activity via C-terminal inhibition (Soti et al. 2002; Yin et al. 2009). Cisplatin has been used as a conventional chemotherapy for decades due to its ability to form DNA adducts and therefore prevent DNA transcription. By binding to the C-terminal, cisplatin hinders

nucleotide binding. Treatment of a human ovarian cell line with EGCG was found to inhibit HSPC1 activity and therefore result in the degradation of several client proteins including ErbB2, Raf-1, and phosphorylated Akt (Yin et al. 2009).

C-terminal inhibitors appear to have many non-specific effects and as a result have not been entered into clinical trials (Sreedhar et al. 2004). However, recent research has revealed that C-terminal inhibition, unlike N-terminal inhibition, does not result in the enhanced activity of HSF-1 (Conde et al. 2009; Holzbeierlein et al. 2010). Indeed, treatment of Xenopus oocytes with heat shock in the presence of novobiocin results in a dose dependent decrease in HSF-1 transcriptional activity (Conde et al. 2009). In contrast similar treatment of these cells in the presence of GA resulted in a dose-dependent increase in HSF-1 activity. This lack of HSF-1 induction is a very attractive attribute of C-terminal inhibitors and therefore research and development into synthetic C-terminal inhibitors continues.

7.3 Important HSPC1 client proteins in CLL

Research into the involvement of HSPC1 in cancer progression has identified a wide variety of client proteins that may contribute to the progression of both solid tumours and haematological malignancies. There are a number of HSPC1 clients that have been implicated in the development and progression of CLL in particular (Johnson et al. 2007) and therefore the use of HSPC1 inhibitors as a treatment option appears attractive. The list of HSPC1 client proteins is continually expanding suggesting that novel therapeutic targets in CLL may present themselves in the future.

The tyrosine kinase Zeta-associated Protein-70 (ZAP-70) is a well established poor prognostic factor and an HSPC1 client (Bartis et al. 2007). In normal T-cells, ZAP-70 is associated with the TCR where it functions in downstream TCR signalling (Cruse et al. 2007). However, although ZAP-70 is present in normal pre-B-cells, its expression should be lost on maturation of the cell. Hence, the presence of ZAP-70 in CLL cells is indicative of an immature clone. A study by Crespo et al. (2003) found that ZAP-70 expression in CLL correlates with disease progression and survival, and also correlates with another well established CLL prognostic factor, IgV_H mutational status. Castro et al. (2005) demonstrated that ZAP-70 in CLL cells co-immunoprecipitates with HSPC1, while ZAP-70 from normal T-cells does not. In addition, treatment of ZAP-70^+ CLL cells with HSPC1 inhibitors resulted in degradation of ZAP-70, while treatment of T-cells from CLL and control patients with HSPC1 inhibitors did not affect the expression of ZAP-70. Manipulation of ZAP-70^- CLL cells to express ZAP-70 was shown to activate HSPC1 and induce sensitivity to 17-AAG.

The tumour suppressor protein p53, encoded by the *TP53* gene is frequently mutated in CLL, and other cancer types. Mutant $p53^+$ CLL patients show increased resistance to a wide range of chemotherapeutic agents (Sturm et al. 2003) and therefore are often treated as a distinct sub-group of patients. Indeed agents that act independently of the p53 pathway, such as the monoclonal antibodies rituximab and alemtuzumab and other agents such as lenalidomide, are often used as alternative first-line treatments for patients with a 17p deletion (mutated p53 status). HSPC1 inhibitor treatment of CLL cells results in a dose-dependent reduction in mutant p53 levels, a simultaneous up-regulation of wild type p53 and consequent cytotoxicity (Lin et al. 2007). This treatment also results in an increase in p21, an inducer of cell cycle arrest.

It should be noted that this same study showed that CLL cells without a p53 mutation were also sensitive to HSPC1 inhibitor treatment. However, this could be due to activation of wild-type p53 and consequent p53-dependent apoptosis. Further research has shown a synergistic effect between 17-DMAG and Doxorubicin, demonstrating a sensitisation of p53 mutated cells to Doxorubicin-induced cell death (Robles et al. 2006).

HSPC1 chaperones a broad array of protein kinases involved in signal transduction pathways including phosphorylated Akt, Lyn, B-Raf and IκK (Broemer et al. 2004; da Rocha Dias et al. 2005; Sato et al. 2000; Trentin et al. 2011). The interaction of HSPC1 with these phosphorylated kinases prevents dephosphorylation of the kinase and its subsequent inactivation, therefore maintaining its activity. The over-activity of Akt (Ringshausen et al. 2002), Lyn (Contri et al. 2005), and implied elevation of IκK (Hertlein et al. 2010) in CLL cells has been previously observed and thought to contribute to cell survival and activation of downstream kinases. Indeed, as IκK regulates the activation of the NF-κB family of transcription factors, its over-activity has implications for a large number of NF-κB target genes including Bcl-2, X-IAP, c-FLIP and Mcl-1 (Hertlein et al. 2010). In fact, a number of these proteins have been implicated in the progression of CLL and have been found to correlate with poor prognosis (Pepper et al. 2008; Pepper et al. 2009) which may suggest a clear link to over-activity of NF-κB. Nevertheless, HSPC1 inhibitor treatment has been shown to destabilise Akt, Lyn and IκK (Hertlein et al. 2010; Johnson et al. 2007; Jones et al. 2004; Lin et al. 2007; Trentin et al. 2008), in CLL cells resulting in proteosomal degradation of these HSPC1 clients and ultimately apoptosis. A study by McCraig et al. (2011) showed that CD40 stimulation of CLL cells *in-vitro* increased the expression of the anti-apoptotic protein Mcl-1 and enhanced cell survival, suggesting that Mcl-1 may be responsible for *in-vivo* survival of CLL cells following CD40 stimulation by T-cells. 17-DMAG treatment of CLL cells was found to result in down-regulation of Mcl-1 even in the presence of CD40 stimulation and inhibit *in-vitro* survival (Hertlein et al. 2010; McCraig et al. 2011) suggesting that *in-vivo* treatment with 17-DMAG may prevent CLL survival associated with T-cell stimulation in lymph nodes.

The B-cell restricted enzyme, Activation-Induced Cytidine Deaminase (AID) has been shown to have prognostic significance in CLL (Heintel et al. 2004; Palacios et al. 2010). This enzyme is required in normal B-cells for somatic hypermutation and class switch recombination (CSR) and is induced following interaction of CD40 with CD40L on T-cells in germinal centres. However, AID expression has been found in CLL cells circulating in peripheral blood and has been found to correlate with unmutated IgV_H status and unfavourable cytogenetics (Heintel et al. 2004). AID expressing CLL cells were also shown to expresses high levels of anti-apoptotic proteins and proliferation factors (Palacios et al. 2010). It was proposed that these high levels of AID and ongoing CSR are a consequence of recent contact with the proliferation centres and provides further evidence that the microenvironment plays a critical role in CLL cell survival and disease progression. Interestingly, AID is an HSPC1 client and chemical inhibition of HSPC1 activity results in destabilisation and proteosomal degradation of AID and reduced antibody diversification (Orthwein et al. 2010).

It would appear that HSPC1 inhibitor treatment of CLL cells targets a wide variety of client proteins, many of which have been implicated in the progression of the disease.

Furthermore, HSPC1 inhibitor-induced cell death appears to be p53 independent suggesting that it may also be useful in the treatment of patients with a mutated p53 status. However, it is well established that CLL has an extremely heterogeneous clinical course and as a result patients show great variation in their responses to specific treatments. Results from our lab have shown that CLL patients also show great heterogeneity in HSP levels with some patients expressing very high levels of a particular HSP and some patients expressing extremely low levels (Dempsey et al. 2010a). These results indicate that although targeting HSPs may appear an attractive strategy, it may not be successful in all patients. Therefore, HSP analysis may prove useful in providing a personalised treatment.

7.4 Effect of HSPC1 inhibition on normal cells

As HSPC1 is abundant in non-tumour cells, the use of HSPC1 inhibitors as anti-cancer therapies may seem impractical. Indeed, the survival of normal cells following exposure to stressors such as ionising radiation or cytotoxic drugs is dependent upon activation of signal transduction pathways, the components of which are HSPC1 client proteins. This is important as in the clinical setting, both tumour and non-tumour cells may have been pre-exposed to cytotoxic drugs or ionising radiation as part of previous therapy. Little is known about the effects of HSPC1 inhibition on signal transduction pathways in stressed non-tumour cells and whether the response to HSPC1 inhibitors depends upon the pre-existing levels of HSPA1A and HSPB1 in target cells. Although HSPC1 inhibitors have been shown to be highly tumour-specific, this has recently been questioned (Gooljarsingh et al. 2006). If correct, introduction of these inhibitors into the systemic circulation, where they will have access to both tumour and non-tumour cells, may introduce complications and this issue warrants further investigation.

7.5 Targeting HSPA1A

At present, the modulation of HSPA1A activity using chemical inhibitors does not appear to be achievable in the clinical setting. Although, several compounds are able to inhibit the activity of HSF-1 and therefore regulate HSPA1A, the associated toxicity with HSF-1 and HSPA1A inhibitors is too severe. Furthermore, the inhibition of HSF-1 appears to have a number of associated effects such as the up-regulation of Hsp32, which itself is anti-apoptotic (Lin et al. 2004; Yao et al. 2007). The flavanoid quercetin and the benzylidene lactam KNK-437 have both been shown to inhibit HSPA1A and HSPB1 and sensitise cells to chemotherapeutic drugs or hyperthermia (Taba et al. 2011; Sahin et al. 2011; Zanini et al. 2007). Furthermore, the ability of KNK-437 to enhance the anti-tumour activity of HSPC1 inhibitors has been demonstrated (Guo et al. 2005), although as yet, this has not been tested on primary cells. These data suggest that if the solubility issues and non-specific effects of these inhibitors can be resolved, HSF-1 and/or HSPA1A may prove effective therapeutic targets. Recent work by Zaarur et al. (2006) used a high throughput screening programme to identify chemicals with the capacity to inhibit the heat shock response. Although a number of compounds were identified, one in particular, Emunin, showed very low levels of toxicity while sensitising cells to HSPC1 inhibitors and proteosome inhibitors. Interestingly, this compound appeared to have a novel mode of action as it did not affect HSF-1 activity. Although its exact mechanism remains to be determined, it appears to possess a specific regulatory affect on HSP protein translation. A further benefit of using this type of

compound is that if, as in some cases, the over-expression of HSPs in a tumour is HSF-1 independent (Zaarur et al. 2006), manipulating the stress response in this way may still prove advantageous.

The surface expression of HSPA1A on tumour cells provides a novel focus for anti-cancer therapies. The ability of membrane embedded HSPA1A to bind and stimulate NK-cells and induce killing of sHSPA1A+ tumour cells (Gross et al. 2003; Gross et al. 2008; Multhoff et al. 1999) has led to trials involving *ex-vivo* stimulation of autologous NK-cells with TKD peptide and low dose IL-2. This patient-specific technique involves obtaining leukocyte concentrates from the patient by leukapheresis, purifying PBMCs and stimulating them with TKD peptide/low dose IL-2 for four days. Following stimulation, the activated PBMC preparation is re-infused into the patient. Re-infusion is repeated every fortnight for a maximum of five doses (Krause et al. 2004). This technique has been shown to eliminate the primary tumour, prevent metastasis and significantly increase life expectancy in tumour mouse models (Mutlhoff et al. 2000; Stangl et al. 2006). Furthermore, use of this technique in patients with lower rectal carcinoma and non-small cell lung carcinoma was found to increase NK-cell cytotoxicity against sHSPA1A+ tumour cells (Krause et al. 2004) and was found to be well tolerated. However, it should be noted that patients included in this study did not show complete remission following treatment but these were patients with advanced disease and were refractory to standard chemotherapy. A subsequent case study using another advanced disease patient was unsuccessful in attaining remission, but did demonstrate the maintenance of NK-cell cytolytic activity following TKD-IL-2 stimulation (Milani et al. 2009). These data suggest that *ex-vivo* TKD/IL-2 stimulation of autologous NK-cells may have therapeutic potential.

As an alternative strategy to inhibiting the activity of HSPs, recent work from our lab attempted to manipulate the cellular location of HSPs in order to sensitise CLL cells to chemotherapeutic agents (Dempsey et al. 2010b). As mentioned earlier, a number of HSPs associate with lipid rafts and incorporate into the plasma membrane (Gastpar et al. 2005; Nagy et al. 2007; Vega et al. 2008) where they may remain or may be released into the extracellular environment. Vigh et al. (2007b) have proposed a 'membrane sensor model' in which cell stress may be detected at the membrane level and the cell is able to produce a stress response. It is proposed that stress signals originating from the cell membrane result in activation of specific HSP genes and movement of these newly synthesised HSPs to the membrane where they facilitate in stabilising the cell membrane (Vigh et al. 2007a) and therefore facilitating cell survival (Figure 2). It is proposed that a change in membrane fluidity and microdomain organisation (Torok et al. 2003; Vigh et al. 2007a) may be sufficient to result in such membrane stress signals. Our group used several membrane fluidising treatments including aliphatic alcohol, local anaesthetic or mild hyperthermia to fluidise the cell membrane and induce movement of HSPs to the cell surface (Dempsey et al. 2010b). The result was a change in the cellular localisation of HSPA1A, HSPD1, and to a lesser extent, HSPB1 and HSPC1. We found that this movement to the cell surface resulted in a transient decrease in internal levels of HSPs. This temporary decrease in anti-apoptotic HSPs allowed a number of chemotherapeutic agents including Doxorubicin, Cyclophosphamide and TRAIL to act on the cells resulting in significant cytotoxicity of CLL cells. Interestingly, by combining membrane fluidising treatments with chemotherapeutic agents, low doses of drugs were able to cause considerable apoptosis. These low doses were

unable to cause cytotoxicity in isolation. We found that inhibiting the movement of HSPs using methyl-β-cyclodextrin (mβcd), a cholesterol sequestering agent, and inhibitor of lipid raft transport, prior to combination treatment, prevented the synergistic effect of fluidising treatment and chemotherapeutic drug. The results suggest that manipulation of HSP cellular location may be an attractive strategy for enhancing the chemotherapeutic treatment of CLL. Furthermore, this method does not rely on CLL patients expressing similar levels of internal HSPs and therefore is an attractive therapeutic approach for a heterogeneous disease. Furthermore, the data may point to a revival of targeted hyperthermia to induce HSP translocation in combination with other therapeutic agents.

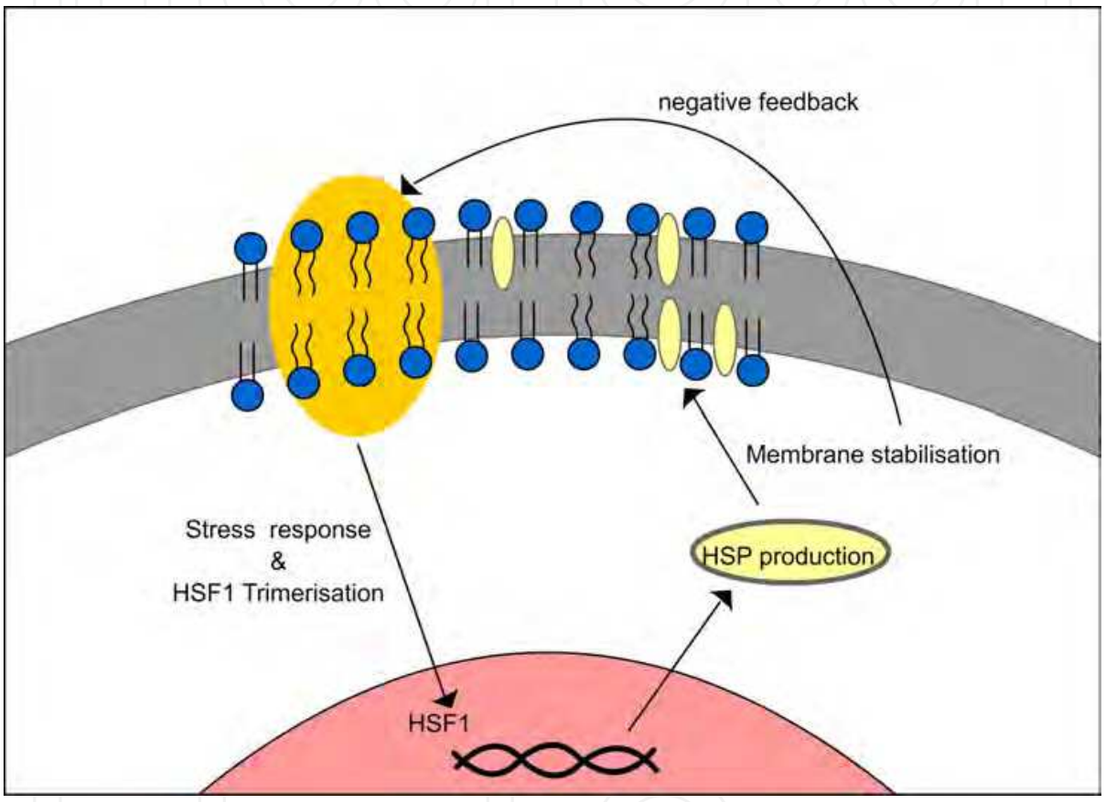


Fig. 2. Induction of an HSP Response via Alteration in Membrane Fluidity (Taken from Dempsey, 2009). Change in membrane fluidity is detected at the membrane, activating a membrane signal which results in the transcription of HSP genes. Newly synthesised HSPs then move to the membrane resulting in membrane stabilisation and re-established membrane-lipid order. This stabilisation effect may itself be a negative feedback regulator to turn off HSP transcription.

8. Conclusion

The up-regulation of HSPs in cancer has led to a large variety of studies investigating their prognostic and therapeutic potential. Due to the stressful nature of tumour development, it is not surprising that these anti-apoptotic proteins remain elevated in cancer cells. The anti-apoptotic characteristics of these proteins enable tumour cells to survive in otherwise lethal conditions and hence have been associated with a poor prognosis and resistance to therapy.

These elements alone suggest HSPs may be suitable targets for therapy. However after considering that a number of HSPs are involved in the modifications required for cancer development (the six 'hallmarks of cancer'), they become particularly attractive. Further work on inhibiting HSP activity is required before such compounds can be considered as routine therapies. Extracellular HSPs have been shown to have critical roles in cancer progression with surface-bound HSPC1 in particular contributing to cell invasion and metastasis. Conversely, sHSPA1A has been shown to be detrimental to the tumour, stimulating NK-cell responses and resulting in tumour regression, while a number of extracellular HSPs present tumour-derived peptides to APCs resulting in antigen cross-presentation and T-cell responses. The beneficial effects of these extracellular HSPs have also formed the basis of research into HSP-based therapies focusing on increasing the immune response against tumours. Increasing HSP translocation via cell membrane fluidisation may therefore have the double benefit of increasing the susceptibility of tumours to chemotherapeutic agents and stimulating the immune response. Our work on CLL has found that HSPA1A, HSPB1 and HSPC1 are located inside CLL cells, on the cell surface and also in the serum of CLL patients. However, the pattern of patient variability within and between specific HSPs suggests that analysis of patient HSP profile would be beneficial in directing therapy.

9. References

Adams, D.J. & McGuire, W.L. (1983). Quantitative Enzyme-linked Immunosorbent Assay for the Estrogen-regulated Mr 24,000 Protein in Human Breast Tumors: Correlation with Estrogen and Progesterone Receptors. *Cancer Research*. 45(6):2445-2449

An, W.G., Schulte, T.W. & Neckers, L.M. (2000). The heat shock protein 90 antagonist geldanamycin alters chaperone association with p210(bcr-abl) and v-src proteins before their degradation by the proteasome. *Cell Growth & Differentiation*. 11(7):355-360

Arnold-Schild, D., Hanau, D., Spehner, D., Schmid, C., Rammensee, H.G., de la Salle, H. & Schild, H. (1999). Cutting Edge: Receptor-Mediated Endocytosis of Heat Shock Proteins by Professional Antigen-Presenting Cells. *The American Association of Immunologists*. 162(7):3757-3760

Asea, A., Kraeft, S.K., Kurt-Jones, E.A., Stevenson, M.A., Chen, L.B., Finberg, R.W., Koo, G.C. & Calderwood, S.K. (2000). HSP70 stimulates cytokine production through a CD14-dependant pathway, demonstrating its dual role as a chaperone and cytokine. *Nature Medicine*. 6(4):435-442

Bailey, S., A. G. Hall, A. D. J. Pearson and C. P. F. Redfern (2001). The role of AP-1 in glucocorticoid resistance in leukaemia. *Leukaemia Research.* 15(3):391-397.

Barreto, A., Gonzalez, J.M., Kabingu, E.K., Asea, A. & Fiorentino, S. (2003). Stress-induced release of HSC70 from human tumors. *Cellular Immunology*. 222(2):97–104

Bartis, D., Boldizsar, F., Kvell, K., Szabo, M., Palinkas, L., Nemeth, P., Monostori, E. & Berki, T. (2007). Intermolecular relations between the glucocorticoid receptor, ZAP-70 kinase, and Hsp-90. *Biochemical and Biophysical Research Communications*. 354(1):253-258

Basu, S., Binder, R., Ramalingam, T. & Srivastava, P. (2001). CD91 is a common receptor for Heat Shock Proteins gp96, hsp90, hsp70, and calreticulin. *Immunity*. 14(3):303-313

Bausero,M.A., Gastpar, R., Multhoff, G. & Asea, A. (2005). Alternative Mechanism by which IFN-γ Enhances Tumor Recognition: Active Release of Heat Shock Protein 72. *Journal of Immunology*. 175(5):2900-2912

Becker, B., Multhoff, G., Farkas, B., Wild, P.J., Landthaler, M., Stolz, W. & Vogt, T. (2004). Induction of Hsp90 protein expression in malignant melanomas and melanoma metastases. *Experimental Dermatology*.13(1):27–32.

Beere, H.M. & Green, D.R. (2001). Stress management – heat shock protein-70 and the regulation of apoptosis. *Trends in Cell Biology*. 11(1):6-10

Bolhassani, A. & Rafati, S. (2008). Heat-shock proteins as powerful weapons in vaccine development. *Expert Review Vaccines*. 7(8):1185-1189

Boudeau, J., Deak, M., Lawlor, M.A., Morrice, N.A. & Alessi, D.R. (2003). Heat-shock protein 90 and Cdc37 interact with LKB1 and regulate its stability. *Journal of Biochemistry*. 370(3):849-857

Brameshuber, M., Weghuber, J., Ruprecht, V., Gombos, I., Horvath, I., Vigh, L., Eckerstorfer, P., Kiss, E., Stockinger, H. & Schutz, G.J. (2010). Imaging of Mobile Long-lived Nanoplatforms in the Live Cell Plasma Membrane. *The Journal of Biological Chemistry*. 285(53):41765-41771

Broemer, M., Krappmann, D. & Scheidereit, C. (2004). Requirment of Hsp90 acitvity for IκB kinase (IKK) biosynthesis and for constitutive and inducible IKK and NF-κB function. *Oncogene*. 23(31):5378-5386

Calderwood, S.K., Khaleque, M. A., Sawyer, D.B. & Ciocca, D.R. (2006). Heat shock proteins in cancer: chaperones of tumorigenesis. *Trends in Biochemical Sciences*. 31(3):164-172

Calderwood, S.K., Mambula, S.S., Gray Jr., P.J. & Theriault, J.R. (2007). Extracellular heat shock proteins in cell signalling. *FEBS Letters*. 581(19):3689-3694

Campos, L., Chautard, S., Viallet, A. & Guyotat, D. (1999). Expression of heat-shock proteins in acute leukaemia cells. *Haematologica*. 84:13.

Castro, J.E., Prada, C.E., Loria, O., Kamal, A., Chen, L., Burrows, F.J. & Kipps, T.J. (2005). ZAP-70 is a novel conditional heat shock protein 90 (Hsp90) client: inhibition of Hsp90 leads to ZAP-70 degradation, apoptosis, and impaired signaling in chronic lymphocytic leukemia. *Blood*. 106(7):2506-2512

Chant, I. D., Rose, P. E., & Morris, A. G. (1995). Analysis of Heat-Shock Protein Expression in Myeloid-Leukemia Cells by Flow-Cytometry. *British Journal of Haematology*. 90(1):163-168.

Chant, I. D., Rose, P. E., & Morris, A. G. (1996). Susceptability of AML cells to in vitro apoptosis correlates with heat shock protein 70 (hsp70) expression. *British Journal of Haematology*. 93(4):898-902.

Charette, S.J., Lavoie, J.N., Lambert, H. & Landry, J. (2000). Inhibition of Daxx-mediated apoptosis by heat shock protein 27. *Molecular and Cellular Biology*. 20(20):7602-7612

Chauhan, D., Li, G., Hideshima, T., Podar, K., Mitsiades, C., Mitsiades, N., Catley, L., Tai, Y.T., Hayashi, T., Shringarpure, R., Burger, R., Munshi, N., Ohtake, Y., Saxena, S. & Anderson, K.C. (2003a). Hsp27 inhibits release of mitochondrial protein Smac in multiple myeloma cells and confers dexamethasone resistance. *Blood*. 22(3):816-834

Chauhan D, Li GL, Shringarpure R, Podar K, Ohtake Y, Hideshima T, Anderson KC (2003b). Blockade of Hsp27 overcomes bortezomib/proteasome inhibitor PS-341 resistance in lymphoma cells. *Cancer Research*. 63(19):6174-6177

Chen, X., Tao, Q., Yu, H., Zhang, L. & Cao, X. (2002a). Tumor cell membrane-bound heat shock protein 70 elicits antitumor Immunity. *Immunology Letters*. 84(2):81-87

Chen, L.G., Widhopf, G., Huynh, L., Rassenti, L., Rai, K.R., Weiss, A. & Kipps, T.J. (2002b). Expression of ZAP-70 is associated with increased B-cell receptor signaling in chronic lymphocytic leukemia. *Blood*. 100(13):4609-4614

Ciocca, D.R. & Calderwood, S.K. (2005). Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications. *Cell Stress & Chaperones*. 10(2):86-103

Ciocca, D.R. & Calderwood, S.K. (2005). Heat shock proteins in cancer: diagnostic, prognostic, predictive and treatment implications. *Cell Stress & Chaperones*. 10(2):86-103

Ciocca, D.R. & Luque, E.H. (1991). Immunological Evidence for the Identity Between the Hsp27 Estrogen-Regulated Heat-Shock Protein and the P29 Estrogen Receptor-Associated Protein in Breast and Endometrial Cancer. *Breast Cancer Research and Treatment*. 20(1):33-42

Clayton, A., Turkes, A., Navabi, H., Mason, M.D. & Tabi, Z. (2005). Induction of heat shock proteins in B-cell exosomes. *Journal of Cell Science*. 118(16):3631-3638

Conde, R., Belak, Z.R., Nair, M., O'Carroll, R.F. & Ovsenek, N. (2009). Modulation of Hsf1 activity by novobiocin and geldanamycin. *International journal of Biochemistry and Cell Biology*. 87(6):845-851

Contri, A. Brunati, A.M., Trentin, L., Cabrelle, A., Miorin, M., Cesaro, L., Pinna, L.A., Zambello, R., Semenzato, G. & Donella-Deana, A. (2005). Chronic lymphocytic leukaemia B Cells contain anomalous Lyn tyrosine kinase, a putative contribution to defective apoptosis. *The Journal of Clinical Investigation*. 115(2):369-378

Cornford, P.A., Dodson, A.R., Parsons, K.F., Desmond, A.D., Woolfenden, A., Fordham, M., Neoptolemos, J.P., Ke, Y. & Foster, C.S. (2000). Heat Shock Protein Expression Independently Predicts Clinical Outcome in Prostate Cancer. *Cancer Research*. 60(24):7099-7105

Crespo, M., Bosch, F., Villamor, N., Bellosillo, B., Colomer, D., Rozman, M., Marce, S., Lopez-Guillermo, A., Campo, E. & Montserrat, E. (2003). Zap-70 expression as a surrogate for immunoglobulin-variable-region mutations in chronic lymphocytic leukemia. *N. Engl. J. Med*. 348, 1764–1775

Cruse, J.M., Lewis, R.E., Webb, R.N., Sanders, C.M. & Suggs, J.L. (2007). Zap-70 and CD38 as predictors of IgVH mutation in CLL. *Experimental and Molecular Pathology*. 83(3):459-461

Dakappagari, N. Neely, L. Tangri, S. Lundgren, K. Hipolito, L. Estrellado, A. Burrows, F & Zhang, H. (2010). An investigation into the potential use of Hsp70 as a novel tumour biomarker for Hsp90 inhibitors. *Biomarkers*. 15(1):31-38

Dempsey, N.C. (2009). *Heat Shock Protein Localisation in Haematological Malignancies*. Doctoral Thesis. University of Liverpool. Liverpool, UK.

Dempsey, N.C., Leoni, F., Ireland, H.E., Hoyle, C., & Williams, J.H.H. (2010). Differential Heat Shock Protein Localization in Chronic Lymphocytic Leukemia. *Journal of Leukocyte Biology*. 87(2):467-476.

Dempsey, N.C., Ireland, H.E., Smith, C.M., Hoyle, C.F. & Williams, J.H.H. (2010). Heat Shock Protein translocation induced by membrane fluidization increases tumor-cell sensitivity to chemotherapeutic drugs. *Cancer Letters*. 296(2):256-267

Duval, A., Olaru, D., Campos, L., Flandrin, P., Nadal, N. & Guyotat, D. (2006). Expression and prognostic significance of heat-shock proteins in myelodysplastic Syndromes. *Haematologica*. 91(5):713-714

Eccles, S.A., Massey, A., Raynaud, F.I., Sharp, S.Y., Box, G., Valenti, M.V., et al. (2008). NVP-AUY922: A Novel Heat Shock Protein 90 Inhibitor Active against Xenograft Tumor Growth, Angiogenesis, and Metastasis. *Cancer Research*. 86(8):2850-2860

Eustace BK, Sakurai T, Stewart JK, Yimlamai D, Unger C, Zehetmeier C, Lain B, Torella C, Henning SW, Beste G, Scroggins BT, Neckers L, Ilag LL, Jay DG. (2004). Functional proteomic screens reveal an essential extracellular role for hsp90 alpha in cancer cell invasiveness. *Nature Cell Biology*. 3(9):1098-1100

Faried, A., Sohda, M., Nakajima, M., Miyazaki, T., Kato, H. & Kuwano, H. (2004). Expression of heat-shock protein Hsp60 correlated with the apoptotic index and patient prognosis in human oesophageal squamous cell carcinoma. *European Journal of Cancer*. 40(18):2804-2811

Ferrarini, M., Heltai, S., Zocchi, M.R. & Rugarli, C. (1992). Unusual Expression and Localisation of Heat-Shock Proteins in Human Tumor-Cells. *International Journal of Cancer*. 51(4):613-619

Flaherty, K.T., Puznov, I., Kim, K.B., Ribas, I., McArther, G.A., Sosman, J.A., O'Dwyer, P.J., Lee, R.J., Grippo, J.F., Nolop, K. &Chapman, P.B (2010). Inhibition of Mutated, Activated BRAF in Metastatic Melanoma. The New England Journal of Medicine. 363(9):809-819

Garrido, C., Gurbuxani, S., Ravagnan, L. & Kroemer, G. (2001). Heat Shock Proteins: Endogenous Modulators of Apoptotic Cell Death. *Biochemical & Biophysical Research Communications*. 286(3):433-442

Gastpar, R., Gehrmann, M., Bausero, M.A., Asea, A., Gross, C., Schroeder, J.A. & Multhoff, G. (2005). Heat Shock Protein 70 Surface-Positive Tumor Exosomes. *Cancer Research*. 5;65(12):5238-47.

Gehrmann, M., H. Schmetzer, G. Eissner, T. Haferlach, W. Hiddemann and G. Multhoff (2003). Membrane-bound heat shock protein 70 in acute myeloid leukemia: a tumor-specific recognition structure for the cytolytic activity of autologous natural killer cells. *Haematologica*. 88(4): 474-476.

Gehrmann, M., Radons, J., Molls, M. & Multhoff, G. (2008). The therapeutic implications of clinically applied modifiers of heat shock protein 70 (Hsp70) expression by tumor cells. *Cell Stress & Chaperones*. 13(1):1-10

Geisler, J.P., Tammela, J.E., Manahan, K.J., Geisler, H.E., Miller, G.A., Zhou, Z. & Wiemann, M.C. (2004). HSP27 in patients with ovarian carcinoma: still an independent prognostic indicator at 60 months follow-up. *European Journal of Gynaecological Oncology*. 25(2):165-168

Giaginis, C., Daskalopoulou, S.S., Vgenopoulou, S., Sfiniadakis, I., Kouraklis, G. & Theocharis, S.E. (2009). Heat Shock Protein-27, -60 and -90 expression in gastric cancer: association with clinicopathological variables and patient survival. *BMC Gastroenterology*. 9:1-10

Glaessgen, A., Jonmarker, S., Lindberg, A., Nilsson, B., Lewensohn, R., Ekman, P., Valdman, A. & Egevad, L. (2008). Heat shock proteins 27, 60 and 70 as prognostic markers of prostate cancer. *APMIS*. 116(10):888-895

Gooljarsingh, L.T. Fernandes, C. Yan, K. Zhang, H. Grooms, M. Johanson, K. Sinnamon, R.H. Kirkpatrick, R. B. Kerrigan, J. Lewis, T. Arnone, M. King, A.J. Lai, Z. Copeland, R.A. & Tummino, P.J. (2006). A Biochemical rationale for the anticancer effects of Hsp90 inhibitors: slow, tight binding inhibition by geldanamycin and its analogues. *PNAS*. 103(20):7625-7630

Gross, C., Hansch, D., Gastpar, R. & Multhoff, G. (2003). Interaction of Heat Shock Protein 70 Peptide with NK Cells Involves the NK Receptor CD94. *Biological Chemistry*. 384(2):267 – 279

Gross, C., Holler, E., Stangl, S., Dickinson, A., Pockley, A.G., Asea, A.A., Mallappa, N., & Multhoff, G. (2008). An Hsp70 peptide initiates NK cell killing of leukemic blasts after stem cell transplantation. *Leukemia Research*. 32(4):527-534

Guo, F., Sigua, C., Bali, P., George, P., Fiskus, W., Scuto, A. & Annavarapu, S. (2005). Mechanistic role of heat shock protein 70 in Bcr-Abl–mediated resistance to apoptosis in human acute leukemia cells. *Blood*. 105(3):1246-1255

Hanahan, D. & Weinberg, R.A. (2000). The Hallmarks of Cancer. *Cell*. 100(1):57–70

Hanahan, D. & Weinberg, R.A. (2011). Hallmarks of Cancer: The Next Generation. *Cell*. 144(5):646-674

Hanson, B.E. & Vesole, D.H. (2009). Retaspimycin hydrochloride (IPI-504): a novel heat shock protein inhibitor as an anti-cancer agent. *Expert Opinion on Investigational Drugs*. 18(9):1375-1383

Heintel, D., Kroemer, E., Kienle, D., Schwarzinger, I., Gleiss, a, Schwarzmeier, J., Marculescu, R., et al. (2004). High expression of activation-induced cytidine deaminase (AID) mRNA is associated with unmutated IGVH gene status and unfavourable cytogenetic aberrations in patients with chronic lymphocytic leukaemia. *Leukemia*. 18(4):756-62

Hertlein, E. Wagner, A.J. Jones, J. Lin, T.S. Maddocks, K.J. Towns, W.H. Goettl, V.M. Zhang, X. Jarjoura, D. Raymond, C.A. West, D.A. Croce, C.M. Byrd, J.C. & Johnson, A.J. (2010). 17-DMAG targets the Nuclear-Factor-{kappa}B family of proteins to induce apoptosis in Chronic Lymphocytic Leukaemia: clinical implications of Hsp90 inhibition. *Blood*. 116(1):45-53

Holzbeierlein, J.M., Windsperger, A. & Vielhauer, G. (2010). Hsp90: A Drug Target? *Current Oncology Reports*. 12:95-101

Hightower, L. E., Guidon, P. T. (1989) Selective release from cultured mammalian cells of heat-shock (stress) proteins that resemble glia-axon transfer proteins. *Journal Cellular Physiology*. 138:257–266.

Hunter-Lavin, C., Davies, E. L., Bacelar, M., Marshall, M. J., Andrew, S. M. and Williams, J. H. H. (2004). Hsp70 release from peripheral blood mononuclear cells. *Biochemical and Biophysical Research Communications.* 324(2):511-517.

Huntoon, C.J., Nye, M.D., Geng, L., Peterson, K.L., Flatten, K.S., Haluska, P., Kaufmann, S.H., Karnitz, L.M. (2010). Heat shock protein 90 inhibition depletes LATS1 and LATS2, two regulators of the mammalian hippo tumor suppressor pathway. *Cancer Research*. 70(21):8642-8650

Iwaya, K., Tsuda, H., Fujita, S., Suzuki, M. & Hirohashi, S. (1995). Natural State of Mutant P53 Protein and Heat-Shock Protein-70 in Breast-Cancer Tissues. *Laboratory Investigation*. 72(6):707-714

Jaattela, M. (1999). Escaping Cell Death: Survival Proteins in Cancer. *Experimental Cell Research*. 248:30-43

Johnson, A.J., Wagner, A.J., Cheney, C.M., Smith, L.L., Lucas, D.M., Guster, S.K., Grever, M.R., Lin, T.S. & Byrd, J.C. (2007). Rituximab and 17-allylamino-17-demethoxygeldanamycin induce synergistic apoptosis in B-cell chronic lymphocytic leukaemia. *British Journal of Haematology*. 139(5):837-844

Jones, D.T., Addison, E., North, J.M., Lowdell, M.W., Hoffbrand, A.V., Mehta, A.B., Ganeshaguru, K., Folarin, N.I. & Wickremasinghe, R.G. (2004). Geldanamycin and herbimycin A induce apoptotic killing of B chronic lymphocytic leukemia cells and augment the cells' sensitivity to cytotoxic drugs. *Blood*. 103(5):1855-1861

Kampinga, H.H., Hageman, J., Vos, M.J., Kubota, H., Tanguay, R.M., Bruford, E.A., Cheetham, M.E., Chen, B. & Hightower, L.E. (2009). Guidelines for the nomenclature of the human heat shock proteins. *Cell Stress and Chaperones*. 14(1):105-111

Kantarjian, H.M.,O'Brien, S.,Cortes, J., Giles, F.J.,Rios, M.B., Shan, J.Q., Faderl, S., Garcia-Manero, G., Ferrajoli, A., Verstovsek, S., Wierda, W., Keating, M. & Talpaz, M. (2003). Imatinib mesylate therapy improves survival in patients with newly diagnosed Philadelphia chromosome-positive chronic myelogenous leukemia in the chronic phase - Comparison with historic data. *Cancer*. 98(12):2636-2642

Kawanishi, K., Shiozaki, H., Doki, Y., Sakita, I., Inoue, M., Yano, M., Tsujinaka, T., Shamma, A. & Monden, M. (1999). Prognostic significance of heat shock proteins 27 and 70 in patients with squamous cell carcinoma of the esophagus. *Cancer*. 85(8):1649-1657

Khaleque, M.A., Bharti, A., Sawyer, D., Gong, J.L., Benjamin, I.J., Stevenson, M.A. & Calderwood, S.K. (2005). Induction of heat shock proteins by heregulin beta 1 leads to protection from apoptosis and anchorage-independent growth. *Oncogene*. 24(43): 6564-6573

Kim, Y. S. Alarcon, S. V. Lee, S. Lee, M.-J. Giaccone, G. Neckers, L. & Trepel, J. B. (2009). Update on Hsp90 inhibitors in clinical trial. *Current Topics in Medicinal Chemistry*. 9(15):1479-1492

Kleinjung, T., Arndt, O., Feldmann, H., Bockmühl, U., Gehrmann, M., Zilch, T., Pfister, K., Schönberger, J., Marienhagen, J. & Eilles, C. (2003). Heat shock protein 70 (Hsp70) membrane expression on head-and-neck cancer biopsy - a target for natural killer (NK) cells. *International Journal of Radiation Oncology Biology Physics*. 57(3):820-826

Krause, S.W., Gastpar, R., Andreesen, R., Gross, C., Ullrich, H., Thonigs, G., Pfister, K. & Multhoff, G. (2004). Treatment of colon and lung cancer patients with ex vivo heat shock protein 70-peptide-activated, autologous natural killer cells: a clinical phase I trial. *Clinical Cancer Research*. 10(11):3699-3707

Krukenberg, K.A., Street, T.O., Lavery, L.A. & Agard, D.A. (2011). Conformational dynamics of the molecular chaperone Hsp90. *Quarterly Reviews of Biophysics*. 44(2):229-255

Kuppner, M.C., Gastpar, R., Gelwer, S., Nossner, E., Ochmann, O., Scharner, A. & Issels, R.D. (2001). The role of heat shock protein (hsp70) in dendritic cell maturation: Hsp70 induces the maturation of immature dendritic cells but reduces DC differentiation from monocyte precursors. *European Journal of Immunology*. 31(5):1602-1609

Kurotaki, T., Tamura, Y., Ueda, G., Oura, J., Kutomi, G., Hirohashi, Y., Sahara, H., Torigoe, T., Hiratsuka, H., Sunakawa, H., Hirata, K. & Sato, N. (2007). Efficient cross-presentation by heat shock protein 90-peptide complex-loaded dendritic cells via an endosomal pathway. *Journal of Immunology*. 179(3):1803-1813

Lanneau, D., Brunet, M., Frisan, E., Solary, E., Fontenay, M., Garrido, C. (2008). Heat shock proteins: essential proteins for apoptosis regulation. *Journal of Cellular and Molecular Medicine*. 12(3):743-761

Le Blanc, A.C. (2003). Natural cellular inhibitors of caspases. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*. 27:215-229

Lehman, T.A., Bennett, W.P., Metcalf, R.A., Welsh, J.A., Ecker, J., Modali, R.V., Ullrich, S., Romano, J.W., Appella, E., Testa, J.R., Gerwin, B.I. & Harris, C.C. (1991). P53 Mutations, Ras Mutations and P53-Heat Shock 70 Protein complexes in Human Lung-Carcinoma Cell-Lines. *Cancer Research*. 51(15):4090:4096

Li, Y., Zhang, T., Schwartz, S.J. & Sun, D. (2009). New developments in Hsp90 inhibitors as anti-cancer therapeutics: Mechanisms, clinical perspective and more potential. *Drug Resistance Updates*. 12(1-2)17-27

Lin, K., Rockliffe, N., Johnson, G.G., Sherrington, P.D. & Pettitt, A.R. (2007). Hsp90 inhibition has opposing effects on wild-type and mutant p53 and induces p21 expression and cytotoxicity irrespective of p53/ATM status in chronic lymphocytic leukaemia cells. *Oncogene*. 27(17): 2445-2455

Love, S. & King, R.J.B. (1994). A 27kDa Heat-Shock-Protein that has anomalous prognostic powers in early and advanced breast-cancer. *British Journal Of Cancer*. 69(4):743-748

Lundgren, K., Zhang, H., Brekken, J., Huser, N., Powell, R.E., Timple, N., Busch, D.J., Neely, L., Sensintaffar, J.L., Yang, Y.C., McKenzie, A., Friedman, J., Scannevin, R., Kamal, A., Hong, K., Kasibhatla, S.R., Boehm, M.F., Burrows, F.J. (2009). BIIB021, an orally available, fully synthetic small-molecule inhibitor of the heat shock protein Hsp90. *Molecular Cancer Therapeutics*. 8(4):921-929

Mambula, S.S. & Calderwood, S.K. (2006). Heat induced release of Hsp70 from prostate carcinoma cells involves both active secretion and passive release from necrotic cells. *International Journal of Hyperthermia*. 22(7):575-585

Matysiak, M., B. Makosa, A. Walczak and K. Selmaj (2008). Patients with multiple sclerosis resisted to glucocorticoid therapy: abnormal expression of heat-shock protein 90 in glucocorticoid receptor complex. *Multiple Sclerosis* 14(7): 919-926

McCraig, A.M., Cosimo, E., Leach, M.T. & Michie, A.M. (2011). Dasatinib inhibits B cell receptor signalling in chronic lymphocytic leukaemia but novel combination approaches are required to overcome additional pro-survival microenvironmental signals. *British Journal of Haematology*. 153:199-211

McCready, J., Sims, J.D., Chan, D. & Jay, D.J. (2010). Secretion of extracellular Hsp90α via exosomes increases cancer cell motility: a role for plasminogen activation. *BMC Cancer*. 10:294-304

McLaughlin, S.H., Smith, H.W. & Jackson, S.E. (2002). Stimulation of the Weak ATPase Activity of Human Hsp90 by a Client Protein. *Journal of Molecular Biology*. 315:787-798

Milicevic, Z. T., Petkovic, M. Z., Drndarevic, N. C., Pavlovic, M. D., & Todorovic, V. N. (2007). Expression of heat shock protein 70 (HSP70) in patients with colorectal adenocarcinoma - immunohistochemistry and Western blot analysis. *Neoplasma*. 54(1):37-45.

Minami, Y., Kiyoi, H., Yamamoto, Y., Ueda, R., Saito, H. & Naoe, T. (2002). Selective apoptosis of tandemly duplicated FLT3-transformed leukemia cells by Hsp90 inhibitors. *Leukemia*. 16(8): 1535-1540

Modi, S., Stopeck, A.T., Gordon, M.S., Mendelson, D., Solit, D.B., Bagatell, R., Ma, W., Wheler, J., Rosen, N., Norton, L., Cropp, G.F., Johnson, R.G., Hannah, A.L. & Hudis, C.A. (2007). Combination of Trastuzumab and Tanespimycin (17-AAG, KOS-953) Is Safe and Active in Trastuzumab-Refractory HER-2–Overexpressing Breast Cancer: A Phase I Dose-Escalation Study. *Journal of Clinical Oncology*. 25(34):5410-5417

Murshid, A., Gong, J. & Calderwood, S.K. (2008). Heat-shock proteins in cancer vaccines: agents of antigen cross presentation. *Expert Reviews in Vaccines*. 7(7):1019-1030

Nagy, E., Balogi, Z., Gombos, I., Akerfelt, M., Bjorkbom, A., Balogh, G., Torok, Z., Maslyanko, A., Fiszer-Kierzkowska, A., Lisowska, K., Slotte, P.J., Sistonen, L., Horvath, I. & Vigh, L. (2007). Hyperfluidisation-coupled membrane microdomain reorganisation is linked to activation of the heat shock response in a murine melanoma cell line. *PNAS*. 104(19):7945-7950

Nazarian, R., Shi, H., Wang, Q., Kong, X., Koya, R.C., Lee, H., Chen, Z., Lee, M.K., Attar, N., Sazegar, H., Chodon, T., Nelson, S.F., McArthur, G., Sosman, J.A., Ribas, A. & Lo, R.S. (2010). Melanomas acquire resistance to B-RAF(V600E) inhibition by RTK or N-RAS upregulation. *Nature*. 468(7326):973-977

Neckers, L. (2007). Heat shock protein 90: the cancer chaperone. *Journal of Bioscience*. 32(3):517–530

Nimmanapalli, R., O'Bryan, E., Huang, M., Bali, P., Burnette, P.K., Loughran, T., Tepperberg, J., Jove, R. & Bhalla, K. (2002). Molecular Characterization and Sensitivity of STI-571 (Imatinib Mesylate, Gleevec)- resistant, Bcr-Abl-positive, Human Acute Leukemia Cells to SRC Kinase Inhibitor PD180970 and 17-Allylamino-17-demethoxygeldanamycin. *Cancer Research*. 62:5761–5769,

Nylandsted, J., Brand, K., & Jaattela, M. (2000). Heat shock protein 70 is required for the survival of cancer cells. *Mechanisms of Cell Death*. 926:122-125.

Okawa, Y., Hideshima, T., Steed, P., Vallet, S., Hall, S., Huang, K., Rice, J., Barabasz, A., Foley, B., Ikeda, H., Raje, N., Kiziltepe, T., Yasui, H., Enatsu, S., Anderson, K.C. (2009). SNX-2112, a selective Hsp90 inhibitor, potently inhibits tumor cell growth, angiogenesis, and osteoclastogenesis in multiple myeloma and other hematologic tumors by abrogating signaling via Akt and ERK. *Blood*. 113(4):846-855

Orthwein, A., Patenaude, A.-M., Affar, E. B., Lamarre, A., Young, J. C., & Di Noia, J. M. (2010). Regulation of activation-induced deaminase stability and antibody gene diversification by Hsp90. *The Journal of experimental medicine*. 207(12):2751-65

Palacios, F., Moreno, P., Morande, P., Abreu, C., Correa, A., Porro, V., Landoni, A. I., et al. (2010). High expression of AID and active class switch recombination might account for a more aggressive disease in unmutated CLL patients: link with an activated microenvironment in CLL disease. *Blood*. 115(22):4488-96

Pashtan, I., Tsutsumi, S., Wang, S.Q., Xu, W.P., Neckers, L. (2008). Targeting Hsp90 prevents escape of breast cancer cells from tyrosine kinase inhibition. *Cell Cycle*. 7(18): 2936-2941

Paul, C., Manero, F., Gonin, S., Kretz-Remy, C., Virot, S. & Arrigo, A.P. (2002). Hsp27 as a negative regulator of cytochrome c release. *Molecular and Cellular Biology*. 22(3):816-834

Peng, C., Li, D. & Li, S. (2007). Heat Shock Protein 90. A Potential Therapeutic Target in Leukemic Progenitor and Stem Cells Harboring Mutant BCR-ABL Resistant to Kinase Inhibitors. *Cell Cycle*. 6(18):2227-2231

Pepper, C., Lin, T.T., Pratt, G., Hewamana, S., Brennan, P., Hiller, L., Hills, R., Ward, R., Starczynski, J., Austen, B., Hooper, L., Stankovic, T. & Fegan, C. (2008). Mcl-1 expression has in vitro and in vivo significance in chronic lymphocytic leukemia and is associated with other poor prognostic markers. *Blood*. 112(9):3807-3817

Pepper, C., Hewamana, S., Brennan, P. & Fegan, C. (2009). NF-kappaB as a prognostic marker and therapeutic target in chronic lymphocytic leukemia. *Future Oncology*. 5(7):1027-37

Pfister, K., Radons, J., Busch, R., Tidball, J.G., Pfeifer, M., Freitag, L., Feldmann, H.J., Milani, V., Issels, R. & Multhoff, G. (2007). Patient survival by Hsp70 membrane phenotype. *American Cancer Society*. 110(4):926-935

Pick, E., Kluger, Y., Giltnane, J.M., Moeder, C., Camp, R.L., Rimm, D.L. & Kluger, H.M. (2007). High HSP90 expression is associated with decreased survival in breast cancer. *Cancer Research*. 67(7): 2932-7.

Ramalingam, S.S., Egorin, M.J., Ramanathan, R.K., Remick, S.C., Sikorski, R.P., …Lagattuta, T.F., Chatta, G.S., Friedland, D.M., Stoller, R.G., Potter, D.M., Ivy, S.P. & Belani, C.P. (2008). A phase I study of 17-allylamino-17-demethoxygeldanamycin combined with paclitaxel in patients with advanced solid malignancies. *Clinical Cancer Research*. 14(11): 3456-3461

Ramp, U., Mahotka, C., Heikaus, S., Shibata, T., Grimm, M.O., Willers, R. & Gabbert, H.E. (2007). Expression of heat shock protein 70 in renal cell carcinoma and its relation to tumor progression and prognosis. *Histology and Histopathology*. 22:1019-1107

Ravagnan, L., Gurbuxani, S., Susin, A., Maisse, C., Daugas, E., Zamzami, N., Mak, T., Jaatella, Penninger, J.M., Garrido, C. & Kroemer, G. (2001). Heat-shock protein 70 antagonizes apoptosis-inducing factor. *Nature Cell Biology*. 3(9): 839-843

Richards, E.H., Hickey, E., Weber, L., Masters, J.R.W. (1996). Effect of overexpression of the small heat shock protein HSP27 on the heat and drug sensitivities of human testis tumor cells. *Cancer Research*. 56(10):2446-2451

Richardson, P.G., Badros, A.Z., Jagannath, S., Tarantolo, S., Wolf, J.L., Albitar, M., Berman, D., Messina, M. & Anderson, K.C. (2010). Tanespimycin with bortezomib: activity in relapsed/refractory patients with multiple myeloma. *British Journal of Haematology*. 150(4):428-437

Ringshausen, I., Schneller, F., Bogner, C., Hipp, S., Duyster, J., Peschel, C., Decker, T. (2002). Constitutively activated phosphatidylinositol-3 kinase (PI-3K) is involved in the defect of apoptosis in B-CLL: association with protein kinase Cdelta. *Blood*. 100(10):3741-3748.

Robles, A.I., Wright, M.H., Gandhi, B., Feis, S.S., Hanigan, C.L., Wiestner, A. & Varticovski, L. (2006). Schedule-dependent synergy between the heat shock protein 90 inhibitor 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin and doxorubicin restores apoptosis to p53-mutant lymphoma cell lines. *Clinical Cancer Research*. 12(21): 6547-6556

Romani, A.A., Crafa, P., Desenzani, S., Graiani, G., Lagrasta, C., Sianesi, M., Soliani, P. & Borghetti, A.F. (2007). The expression of HSP27 is associated with poor clinical outcome in intrahepatic cholangiocarcinoma. *BMC Cancer*. 7(232):232

Sagol, O., Tuna, B., Coker, A., Karademir, S., Obuz, F., Astarcioglu, H., Kupelioglu, A., Astarcioglu, I. & Topalak, O. (2002). Immunohistochemical detection of PS2 protein and heat shock protein-70 in pancreatic adenocarcinomas. relationship with disease extent and patient survival. *Pathology Research & Practice*.198(2):77-84.

Sahin, E., Sahin, M., Sanlioğlu, A.D. & Gümüslü, S. (2011). KNK437, a benzylidene lactam compound, sensitises prostate cancer cells to the apoptotic effect of hyperthermia. *International Journal of Hyperthermia*. 27(1):63-73

Samali, A., Cai, J., Zhivotovsky, B., Jones, D.P. & Orrenius, S. (1999). Presence of a pre-apoptotic complex of pro-caspase-3, Hsp60 and Hsp10 in the mitochondrial fraction of Jurkat cells. *EMBO Journal*. 18(8):2040-2048

Samali, A., Robertson, J.D., Peterson, E., Manero, F., van Zeijl, L., Paul, C., Cotgreave, I.A., Arrigo, A.P. & Orrenius, S. (2001). Hsp27 protects mitochondria of thermotolerant cells against apoptotic stimuli. *Cell Stress and Chaperones*. 6(1):49-58

Santarosa, M., Favaro, D., Quaia, M. & Galligioni, E. (1997). Expression of heat shock protein 72 in renal cell carcinoma: Possible role and prognostic implications in cancer patients. *European Journal of Cancer*. 33(6):873-877

Sato, S., Fujita, N. & Tsuruo, T. (2000). Modulation of Akt kinase activity by binding to Hsp90. *PNAS*. 97(20): 10832–10837

Schepers, H., Geugien, M., van der Toorn, M., Bryantsev, A.L., Kampinga, H.H., Eggen, B.J.L. & Vellenga, E. (2005). HSP27 protects AML cells against VP-16-induced apoptosis through modulation of p38 and c-Jun. *Experimental Haematology*. 33(6):660-670

Schilling, D., Gehrmann, M., Steinem, C., De Maio, A., Pockley, A.G., Abend, M., Molls, M. Multhoff, G. (2007). Binding of heat shock protein 70 to extracellular phosphatidylserine promotes killing of normoxic and hypoxic tumor cells. *FASEB Journal*. 23(8):2467-77

Schneider, J., Jimenez, E., Marenbach, K., Romero, H., Marx, D. & Meden, H. (1999). Immunohistochemical detection of HSP60-expression in human ovarian cancer. Correlation with survival in a series of 247 patients. *Anticancer Research*. 19(3A): 2141-2146

Shah, N.P., Nicoll, J.M., Nagar, B., Gorre, M.E., Paquette, R.L., Kuriyan, J. & Sawyers, C.L. (2002). Multiple BCR-ABL kinase mutations confer polyclonal resistance to the tyrosine kinase inhibitor Imatinib (STI571) in chronic phase and blast crisis chronic myeloid leukemia. *Cancer Cell*. 2:117-125

Shahidi, H., A. Vottero, C. A. Stratakis, S. E. Taymans, M. Karl, C. A. Longui, G. P. Chrousos, W. H. Daughaday, S. A. Gregory and J. M. D. Plate (1999). Imbalanced expression of the glucocorticoid receptor isoforms in cultured lymphocytes from a patient with systemic glucocorticoid resistance and chronic lymphocytic leukemia. *Biochemical and Biophysical Research Communications.* 254(3): 559-565.

Shan, Y.X., Liu, T.J., Su, H.F., Samsamshariat, A., Mestril, R. & Wang, P.H. (2003). Hsp10 and Hsp60 modulate Bcl-2 family and mitochondria apoptosis signaling induced by doxorubicin in cardiac muscle cells. *Journal of Molecular and Cellular Cardiology*. 35(9):1135-1143

Sherman, M. & Multhoff, G. (2007). Heat Shock Proteins in Cancer. *Annals of the New York Academy of Sciences*. 1113:192-201

Shotar, A. M. (2005). P53 and heat shock protein 70 expressions in colorectal adenocarcinoma. *Saudi Medical Journal*. 26(10):1602-1606.

Sidera, K., Gaitanou. M., Stellas, D., Matsas, R. & Patsavoudi, E. (2008). A Critical Role for HSP90 in Cancer Cell Invasion Involves Interaction with the Extracellular Domain of HER-2. *The Journal of Biological Chemistry*. 283(4):2031–2041

Shin BK, Wang H, Yim AM, Le Naour F, Brichory F, Jang JH, Zhao R, Puravs E, Tra J, Michael CW, Misek DE, Hanash SM. (2003). Global profiling of the cell surface proteome of cancer cells uncovers an abundance of proteins with chaperone function. *Journal of Biological Chemistry*. 278(9):7607-1

Sorger, P.K. (1991). Heat Shock Factor and the Heat Shock Response. *Cell*. 65(3):363-366

Soti, C., Racz, A. & Csermely, P. (2002). A Nucleotide-Dependent Molecular Switch Controls ATP Binding at the C-Terminal Domain of Hsp90. *The Journal of Biological Chemistry*. 277(9):7066-7075

Sreedhar, A.S., Soti, C. & Csermely, P. (2004). Inhibition of Hsp90: a new strategy for inhibiting protein kinases. *Biochemica & Biophysica Acta*. 1697:233-242

Steiner, K., Graf, M., Hecht, K., Reif, S., Rossbacher, L., Pfister, K., Kolb, H.J., Schmetzer, H.M. & Multhoff, G. (2006). High HSP70-membrane expression on leukemic cells from patients with acute myeloid leukemia is associated with a worse prognosis. *Leukemia*. 20:2076–2079

Sturm, I., Bosanquet, AG., Hermann, S., Guner, D., Dorken, B. & Daniel, P.T. (2003). Mutation of p53 and consecutive selective drug resistance in B-CLL occurs as a

consequence of prior DNA-damaging chemotherapy. *Cell Death & Differentiation*. 10:477-484

Taba, K., Kuramitsu, Y., Ryozawa, S.,Yoshida, K., Tanaka, T., Mori-Iwamoto, S., Maehara, S., Maehara, Y., Sakaida, I., Nakamura, K. (2011). KNK437 Downregulates Heat Shock Protein 27 of Pancreatic Cancer Cells and Enhances the Cytotoxic Effect of Gemcitabine. *Chemotherapy*. 57(1):12-16

Taldone, T., Gozman, A., Maharaj, R. & Chiosis, G. (2008). Targeting Hsp90: small-molecule inhibitors and their clinical development. *Current Opinion in Pharmacology*. 8(4):370-4

Tauchi, K., Tsutsumi, Y., Hori, S., Yoshimura, S., Osamura, R.Y. & Watanabe, K. (1991). Expression of Heat-Shock Protein-70 and C-Myc Protein in Human Breast-Cancer – An Immunohistochemical Study. *Japanese Journal of Clinical Oncology*. 21(4):256-263

Thomas, X., Campos, L., Mounier, C., Cornillon, J., Flandrin, P., Le, Q.H., Piselli, S. & Guyotat, D. (2005). Expression of heat-shock proteins is associated with major adverse prognostic factors in acute myeloid leukemia. *Leukaemia Research*. 29:1049-1058

Todryk, S., Melcher, A.A., Harwick, N., Linardakis, E., Bateman, A., Colombo, M.P., Stoppacciaro, A. & Vile, R.G. (1999). Heat shock protein 70 induced during tumor cell killing induces Th1 cytokines and targets immature dendritic cell precursors to enhance antigen uptake. *Journal of Immunology*. 163(3):1398-1408

Todryk, S., Melcher, A.A., Dalgleish, A.G., Vile, R.G. (2000). Heat shock proteins refine the danger theory. *Immunology*. 99:334-337

Torok, Z., Tsvetkova, N.M., Balogh, G., Horvath, I., Nagy, E., Penzes, Z., Hargitai, J., Bensaude, O., Csermely, P., Crowe, J.H., Maresca, B. & Vıgh, L. (2003). Heat shock protein coinducers with no effect on protein denaturation specifically modulate the membrane lipid phase. *PNAS*. 100(6):3131–3136

Trentin, L., Frasson, M., Donella-Deana, A., Frezzato, F., Pagano ,M.A., Tibaldi, E., Gattazzo, C., Zambello, R., Semenzato, G. & Brunati, A.M. (2008). Geldanamycin-induced Lyn dissociation from aberrant Hsp90-stabilized cytosolic complex is an early event in apoptotic mechanisms in B-chronic lymphocytic leukemia. *Blood*. 112(12):4665-74

Tsutsumi, S., Scroggins, B., Koga, F., Lee, M.J., Trepel, J., Felts, S., Carreras, C. & Neckers, L. (2008). A small molecule cell-impermeant Hsp90 antagonist inhibits tumor cell motility and invasion. *Oncogene*. 27: 2478–2487

Urushibara, M., Kageyama, Y., Akashi, T., Otsuka, Y., Takizawa, T,. Koike, M. & Kihara, K. (2007). HSP60 may predict good pathological response to neoadjuvant chemoradiotherapy in bladder cancer. *Japanese Journal of Clinical Oncology*. 37(1):56-61

Vargas-Roig, L.M., Gago, F.E., Tello, O., Anzar, J.C. & Ciocca, D.R. (1998). Heat Shock Protein Expression and Drug Resistance in breast cancer patients treated with induction chemotherapy. *International Journal of Cancer*. 79:468-475

Vega, V.L., Rodrıguez-Silva, M., Frey, T., Gehrmann, M., Diaz, J.C., Steinem, C., Multhoff, G., Arispe, N. & De Maio, A. (2008). Hsp70 Translocates into the Plasma Membrane after Stress and Is Released into the Extracellular Environment in a Membrane-

Associated Form that Activates Macrophages. *Journal of Immunology*. 180(6):4299-4307

Vigh, L., Horvath, I., Maresca, B. & Harwood, J.L. (2007a). Can the stress protein response be controlled by 'membrane-lipid therapy'? *Trends in Biochemical Sciences*. 32(8):357-363

Vigh, L., Nakamoto, H., Landry, J., Gomez-Munoz, A., Harwood, J.L. & Horvath, I. (2007b). Membrane Regulation of the Stress Response from Prokaryotic Models to Mammalian Cells. *Annals of the New York Academy of Sciences*. 1113:40–51

Walerych, D., Kudla, G., Gutkowska, M., Wawrzynow, B., Muller, L., King, F.W., Helwak, A., Boros, J., Zylicz, A., Zylicz, M. (2004). Hsp90 chaperones wild-type p53 tumor suppressor protein. *Journal of Biological Chemistry*. 279(47):48836-45

Wei, Y.Q., Zhao, X., Kariya, Y., Fukata, H., Teshigawara, K. & Uchida, A. (1996). Induction of autologous tumor killing by heat treatment of fresh human tumor cells: involvement of gamma delta T cells and heat shock protein 70. *Cancer Research*. 56(5):1104–1110.

Whitesell, L., Mimnaugh, E.G., De Costa, B., Myers, C.E. & Necker, L.M. (1994). Inhibition of heat shock protein HSP90-pp60 heteroprotein complex formation by benzoquinone ansamycins: Essential role for stress proteins in oncogenic transformation. *PNAS*. 91:8324-8328

Williams, J. H. H. and Ireland, H. E. (2008). Sensing danger - Hsp72 and HMGB1 as candidate signals. *Journal of Leukocyte Biology*. 83(3):489-492.

Wu, C. (1995). Heat Shock Treanscription Factors. Structure & Regulation. *Annual Review of Cell and Developmental Biology*. 11:441-469

Wu, X., Wanders, A., Wardega, P., Tinge, B., Gedda, L., Bergstrom, S., Sooman, L., Gullbo, J., Bergqvist, M., Hesselius, P., Lennartsson J. & kman, S. (2009). Hsp90 is expressed and represents a therapeutic target in human oesophageal cancer using the inhibitor 17-allylamino-17-demethoxygeldanamycin. *British Journal of Cancer*. 100, 334–343

Xanthoudakis, S., Roy, S., Rasper, D., Hennessey, T., Aubin, Y., Cassady, R., Tawa, P., Ruel, R., Rosen, A. & Nicholson, D.W. (1999). Hsp60 accelerates the maturation of pro-caspase-3 by upstream activator proteases during apoptosis. *EMBO Journal*. 18(8):2049-2056

Yaglom, J.A., Gabai, V.L. & Sherman, M.Y. (2007). High Levels of Heat Shock Protein Hsp72 in Cancer Cells Suppress Default Senescence Pathways. *Cancer Research*. 67(5):2373-2381

Yao, P., Nussler, A., Liu, L., Hao, L., Song, F., Schirmeier, A. & Nussler, N. (2007). Quercetin protects human hepatocytes from ethanol-derived oxidative stress by inducing heme oxygenase-1 via the MAPK/Nrf2 pathways. *Journal of Hepatology*. 47(2):253-61

Yin, Z., Henry, E.C. & Gasiewicz, T.A. (2009). (-)-Epigallocatechin-3-gallate is a novel Hsp90 inhibitor. *Biochemistry*. 48(2):336-45.

Zaarur, N., Gabai, V.L., Porco, J.A. Jr., Calderwood, S. & Sherman, M.Y. (2006). Targeting Heat Shock Response to Sensitise Cancer Cells to Proteosome and Hsp90 Inhibitors. *Cancer Research*. 66(3): 1783-1791

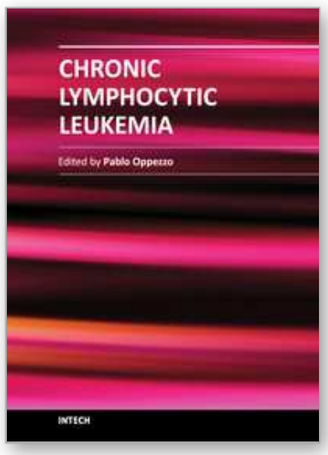
Zagouri, F., Sergentanis, T.N., Nonni, A., Papadimitriou, C.A., Michalopoulos, N.V., Domeyer, P., Theodoropoulos, G., Lazaris, A., Patsouris, E., Zogafos, E., Pazaiti, A. & Zografos

G.C. (2010). Hsp90 in the continuum of breast ductal carcinogenesis: Evaluation in precursors, preinvasive and ductal carcinoma lesions. *BMC Cancer*. 10:353-341

Zanini, C., Giribaldi, G., Mandili, G., Carta, F., Crescenzio, N., …Bisaro, B., Doria, A., Foglia, L., Di Montezemolo, C., Timeus, F. & Turrini, F. (2007). Inhibition of heat shock proteins (HSP) expression by quercetin and differential doxorubicin sensitization in neuroblastoma and Ewing's sarcoma cell lines. *Journal of Neurochemistry*. 103(4):1344-1354

Zhao, Z.G. & Shen, W.L. (2005). Heat shock protein 70 antisense oligonucleotide inhibits cell growth and induces apoptosis in human gastric cancer cell line SGC-7901. *World Journal of Gastroenterology*. 11(1):73-78

Zou, J.Y., Guo, Y.L., Guettouche, T., Smith, D.F., Voellmy, R. (1998). Repression of heat shock transcription factor HSF1 activation by HSP90 (HSP90 complex) that forms a stress-sensitive complex with HSF1. *Cell*. 94(4):471-480



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B-cell chronic lymphocytic leukemia (CLL) is considered a single disease with extremely variable course, and survival rates ranging from months to decades. It is clear that clinical heterogeneity reflects biologic diversity with at least two major subtypes in terms of cellular proliferation, clinical aggressiveness and prognosis. As CLL progresses, abnormal hematopoiesis results in pancitopenia and decreased immunoglobulin production, followed by nonspecific symptoms such as fatigue or malaise. A cure is usually not possible, and delayed treatment (until symptoms develop) is aimed at lengthening life and decreasing symptoms. Researchers are playing a lead role in investigating CLL's cause and the role of genetics in the pathogenesis of this disorder. Research programs are dedicated towards understanding the basic mechanisms underlying CLL with the hope of improving treatment options.

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