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Heat shock proteins as modulators and therapeutic targets of chronic disease: An integrated perspective

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1 **Heat shock proteins as modulators and therapeutic targets of**
2 **chronic disease: an integrated perspective**

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48

49 **Abstract**

50 Many heat shock proteins (HSPs) are essential to survival as a consequence of their role as
51 molecular chaperones, and play a critical role in maintaining cellular proteostasis by
52 integrating the fundamental processes of protein folding and degradation. HSPs are
53 arguably amongst the most prominent classes of proteins that have been broadly linked to
54 many human disorders, with changes in their expression profile and/or
55 intracellular/extracellular location now being described as contributing to the pathogenesis of
56 a number of different diseases. Although the concept was initially controversial, it is now
57 widely accepted that HSPs have additional biological functions over and above their role in
58 proteostasis (so called 'protein moonlighting'). Most importantly, these new insights are
59 enlightening our understanding of biological processes in health and disease, and revealing
60 novel and exciting therapeutic opportunities. This theme issue draws on therapeutic insights
61 from established research on HSPs in cancer and other non-communicable disorders, with
62 an emphasis on how the intracellular function of HSPs contrasts with their extracellular
63 properties and function, and interrogates their potential diagnostic and therapeutic value to
64 the prevention, management and treatment of chronic diseases.
65

66 **1. Introduction**

67 The most extensively studied heat shock proteins (HSPs) are the molecular chaperones that
68 function intracellularly in an ATP-dependent manner and include heat shock protein 60
69 kDa/heat shock protein 10 kDa (HSP60/HSP10; chaperonins) (HSPD/HSPE); HSP40
70 (DNAJ), HSP70 (HSPA); HSP90 (HSPC); HSP100; and HSP110 (HSPH) families. The
71 expression of many of these HSPs is regulated by heat shock transcription factors (HSFs),
72 of which HSF1 is the best studied. Increasing evidence now suggests that these molecular
73 chaperones also have biological properties in the extracellular environment which may be
74 independent of their chaperone functions. In addition to ATP, the molecular chaperone
75 activity of the major HSPs is regulated by a cohort of non-substrate accessory proteins,
76 known as co-chaperones. Co-chaperones are a diverse group of chaperone regulatory
77 proteins which are required, to a greater or lesser degree, by certain chaperones. HSP90,
78 for example, has over 20 co-chaperones that fine tune its function and adapt it to the
79 different stages of the protein folding pathway. Some HSP families, such as HSP40, include
80 members having both chaperone and co-chaperone activity.
81

82 A particularly lively area relates to the evolving insight into the therapeutic potential of
83 targeting HSPs in cancer, and their value as an exciting class of molecular target. Although
84 HSPs and their transcription factors have been the subject of sustained interest in the field of
85 cancer biology, more recently they have been attracting interest in many other chronic
86 conditions such as diabetes, obesity, autoimmune disease, neurodegeneration, muscular
87 dystrophies, psychiatric disorders and chronic heart failure. These studies are revealing that
88 although increased levels of intracellular HSPs may be beneficial for acute conditions, such
89 increases can be detrimental for certain chronic conditions, as exemplified by acute and
90 chronic heart conditions. The contribution of extracellular HSPs to chronic disease is poorly
91 understood. Increased levels of extracellular HSPs appear to be detrimental by enhancing
92 inflammation pathways, and hence for conditions such as diabetes a reduction in the ratio of
93 extracellular to intracellular HSPs is beneficial. In contrast, extracellular HSPs can also be
94 beneficial to certain autoimmune conditions as a consequence of their ability to engage with,
95 and recruit the immunomodulatory activity of regulatory T cell populations. Although the
96 reported dichotomies in functionality of HSPs would appear to be counter-intuitive and has
97 been the subject of great debate and counter-arguments, one needs to consider the context
98 and the temporal nature of disease and its control. What is clear from current knowledge is
99 that HSPs play important biological roles under physiological, stressful and disease
100 conditions.
101

102 The articles in this theme issue highlight how insights (both anticipated and unanticipated)
103 into the biological function of HSPs in cancer have revealed new therapeutic options for the
104 treatment of the disease. The issue also explores how the intracellular function (ATP-rich
105 context) of HSPs contrasts with their extracellular function (ATP-poor context), and their
106 potential diagnostic and therapeutic value to the prevention, management and treatment of
107 chronic diseases. Here we integrate and critique the content of this theme issue, addressing
108 HSP moonlighting in the context of their contrasting intracellular and extracellular roles.
109

110 **2. Heat shock proteins and protein moonlighting**

111 Although the finding that exposure to a non-physiological temperature (37°C *versus* 26°C)
112 induced a new puffing pattern in the polytene chromosomes of *Drosophila* [1] was
113 interesting, the author could not have anticipated the significance and broad reach of this
114 finding, especially given that the 'biological relevance of the findings were unclear' and it
115 proved difficult to publish the findings. However, over 50 years later, we continue to
116 appreciate the importance of this heat shock response (HSR) to the maintenance of cellular
117 homeostasis and protection against a multitude of physical, chemical and biological
118 stressors that exist in the environment [2].
119

120 As the protein folding paradigm and molecular chaperone functions of HSPs were
121 developing in the late 1980s and 1990s, it became apparent that some of these proteins
122 were also present on the surface of cells or in the extracellular fluids. This contradicted the
123 established dogma that these proteins were exclusively intracellular and so it took time for
124 the data to be accepted, the findings to gain traction with the scientific community and for
125 this new field of extracellular HSPs to be accepted and become established. Interest in the
126 biological role(s) and functions of these proteins grew, as did interest into the potential
127 capacity of extracellular HSPs to influence biology and physiology. As discussed in this
128 issue, it was shown that the treatment of cells with purified HSPs resulted in cell activation
129 similar to that induced by pro-inflammatory cytokines. Despite controversy surrounding the
130 possibility that at least some of the pro-inflammatory effects of HSPs might be due to
131 contaminants of the preparations that have been used [3, 4], there is also a wealth of
132 evidence from a number of settings which argues against this concept [5].
133

134 A new paradigm has arisen that at least some HSPs are secreted proteins [6] with pro-
135 (HSP60, HSP70, HSP90) or anti-inflammatory (HSP10, thioredoxin, HSP27, BiP) actions of
136 importance in human diseases such as cancer, coronary heart disease, diabetes and
137 rheumatoid arthritis [7], to name but a few. In addition to having direct effects on cells, HSPs
138 can bind peptides and present them to T cells to modulate immune responses, and this
139 might have implications in a number of disease settings, including cancer [8]. It has become
140 apparent that HSP70 can be present in a membrane expressed form. The significant
141 diagnostic, therapeutic and imaging potential of this finding, and the progress which has
142 been made in exploiting membrane HSP70-based theranostics (i.e. combining diagnostic
143 and therapeutic capabilities into a single agent; a key element of Precision Medicine) for the
144 management and treatment of patients with cancer, is considered in detail in this issue [9].
145

146 Taken together, the findings that HSPs can be present in the extracellular and cell-
147 associated compartments have led to the establishment of a new paradigm which
148 designates these proteins as 'moonlighting proteins' (proteins with more than one function)
149 that have the capacity to 'escape' from cells and interact with different cell types to elicit a
150 range of biological effects. These proteins can even act as receptors for inflammatory
151 mediators called 'inflammogens' [10]. Support for this new paradigm comes from a number
152 of studies that are highlighted in this issue [11], and a large number of studies that have, and
153 continue to reveal, the presence of a number of HSPs in the bodily fluids of humans and
154 animals [12]. The first two contributions in this issue provide a critical overview of
155 extracellular HSPs [11] and the biology of protein moonlighting [13].
156

157 **3. Intracellular versus extracellular heat shock proteins in cancer**

158 The initiation, progression and metastasis of cancer have all been shown to be accompanied
159 by multiple cellular insults arising from both intracellular and extracellular sources. Internal to
160 the cancer cell, the high expression of oncogenic proteins (many of which are mutated),
161 altered cellular metabolism, aneuploidy and genomic instability all contribute to its
162 characteristic stressed phenotype. Moreover, during cancer development, cells are exposed
163 to altered extracellular conditions that can include hypoxic, acidotic, mechanical and nutrient
164 deprived microenvironments, further stimulating the cancer cell to engage highly conserved
165 survival pathways such as the HSR. Consistent with the knowledge that cancer cells are
166 exposed, both internally and externally, to major proteotoxic insults that challenge cellular
167 homeostasis and survival, it is not surprising that cancers constitutively express high levels
168 of HSP family members. In fact, tumour cells have become to be regarded as addicted to
169 HSPs (e.g. HSP90) as well as their transcriptional regulators (e.g. HSF1).

170
171 Increased expression of many HSPs, including HSP27 (HSPB1), HSP72 (HSPA1A,
172 HSPA1B) and HSP90 (HSP90AA1, HSP90B1), have been shown in a wide variety of cancer
173 types such as breast, prostate, lung and melanoma, and are associated with poor patient
174 outcomes. Moreover, HSF1, the master regulator of the HSR has also been shown to be
175 increased in expression and constitutively activated in many cancers. The parallel molecular,
176 genetic and pharmacological investigations that have been performed in relation to HSPs
177 and their signalling and transcriptional regulation, has further confirmed their importance to
178 the growth and progression of many tumour types (reviewed in this issue [14]). For example,
179 the work in targeting and developing HSP90 inhibitors has confirmed the importance of
180 HSP90 to cancer signalling and oncogene driven growth (reviewed in this issue [15]). In a
181 similar manner, the HSR has been shown to be an integral part of the oncogenic network,
182 working through the actions of HSF1 to maintain cancer cell survival and function (reviewed
183 in this issue [16]). Interestingly, it has been shown that within the oncogenic context, the
184 expression of HSF1 is indispensable for the growth and survival of cancer cells, while its loss
185 in non-transformed cells has little to no effect [17].

186
187 HSF1 and many of the HSPs have been shown to play fundamental roles in many aspects of
188 the cancer cell phenotype associated with the hallmarks of cancer [18] including sustained
189 proliferative signalling, evading growth suppression, replicative immortality, angiogenesis,
190 resisting cell death and supporting invasion and metastasis [19]. Moreover, they are also
191 involved in a number of the more recently identified hallmarks of cancer such as the
192 deregulation of cellular energetics, genome instability, avoiding immune destruction and
193 enabling tumour-promoting inflammation. The wide-ranging actions of the HSPs and HSF1
194 are not limited to the cancer cells themselves, but have also been shown to play important
195 roles for accessory cell function within the tumour microenvironment such as the cancer
196 associated fibroblasts (CAFs) and tumour associated macrophages (TAMs), ultimately
197 contributing to cancer cell growth and progression [20].

198
199 Although it was originally proposed that the actions of HSPs were primarily intracellular to
200 cancer cells and other cells of the tumour microenvironment, it is now evident that their
201 presence and functionality are also very important to many molecules and processes
202 external to the cell. For example, HSP90 α (HSP90AA1) is known to exist outside the cell,
203 termed as eHSP90, and has been shown to interact with a number of client proteins,
204 including matrix metalloproteinase 2 (MMP2) through which it enhances the migration and
205 invasion of cancer cells (reviewed in this issue [14, 15]). It has been shown that the functions
206 of extracellular HSPs can have both anti-tumour or pro-tumour effects, ranging from anti-
207 tumour or pro-tumour immunomodulation (HSP90, HSP72, HSC70, HSP60, HSP27),
208 suppression or promotion of tumour cell proliferation (GRP78, HSP20, HSP27), as well as
209 promotion of cancer cell invasion (HSP90, GRP75, HSP27) and angiogenesis (HSC70) [21-
210 26]. Moreover, co-chaperones of HSP90, such as the HSP70/HSP90 organising protein
211 (HOP), HSP40 and p23 have also been shown to be extracellular, and similar to their role

212 internal to the cell, are in complex with HSP90 to elicit extracellular functions such as MMP-2
213 activation and cancer cell invasion and migration [23, 27].

214

215 Our increasing knowledge of the unique roles of HSPs and their co-chaperones external to
216 the cell is leading to novel approaches for the therapeutic targeting of cancers. For example,
217 cell surface HSP70 is currently being used as a target of novel therapies that include
218 nanoparticle-based treatments for cancer, and cell-impermeable HSP90 inhibitors are being
219 examined as to their efficacy in inhibiting cancer migration and invasion (reviewed in this
220 issue [9]). Therefore, our increased understanding of the actions of extracellular HSPs will
221 not only lead us to a better understanding of the biology of cancer and its progression, but
222 will also reveal further therapeutic opportunities for the treatment of advanced cancers.

223

224 **4. Intracellular *versus* extracellular heat shock proteins in chronic diseases**

225 Much of the research into the function of HSPs in chronic disease has been focussed on
226 cancer. However, it is also clear that HSPs are involved in many other chronic conditions,
227 from neurological and muscle-wasting disorders to obesity and post-traumatic stress. This
228 range of chaperonopathies highlights the important and central role which these proteins
229 play in maintenance of correct cellular function.

230

231 Findings from experimental, pharmacological or exercise studies on changes to HSP72
232 expression levels suggest that the manipulation of the extracellular to intracellular ratio of
233 HSP levels represents a useful avenue for the prevention and treatment of diabetes
234 (reviewed in this issue [28]). For example, there is evidence that exercise promotes the
235 release of extracellular HSP72 from certain human cells (brain, [29]; epithelium, [30];
236 immune system, [31]; muscle and adipose tissue, [32]). However, long-term exercise
237 promotes a decrease in extracellular HSP72 and an increase in intracellular skeletal muscle
238 HSP72 [28]. In fact, it is now apparent that the balance of extracellular (pro-inflammatory)
239 *versus* intracellular (anti-inflammatory) HSP72 appears to be a determining factor for the
240 extent of tissue inflammation and hence the pathology associated with diabetes. It is
241 hypothesised that interventions that lower the extracellular to intracellular HSP72 ratio are
242 potentially beneficial in the context of diabetes progression [33]. Hence, carefully constructed
243 exercise regimes that favourably modulate this HSP72 ratio may serve as powerful
244 therapeutic interventions for the prevention and management of diabetes. However, more
245 detailed studies on extracellular HSPs and the effects of exercise are needed, particularly
246 the contribution of different tissues to extracellular HSP expression levels, and the
247 biochemical and physiological mechanisms of action of these HSPs.

248

249 HSPs, and HSP72 in particular, also play an important role in muscle function and are
250 potential therapeutic agents for muscle wasting conditions (reviewed in this issue [34]).
251 HSP90, HSP72, and HSP27 all have a pro-myogenic role in muscle development, albeit via
252 distinct mechanisms. HSPs are also differentially expressed in the muscle progenitor pool
253 that differentiates to give rise to new muscle tissue [34]. HSP72 is the most widely studied
254 HSP in this context and is required for muscle repair after acute injury. Both intracellular and
255 extracellular HSP72 contribute to this process, with extracellular HSP72 functioning primarily
256 via the activation of the immune response. Interestingly, many of the effects of HSP72
257 knockout on muscle regeneration involve the immune response, which suggests that, given
258 that extracellular HSP72 arises from intracellular HSP72, the extracellular functions of
259 HSP72 are more important in this context. Indeed, injection of extracellular HSP72 has been
260 shown to ameliorate many of the effects of muscle injury in HSP72 null mice [35]. With
261 respect to disease, over-expression of intracellular HSP72 had a positive effect and led to
262 improvements in body strength and endurance, diaphragm health, normalised muscle force
263 and reduced markers of muscle damage in a mouse model of Duchenne muscular dystrophy
264 [36]. HSP72 also has a positive effect on muscle function in the context of muscle
265 immobilisation, suggesting that over-expression of this protein may be a therapeutic
266 approach for a range of muscle wasting conditions. It is likely that at least some of the

267 described functions of HSP72 in these conditions are attributed to the extracellular function,
268 but this has not been demonstrated definitively.

269
270 In addition to a role in muscle-related immune responses, experimental models have
271 provided evidence that both intracellular and extracellular HSPs also have a protective
272 function in autoimmune diseases (reviewed in this issue [37]). The application of exogenous
273 extracellular recombinant HSPs and the experimental co-induction of endogenous
274 intracellular HSPs have been shown to lead to production of disease protective regulatory T
275 (Treg) cells [37, 38]. This has stimulated research into the development of therapeutic HSP-
276 based peptide vaccines for the restoration of immune tolerance in inflammatory diseases.

277
278 There is emerging evidence for increased expression of extracellular HSP70, HSP90, and
279 certain associated co-chaperones (e.g. BAG-3) in heart failure, and that their functions are
280 complementary and independent of their intracellular isoforms. The important therapeutic
281 and diagnostic considerations of these findings are reviewed in this issue [39]. Current
282 findings suggest that therapeutic strategies involving the increase of HSP levels may be
283 applicable in the context of acute heart conditions (e.g. acute myocardial infarction/ischemic
284 reperfusion injury), but not chronic heart conditions (e.g. hypertension). Indeed, the
285 pharmacological enhancement of intracellular HSP function has been shown to provide
286 protection against experimental myocardial infarction [40]. With respect to chronic heart
287 conditions, extracellular and intracellular HSPs exert different effects. For example, a
288 decrease in the expression of intracellular HSP70 promotes cardiomyocyte hypertrophy and
289 dysfunction while protecting mice from cardiac fibrosis, whereas inhibition of extracellular
290 HSP70 has been shown to improve hypertension-induced hypertrophy and fibrosis [41]. In
291 the context of chronic heart disease, there are some parallels in the findings for extracellular
292 HSP90 and extracellular HSP70. For example, the decrease in fibronectin levels, collagen
293 production and the associated TGF β signalling pathway via the inhibition of extracellular
294 HSP90 [42, 43] has implications for the fibrosis-related pathology of chronic heart conditions.
295 Although there is great promise for extracellular HSP70 and HSP90 as diagnostic markers of
296 chronic heart disease, a deeper understanding of the mechanism(s) of action of extracellular
297 HSP70 and HSP90 and its co-chaperones is required before effective prevention and
298 treatment can be achieved.

299
300 HSPs are also important in the context of neurodegeneration and neurological dysfunction
301 leading to psychiatric diseases. HSP40s are the largest and most diverse of the HSPs and
302 changes in different HSP40 isoforms all give rise to different, but related forms of
303 neurodegeneration (reviewed in this issue [44]). Although these HSP40 isoforms share
304 structural features such as the J domain, they also contain a number of unique functional
305 domains (particularly since most of the isoforms associated with disease are the more
306 diverse type III HSP40/DNAJC). The redundancy between isoforms in some contexts can
307 also explain why it is possible to ameliorate the disease consequences of a mutation or
308 deficiency of one isoform via over-expression of another. For example, overexpression of
309 DNAJA1 can suppress aggregation of polyQ ataxin associated with neurodegeneration [45].
310 Interestingly, there are no neurological disorders associated with mutations in type I HSP40s
311 like DNAJA1, presumably because many of these proteins are essential and loss of function
312 cannot therefore be tolerated. With respect to psychiatric disorders, the co-chaperone
313 FKBP51, acting via HSP90, is both a causative agent and biomarker for various forms of the
314 disease (reviewed in this issue [46]). Increased levels of FKBP51 lead to glucocorticoid
315 resistance by retarding the recruitment of glucocorticoid receptor (GR) to the nucleus and
316 perturbing signalling via the hypothalamic-pituitary-adrenal (HPA) axis that culminates in a
317 poor stress coping phenotype [46]. Specific single nucleotide polymorphisms that result in
318 methylation changes which alter levels of FKBP51 may be a risk or prognostic factor for
319 anxiety or suicide risk [47, 48]. This suggests that modulation of FKBP51 levels may be a
320 relevant therapeutic strategy. However, in the context of both HSP40-related
321 neurodegeneration and FKBP51-related psychiatric disorders, we have limited

322 understanding of the relative contribution of intracellular *versus* extracellular forms of the
323 relevant HSPs due to a paucity of data. Certainly, it is known that both HSP70 and HSP90
324 are extracellular and therefore it is at least theoretically possible that co-chaperones of these
325 two proteins (HSP40 and FKBP51) also exist in functional extracellular forms. In these
326 examples, what we do know is that disease is usually associated with a change in the levels
327 of a particular HSP. For example, mutations or deletions in the HSP40 isoform DNAJC29 is
328 one of the most common causes of ataxia [49]. In some instances, the change in HSP levels
329 are associated with missense mutations, deletions or splicing changes, while in other cases
330 levels change in response to the environment (such as age-induced increases in FKBP51
331 levels which are associated with psychiatric disorders).

332

333 **5. Conclusion**

334 Fundamental insights into how HSPs give rise to disease will be an important component of
335 therapeutic targeting of these proteins. However, many knowledge gaps remain and need to
336 be addressed. Importantly, with cancer and autoimmune disease being the exceptions, there
337 is limited insight into the role played by extracellular HSPs in chronic diseases such as
338 neurodegeneration or psychiatric disorders. In addition, while much is known about the
339 mechanism of action of specific intracellular HSP networks, such as the HSP90-HOP-HSP70
340 or HSP70-HSP40 complexes, the genesis and function of these HSP complexes in the
341 extracellular milieu is poorly understood and raises many fundamental questions that need
342 to be answered before therapeutic applications can be properly developed. Like the HSPs
343 they regulate, co-chaperones like HOP appear to also be secreted via exosomes [50].
344 However, it is not known if HOP is secreted together with HSP90 and HSP70 as a functional
345 complex, or if it is secreted separately and then forms a complex with the HSPs [51].
346 Therefore, the major questions that need to be answered for these extracellular HSP
347 complexes and many other extracellular HSPs include the following:

348

- 349 1. What is the origin of extracellular HSPs, and which isoforms are structurally and
350 functionally distinct from their intracellular counterparts, and which isoforms are
351 derived from their intracellular counterparts?
- 352 2. Which isoforms of extracellular HSPs are encoded by separate genes and which are
353 encoded by splice variants of the same gene?
- 354 3. Are there receptors associated with extracellular HSPs?
- 355 4. As a general principle, is the ratio of extracellular to intracellular HSP levels important
356 for cellular and physiological homeostasis?
- 357 5. What stimuli, mechanisms and pathways are required for the secretion of
358 extracellular HSPs?
- 359 6. Do extracellular (exosomal) HSPs function as molecular chaperones, is their activity
360 regulated by extracellular co-chaperones and what defines extracellular client
361 proteins?

362

363 While there is much work to be done before we can more fully define the true biological role,
364 therapeutic potential and significance of extracellular HSPs, we can draw inspiration from
365 Hippocrates who stated: 'That which drugs fail to cure, the scalpel can cure. That which the
366 scalpel fails to cure, heat can cure. If the heat cannot cure, it must be determined to be
367 incurable'.

368

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370 approval of the article.

371

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392

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