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6 RESEARCH ARTICLE

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9 **Membrane fluidity matters: Hyperthermia from the aspects of lipids and**  
10 **membranes**

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19  
20 **Abstract**

21 Hyperthermia is a promising treatment modality for cancer in combination both with radio- and  
22 chemotherapy. In spite of its great therapeutic potential, the underlying molecular mechanisms  
23 still remain to be clarified. Due to lipid imbalances and ‘membrane defects’ most of the tumour  
24 cells possess elevated membrane fluidity. However, further increasing membrane fluidity to  
25 sensitise to chemo- or radiotherapy could have some other effects. In fact, hyperfluidisation  
26 of cell membrane induced by membrane fluidiser initiates a stress response as the heat  
27 shock protein response, which may modulate positively or negatively apoptotic cell death.  
28 Overviewing some recent findings based on a technology allowing direct imaging of lipid rafts  
29 in live cells and lipidomics, novel aspects of the intimate relationship between the ‘membrane  
30 stress’ of tumour cells and the cellular heat shock response will be highlighted. Our findings  
31 lend support to both the importance of membrane remodelling and the release of lipid signals  
32 initiating stress protein response, which can operate in tandem to control the extent of the  
33 ultimate cellular thermosensitivity. Overall, we suggest that the fluidity variable of membranes  
34 should be used as an independent factor for predicting the efficacy of combinational cancer  
35 therapies.

36  
37 **Introduction**

38 Hyperthermia, mainly as an adjuvant to radiotherapy and  
39 chemotherapy, is an established methodology among the  
40 currently applied cancer treatments. Although its exact  
41 mechanism is still unknown, it is one of the most effective  
42 radiation sensitisers and can additionally enhance the cyto-  
43 toxicity of certain anticancer drugs [1]. Importantly, there is a  
44 tumour-selective effect of hyperthermia in a critical range of  
45 temperature (40–43 °C) *in vivo* [2]. Various strategies and  
46 mechanisms underlying the clinical application of hyperther-  
47 mia in combination with cancer immunotherapy have been  
48 discussed by Repasky et al. [3–5].

49 As a challenge to its therapeutic potential the use of  
50 hyperthermia in cancer therapy has an undesirable and  
51 inevitable side-effect linked to the familiar phenomenon in  
52 thermobiology known as acquisition of thermotolerance  
53 (ATT). A point relevant to the present review is that the  
54 subpopulation of cancer cells which develop thermotolerance  
55 become less sensitive to subsequent hyperthermia-induced  
56 cytotoxicity or various anticancer drugs and radiation [6].  
57 Accordingly, the possibility of preventing thermotolerance

80 **Keywords**

81 Cancer therapy, heat shock protein,  
82 hyperthermia, lipid raft, membrane fluidity,  
83 thermotolerance

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95 development by relatively harmless substances is of high  
96 clinical importance.

97 Thermotolerance is generally associated with the synthesis  
98 and accumulation of heat shock proteins (HSP) molecular  
99 chaperones (especially Hsp70 and Hsp25/27). The exact  
100 sequence and mechanism of hyperthermia-induced events  
101 leading either to cell death or to the activation of cellular  
102 thermotolerance are still largely unexplored. [6]. Acquisition  
103 of thermotolerance is known to induce several other cellular  
104 defences, including the elevation of non-enzymatic and  
105 enzymatic antioxidants or activation the autophagy by  
106 NFκB during the phase of heat shock recovery [7].

107 As highlighted in this review, a large amount of evidence  
108 has also been presented for decades for the involvement of  
109 membranes both in the acquisition of thermotolerance and in  
110 heat lethality. As early as 1924, Heilbrunn proposed that the  
111 physical state of the lipids might be related to the extent of  
112 cell killing by heat [8]. Experimental evidences provided later  
113 by Yatvin and co-workers supported the hypothesis that the  
114 fluidity of membranes might be a major factor contributing to  
115 the death of mammalian cells exposed to hyperthermia [9].

116 Here first we briefly review those evidences, which show  
117 that the dysregulated lipid metabolism and membrane  
118 defects are really common in tumour cells, and that certain  
119 cancer therapies can alter the physicochemical properties of  
120 membranes.

121 Then, based on a novel method which allowed the direct  
122 imaging of nanoscopic long-lived platforms with raft-like  
123 properties diffusing in the live cell plasma membrane, we  
124 discuss what the properties are that allow the surface  
125 membrane to become the key determinant of cellular heat  
126 stress sensing and signalling.

127 The next paragraph overviews how lipidomic fingerprints  
128 revealed that membrane stress achieved either by heat or  
129 membrane fluidiser benzyl alcohol (BA) results in highly  
130 specific alterations in lipid metabolism of melanoma cells.  
131 We emphasise how the activation of certain phospholipases  
132 coupled to the production of specific lipid mediators (such as  
133 arachidonic acid) can refine the expression of HSPs [10,11].

134 Based on new studies next we provide evidence that  
135 isothermal membrane hyperfluidisation can induce an equal  
136 level of cellular thermotolerance with that achieved by heat  
137 priming, and both treatments are accompanied by specific  
138 remodelling of the microdomains of the surface membranes.  
139 The significantly lower levels of major HSPs (Hsp70, Hsp25)  
140 measured in membrane fluidiser treated cells also provided  
141 compelling evidence that the amount of HSPs produced is not  
142 the sole factor in the development of thermotolerance.

143

144

### 145 **Dysregulated lipid metabolism and membrane** 146 **defects are common in tumour cells**

147 Alterations in lipid metabolism have long been recognised as a  
148 hallmark of cancer cells and are discussed in detail elsewhere  
149 [12]. As a consequence of this recognition a number of cancer  
150 drugs are currently under development or in the clinical phase  
151 targeting specific lipid metabolic pathways [13]. As suggested  
152 by Nomura et al., tumour cells undergo a general metabolic  
153 shift towards specific bioenergetic (glycolysis) and anabolic  
154 (protein and lipid synthesis) processes that promote rapid  
155 growth, and it was clearly demonstrated that an increase in the  
156 level of monoacylglycerol (MAG) lipase can drive tumorigen-  
157 esis through the lipolytic release and remodelling of free fatty  
158 acids [14]. Since the influence of oncogene expression on the  
159 early lipidome alterations was unknown, we recently analysed  
160 the changes in the course of ERBB2 expression-mediated  
161 premature senescence induced in lipid profiles by using MCF-7  
162 breast cancer cells. The most marked changes were found in the  
163 levels of PG (34:1), PG (36:1) (increased) and lysophos-  
164 phatidylethanolamine (LPE) (18:1), phosphatidylglycerol (PG)  
165 (40:7) and phosphatidylinositol (PI) (36:1) (decreased).  
166 Statistical analysis revealed a general trend towards shortened  
167 phospholipid acyl chains in senescence, and these changes  
168 were accompanied by increased global membrane fluidity [15].

169 Several solid tumours are characterised by the higher  
170 fluidity of their cell membranes [16–20], correlating with  
171 their proliferative and invasive potentials and their metastatic  
172 abilities [21–23]. A reversal of tumour resistance to apoptotic  
173 stimuli through the alteration of membrane fluidity was  
174 suggested by Baritaki et al. [12]. Melanoma tumour cells with  
175 a high metastatic potential are characterised by an enhanced  
176 lateral mobility of the membrane receptors in metastasis,  
177 while exhibiting a reduced cholesterol/phospholipid ratio  
178 [24]. The plasma membrane (PM)-selective catalytic hydro-  
179 genation of lipids in live murine leukemic GRLS cells (i.e. an  
180 attempt to normalise bulk membrane fluidity by chemical

means) notably increased the expression of a 15 kDa antigen 181  
on the cell surface [25]. It was recently suggested that the 182  
ability of breast tumour stroma to promote the epithelial- 183  
mesenchymal transition, the reduction of cell adhesion, the 184  
migration velocity and directness, and especially an increase 185  
in membrane fluidity, can be viewed as overall progression- 186  
and invasion-promoting effects [26]. 187

### 188 **Cancer therapies can also alter the** 189 **physicochemical properties of membranes:** 190 **the case of cisplatin**

191 Tumour cells treated with cisplatin also exhibit an increase 192  
in PM fluidity, which results from the activation of acid 193  
sphingomyelinase and the subsequent generation of ceramide 194  
(Cer) [27]. The generation of Cer and the redistribution of the 195  
death receptor CD95 into the lipid rafts can promote 196  
the initiation of the apoptotic signal and the elimination of 197  
the malignant cells [27]. As will be discussed later, we have 198  
documented the accumulation of Cer both in heat- and in 199  
BA-pretreated B16 melanoma cells [10]. Most recent studies 200  
by Alvarez-Berrios et al. revealed that magnetic fluid 201  
hyperthermia combined with cisplatin resulted in significantly 202  
enhanced cytotoxicity when compared with hyperthermia 203  
using a water bath. It was shown that hyperthermic potenti- 204  
ation of cisplatin by magnetic nanoparticle heaters is 205  
correlated with an increase in the membrane fluidity, and as 206  
a consequence, elevated passive uptake of the drug in cancer 207  
cells. As was emphasised by the authors, the demonstrated 208  
mechanism in the context of cisplatin could find application 209  
in potentiation of other chemotherapies. 210

211 These and other findings urge a complete revision of our 212  
current concepts of the mode of action of platinum-based 213  
chemotherapy. The examination of transformation incidences 214  
expressed as a function of the surviving fraction revealed that 215  
the combination of heat and cisplatin resulted in fewer 216  
transformants per surviving cell than for cisplatin alone [28]. 217  
In other words, when heat converts sub-lethal damage to 218  
lethal damage in combination with cisplatin, the elevation 219  
of the membrane fluidity and the generation of Cer per se 220  
[10] can act synergistically as a ‘common denominator’ in 221  
hyperthermia and chemotherapy. 222

### 223 **Membranes are key determinants of cellular** 224 **stress adaptation and lethality**

225 It was shown decades ago, that fluidity, organisation and 226  
phase behaviour of membranes are key and strictly controlled 227  
factors in the processes of thermally induced adaptation and 228  
lethality, in both prokaryotic [29–31] and eukaryotic cells 229  
[32–36]. Our early findings, achieved with prokaryotic 230  
models firstly revealed that membranes can act as thermo- 231  
sensors, and there exists a feed-back membrane fluidity 232  
control of certain stress defending genes, such as fatty acid 233  
desaturases in the cold [37]. But how do eukaryotic cells 234  
maintain the physical structure of their membrane lipid 235  
bilayers within optimal and/or tolerable limits? How changes 236  
in plasma membrane physical properties are perceived in a 237  
mammalian cell, and how the abundance of lipids in the 238  
plasma membrane is regulated to balance changing remains 239  
largely unknown. 240

241 The plasma membrane (PM) in mammalian cells has been  
242 hypothesised to contain nanoscopic lipid platforms, which are  
243 discussed in the context of ‘lipid rafts’ or ‘membrane rafts’.  
244 The findings of biochemical and cell biological studies have  
245 prompted the belief that rafts play a crucial role in many  
246 signalling processes [38–40]. Since it has proven difficult to  
247 visualise rafts in living cells, there is currently no consensus  
248 on their size, shape, stability, surface density, composition  
249 and heterogeneity. Very recently, however, we introduced a  
250 method which allowed the direct imaging of nanoscopic long-  
251 lived platforms with raft-like properties diffusing in the live  
252 cell PM [41]. This novel technique, called ‘thinning out  
253 clusters while conserving the stoichiometry of labelling’  
254 (TOCCSL) can sense these platforms through their ability  
255 to assemble a characteristic set of fluorescent marker  
256 proteins or lipids on a time scale of milliseconds. A special  
257 photobleaching protocol was used to reduce the surface  
258 density of labelled mobile platforms down to the level of  
259 well-isolated diffraction-limited spots, without altering the  
260 single spot’s brightness. The statistical distribution of probe  
261 molecules per platform was determined by single molecule  
262 brightness analysis. For demonstration we used the consensus  
263 raft marker glycosylphosphatidylinositol-anchored mono-  
264 meric GFP (mGFP-GPI) and the fluorescent lipid analogue  
265 Bodipy-GM1, which preferentially partitions into liquid  
266 ordered phases. For both markers, we found a cholesterol-  
267 dependent homo-association in the PM of living CHO and  
268 Jurkat T cells in the resting state, thereby demonstrating the  
269 existence of small, mobile, long-lived platforms containing  
270 these probes. We further applied this technology to address  
271 the structural changes in the PM during fever-type heat shock.  
272 At elevated temperatures, the mGFP-GPI homo-association  
273 disappeared, parallel with the increase in the expression  
274 of Hsp27. This finding lent strong support to our earlier  
275 suggestions that PM is involved in the sensing of temperature  
276 elevations through changes in the physical state of the  
277 membrane [36,42,43]. Interestingly, in artificial bilayer sys-  
278 tems, atomic force microscopy studies have shown that  
279 GPI-anchored proteins can be released from the liquid  
280 ordered phase by an increase in temperature [44]. Thus, a  
281 similar mechanism may apparently account for the observed  
282 dissociation of mGFP-GPI homo-associates in the CHO cell  
283 membrane. Taken together, these findings provide direct  
284 support for our hypothesis that fever stress has the potential  
285 to remodel lipid rafts and, via modulating the membrane  
286 microdomains engaged in primary stress sensing and signal-  
287 ling, to enhance the expression of a distinct subclass of HSPs  
288 selectively.

289 By using the TOCCSL technology we next addressed  
290 how the combination of heat shock and a prominent HSP  
291 co-inducer drug candidate, hydroxamic acid BGP-15 [45],  
292 affects the thermosensory properties of membranes. By using  
293 molecular dynamics simulations we provided evidence of the  
294 docking of BGP-15 in model membranes made of sphingo-  
295 myelin-cholesterol. The specific interaction of BGP-15 with  
296 cholesterol (Chol) was further assessed by using a combin-  
297 ation of complementary biophysical approaches. A reduced  
298 rate of Chol depletion by methyl-beta-cyclodextrin (MBCD) in  
299 the presence of BGP-15 was shown *in vitro* by the Langmuir-  
300 Blodgett monolayer technique. The above-described TOCCSL

method allowed the direct imaging of raft integrity during 301  
mild heat stress alone or in combination with the HSP co- 302  
inducer BGP-15. Confocal microscopy allowed us to follow 303  
the redistribution of Chol-rich membrane domains in response 304  
to drug administration with the fPEG-Chol probe. It emerged 305  
that BGP-15 partitions to lipid rafts with a preferential 306  
affinity for Chol. Moreover, BGP-15 was able to remodel 307  
Chol-enriched lipid platforms reminiscent of those observed 308  
earlier following non-lethal heat priming or membrane stress, 309  
and was shown to be obligatory for the generation and 310  
transmission of stress signals [43]. The BGP-15 activation 311  
of HSP expression involved the Rac1 signalling cascade. 312  
Presumably via Rac1 (and other, as yet unrevealed signalling 313  
pathways, bridging the signalling platforms of surface mem- 314  
branes with *hsp* genes via heat shock factors, (HSFs)), we 315  
demonstrated that BGP-15 is able to inhibit the rapid HSF1 316  
acetylation monitored during the early phase of heat stress, 317  
thereby promoting a prolonged duration of HSF1 binding to 318  
heat shock elements [46,47]. Modulation of the heat shock 319  
protein response via drugs, such as BGP-15 acting on the base 320  
of membrane lipid therapy has the potential to be beneficial 321  
in a range of disorders, including cancer [36]. 322

### 323 324 325 326 327 328 **Lipidomics revealed membrane lipid remodelling and release of potential HSP-inducing lipid mediators during early stress responses in murine B16 melanoma cells**

329 Apart from their roles in the structural organisation of  
330 membranes, different membrane lipids can be metabolised  
and give rise to signalling molecules in response to various 331  
stress stimuli. Increasing evidence (relating to sphingolipids 332  
or phospholipase A<sub>2</sub> activation, for instance) links such 333  
signalling processes to membrane microdomains. In turn the 334  
lipid signalling molecules can alter the gene expression and 335  
thereby couple environmental stress or other stimuli to energy 336  
metabolism, cellular aging, for example. 337

338 With the aim of recognising lipid changes as a conse-  
339 quence of heat shock and/or membrane fluidity modulation  
achieved through administration of the non-proteotoxic BA, 340  
we analysed the ESI-MS/MS molecular species data using 341  
a data-mining principal component analysis method [10]. 342  
This allowed a clear differentiation of the experiments into 343  
four non-overlapping clusters, depending on the different 344  
treatments. The first component, which accounted for almost 345  
60% of the variance, clearly distinguished the control and 346  
stress conditions, suggesting that common lipid metabolic 347  
pathways are involved in the stress-mediated lipid alterations. 348  
The second and third components revealed differences 349  
concerning mild or severe heat and the BA-induced mem- 350  
brane stresses, indicating specific changes in the lipidome in 351  
response to these membrane perturbations. 352

353 A key feature of the acute lipid remodelling due to heat  
354 (both mild and severe) or BA-induced membrane perturbation  
observed 60 min after stress intervention was the accumula- 355  
tion of Chol, Cer and saturated PC and PE-P species in the 356  
highly metastatic B16-F10 cells. These lipid species tend 357  
to support the formation of tightly packed subdomains 358  
corresponding to liquid-ordered phases biophysically char- 359  
acterised in model membranes and raft domains in cells [48]. 360



361 The altered microdomain disposition was confirmed by  
362 preliminary results of analysis of the lipid composition of  
363 detergent-resistant membrane domains (DRMs) from  
364 B16-F10 cells as a consequence of stress (Horvath et al.,  
365 unpublished data). This indicated the recruitment of specific  
366 lipids into DRMs during membrane stress. It is known that  
367 elevated Cer levels can displace Chol from membrane/lipid-  
368 'Chol-rafts' and form large, Cer-enriched membrane plat-  
369 forms 'Cer-rafts' [49,50]. Since both Chol and Cer (besides  
370 other raft-component lipids) accumulated during stress in  
371 whole B16-F10 cells, it is conceivable that the rafts undergo  
372 rearrangement and contain different protein components,  
373 thereby altering various signalling pathways, (such as those  
374 involving phosphatidylinositol 3-kinase, Akt and glycogen  
375 synthase kinase 3), which in turn may transmit the stress  
376 signal from the plasma membrane to the nucleus [35,51].  
377 Taken together, these findings may explain our previous  
378 observations concerning heat- or BA-induced Chol-rich PM  
379 microdomain condensation observed by fluorescence micros-  
380 copy in B16-F10 cells [43].

381 The increase in saturated lipids and the concomitant  
382 reduction of polyenes is a clear consequence of stress.  
383 This may highlight common metabolic processes which are  
384 involved in stress responses, whereas the lipid class- or the  
385 lipid species-dependent changes may reflect stressor-specific  
386 alterations. In accordance with the commonly accepted view  
387 [52–54] we suggest that the decrease in polyunsaturated fatty  
388 acid (PUFA)-containing lipids (with special emphasis on the  
389 20:4-containing species) following heat and BA treatments is  
390 due to the action of phospholipases. Such enzymatic activity  
391 is thought to be influenced by membrane fluidity and/or  
392 microheterogeneity for both phospholipase A<sub>2</sub> (PLA<sub>2</sub>) [55]  
393 and phospholipase C (PLC) [56]. These phospholipases are  
394 also known to be stimulated by heat shock (HS) and chemical  
395 stressors [57,58]. The lipid most affected by PUFA removal  
396 was PI (38:4), which can be metabolised mainly by PI-  
397 specific PLA<sub>2</sub> [59] or by PLC. The latter also hydrolyses  
398 PIP<sub>2</sub>, thereby producing two second messengers, diacylgly-  
399 cerol (DAG) and inositol triphosphate (IP<sub>3</sub>) [60]. IP<sub>3</sub> rapidly  
400 mediates the release of Ca<sup>2+</sup> from the endoplasmic reticu-  
401 lum following binding to IP<sub>3</sub> receptors. Interestingly, it has  
402 been reported that, in the initial stage of hyperthermia, the  
403 heat induces the turnover of polyphosphoinositides and the  
404 production of Ca<sup>2+</sup>-mobilising IP<sub>3</sub> [61]. Moreover, a number  
405 of reports have indicated that HS leads to a rapid increase in  
406 the level of intracellular free Ca<sup>2+</sup> from internal stores and  
407 a massive Ca<sup>2+</sup> influx from the extracellular medium. Cell  
408 calcium appears to be critical for the transcriptional activation  
409 of *hsp* genes in B16-F10 [43] and other cell lines [62].

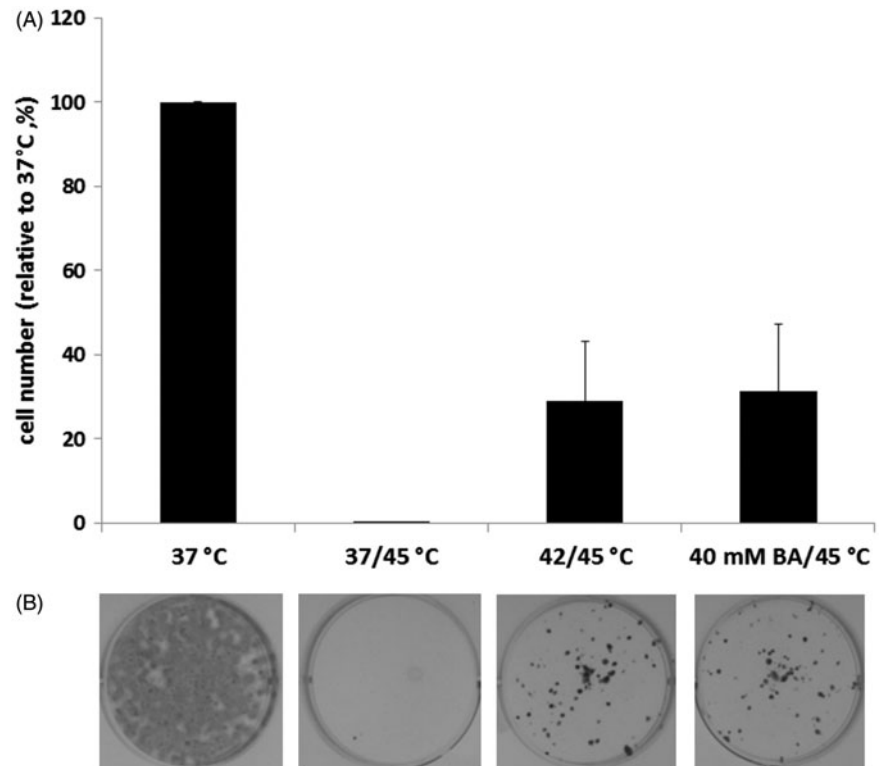
410 Elevated activity of phosphoinositide-specific PLC results  
411 in the formation of DAG which is highly enriched in  
412 arachidonic acid (AA) and may therefore function as second  
413 messenger [63]. It could for example enhance the membrane  
414 association and activation of various isoforms of protein  
415 kinase C (PKC) which have been found to drive the  
416 phosphorylation of HSFs [51]. This is consistent with the  
417 induced expression of Hsp70s in response to the activation of  
418 PKC [35]. Moreover, the heat-induced accumulation of PS  
419 and the BA-induced enhancement of DAG may play a positive  
420 regulatory role in PKC activation and consequently in HS

induction, since both PS and DAG are essential cofactors of  
PKC [64,65], but can be differently affected by the different  
stressors. 20:4-DAG can be subsequently metabolised by  
DAG lipase to 20:4- monoacylglycerol (20:4-MAG) [66,67]  
after which AA can be released through the action of  
monoacylglycerol lipase or fatty acid amide hydrolase action  
[68]. AA released by both PLA<sub>2</sub> and PLC-mediated pathways  
can mediate signal transduction and be recycled via the Lands  
pathway, whereas a portion can be lost to β-oxidation. In fact,  
the addition of AA to HeLa cells stimulated HSF1–DNA  
binding, increased the phosphorylation of HSF1 and, up-  
regulated the transcription of the *hsp70* gene [69] demonstrat-  
ing its HSP modulator ability.

In line with the above findings, it was reasonable to assume  
that the PUFA status and the ability of cells to respond to  
stress are closely interconnected. The modulation in HSP  
expression caused by plating density variation was studied by  
Noonan et al. [70] who observed that the activation of two  
human Hsp70 family members was indeed cell number-  
dependent after heat shock in colon carcinoma cell lines.  
As suggested by Koklic et al. in their 'membrane switch  
hypothesis' [71], the cell density strongly influences the  
lateral domain structure of tumour cell membranes by causing  
the appearance or disappearance of certain membrane domain  
types on the cell surface membranes (thereby acting as a  
'switch'). We recently provided evidence that simply the  
modulation of cell density considerably altered the induci-  
bility of *hsp* genes in B16-F10 cells, and was paralleled by  
pronounced changes in both the Chol level and the size  
distribution of pre-existing Chol-rich plasma membrane  
rafts [46]. When B16-F10 melanoma cells were cultured at  
different initial cell densities, lipidomic analysis revealed a  
profound rearrangement of molecular species composition,  
with around 70% of the lipid molecular species being altered.  
At the same time, different culturing conditions dramatically  
altered the stress inducibility of the major *hsp* genes, *hsp70*  
and *hsp25* [11]. In general, the importance of our findings  
lies in the need for n-3 and n-6 PUFA for the maintenance of  
stress protein responsibility in mammalian cells, which cannot  
synthesise their own, and draw attention to the need for their  
careful control. In fact, tumour cells exhibit a pronounced  
increase in *de novo* fatty acid synthesis, whereas normal cells  
are thought to acquire fatty acids primarily from dietary  
sources [72]. Moreover, our findings lend further support to  
the importance of both the 'quality' of the pre-existing  
membrane microdomains themselves and the release of lipid  
mediators (such as AA and derivatives), together with other  
stress protein-inducing signal transducers, which may act in  
tandem to control the extent of the ultimate cellular stress  
response [11].

A lipidomic approach will be useful for the determination  
of lipidome changes with prospective value as biomarkers and  
to disclose pathways with the potential for therapy [73,74].  
Furthermore, in order to understand the contribution of  
membrane lipid composition to the functionality of mem-  
brane-bound cellular processes (such as operation of surface  
membrane receptors, ion channels, or the mitochondrial  
electron transport chain), comprehensive structural and  
quantitative information on the organellar lipidome is essen-  
tial [75].

Figure 1. Acquisition of thermotolerance with heat and BA.



#### Acquisition of thermotolerance via prior membrane hyperfluidisation in B16-F10 melanoma cells

In a study by Balogh et al., the effects of the administration of the non-proteotoxic [42] membrane fluidiser BA and 'traditional' heat priming were investigated and compared concerning their capacities in thermotolerance development. B16-F10 cells were preconditioned (40 mM BA at 37°C or 42°C) for 1 h, and after a 16 h recovery period, were subjected to a 1 h lethal heat stress at 45°C. As shown in Figure 1, when preconditioned either with mild heat shock or with BA, these melanoma cells acquired a highly elevated thermotolerance. On the other hand, we observed a statistically insignificant difference in the effects induced by prior heat and isothermal membrane hyperfluidisation. Subsequently, we determined the level of the major HSPs, Hsp70 and Hsp25 by western blotting (Figure 2). Remarkably, whereas heat and BA priming exerted similar protective effects, BA treatment evoked weaker HSP response relative to heat priming at a HSP level. Noteworthy, no major differences were found in the levels of major HSPs between the heat sensitive B16 parent line and the heat resistant variants in other work, suggesting that HSPs are not a determining factor in the heat-resistant phenotype of B16 melanoma cells [76]. Thus, the acquired heat tolerance observed in this study should involve other mechanisms (see above) rather than solely the *de novo* synthesis of HSP chaperones. Importantly, if applied at the concentration equipotent in membrane fluidisation with BA, pretreatment with phenethyl alcohol, shown to be ineffective as an *hsp* activator, [43] also caused no measurable change of thermotolerance (unpublished observation).

It is noteworthy that we earlier demonstrated that the acquisition of cellular thermotolerance in BA-primed *Escherichia coli* cells was unrelated to the formation of the major HSPs, such as GroEL (Hsp60) and DnaK (Hsp70).

Instead, remodelling of the membrane lipid composition appeared to be sufficient for the development of short-term bacterial thermotolerance [30]. From studies using yeast unsaturated fatty acid auxotroph lipid mutants, Swan and Watson concluded that the strongly elevated heat sensitivity of unsaturated fatty acid-enriched cells is probably attributable to the membrane damage associated with increases in membrane fluidity independently of HSPs and trehalose [77]. To unravel the possible mechanisms underlying the capability of BA for heat shock gene activation, we earlier revealed that, apart from membrane hyperfluidisation in the deep hydrophobic region, a distinct reorganisation of Chol-sphingomyelin-rich microdomains may also be required for the generation and transmission of stress signals to activate *hsp* genes in B16 cells [43]. B16-F10 cells were next treated either with 40 mM BA or heat-stressed at 42°C for 1 h, and after a 16-h recovery period incubated with the Bodipy FL C<sub>5</sub>-sphingomyelin probe [78] for 10 min. They were then washed and imaged with a custom-made ultrasensitive microscope in total internal reflection mode. The domain size was analysed with the freeware ImageJ software ([www.uhnresearch.ca/facilities/wcif/imagej](http://www.uhnresearch.ca/facilities/wcif/imagej)), with its fast Fourier transform (FFT) bandpass filter and the nucleus counter plug-in (Figure 3). The sphingomyelin probe-labelled domains were sorted into six classes according to their diameters (Figure 3). Whereas the number of smaller domains decreased in response to both heat and BA priming, the larger domains accumulated. Importantly, the amplitude of the effects observed was always more pronounced in the case of BA-induced hyperfluidisation.

#### HSPs are more than simply chaperones

HSPs have multiple functions depending on their location. Some of the intracellular HSPs play an essential role as

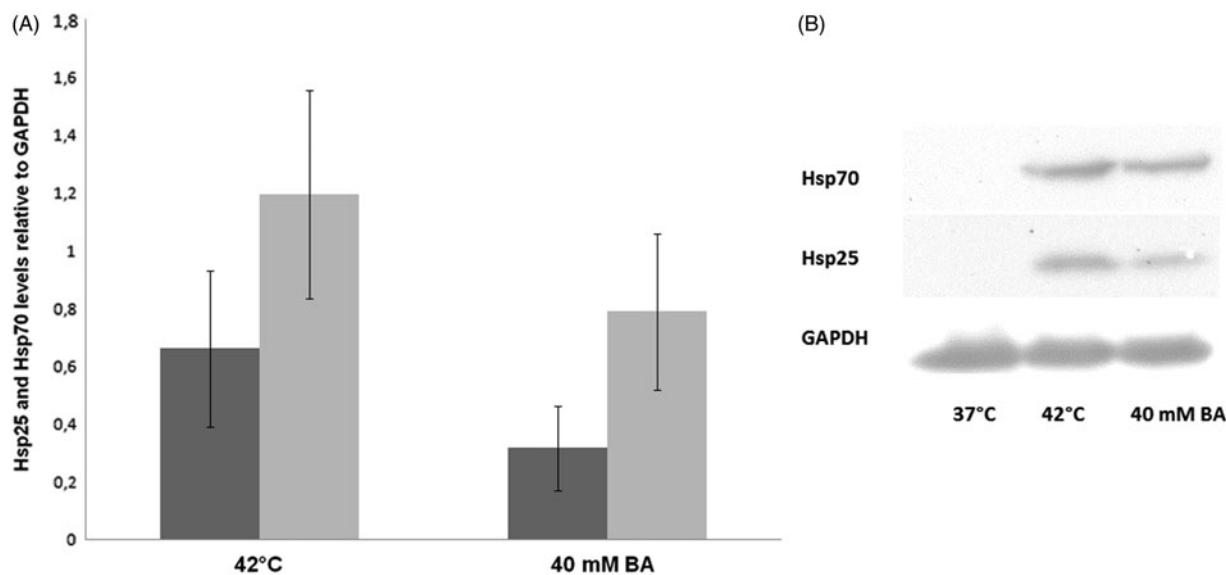
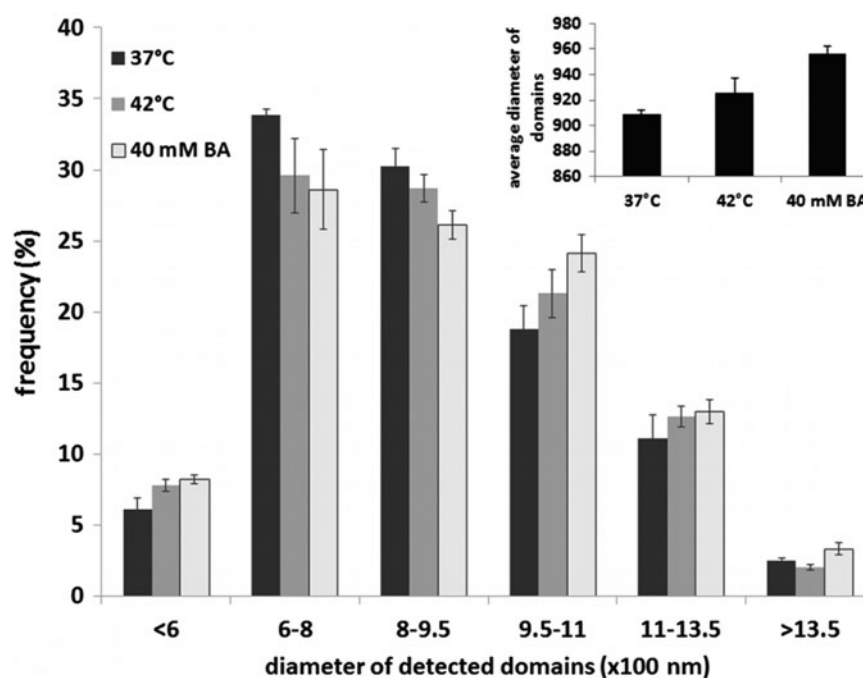


Figure 2. The effects of heat shock-induced or BA-induced membrane fluidisation on HSP expression in B16-F10 cells.

Figure 3. Redistribution of the cholesterol-rich membrane domains on the surface of B16-F10 cells, monitored by a BODIPY FL C5 sphingomyelin probe.



molecular chaperones by assisting the correct folding of nascent and stress-accumulated misfolded proteins, and preventing their aggregation. The protein- and/or lipid-mediated association of a specific set of stress protein molecular chaperones to membranes is a widespread phenomenon that was earlier partially or completely overlooked, and is implicated in a number of physiological and pathological events [64,79–81]. Most relevant to the present review, temporary association of certain HSPs with membranes can reduce the level of fluidity [82–85], elevate bilayer stability [86], and thereby restore the membrane functionality during heat stress conditions. A novel 16.2 kDa human small HSP, HspB11, was shown to inhibit H<sub>2</sub>O<sub>2</sub>, taxol and etoposide-induced cell death through preserving the integrity of the mitochondrial membrane system, the activation of Hsp90, the stabilisation of PM lipid rafts and activation of the PI-3kinase-Akt cytoprotective pathway. We recently provided

evidence for the cholesterol-controlled interaction of HspB11 with lipid rafts [87].

Hsp70 interacts with an anionic phospholipid, bis(monoacylglycerol)phosphate, (BMP) that is predominantly localised to the inner lysosomal membrane. The work of Kirkegaard and co-workers confirmed [88] that the pH-dependent (the interiors of lysosomes are highly acidic) and high-affinity BMP–Hsp70 interaction strongly promotes cell survival. The finding reveals a potential strategy for treating cancer by inhibiting the lysosome-stabilising effects of Hsp70 in tumour cells, thereby promoting lysosome-dependent autophagic cell death, in which the cell digests itself [81]. So molecules that either inhibit Hsp70-related signalling cascades (such as the PI3K/Akt/GSK pathway, which is linked to up-regulated Hsp70 transcription in cancers [36]), or drug candidates that directly block lysosomal localisation of Hsp70, might prove useful in anticancer therapy [89].



721 The association with the plasma membrane seems to  
 722 account for the pleiotropic effect of HSPs (predominantly  
 723 small HSPs) which can contribute to the restoration of  
 724 membrane activity following damage caused by abiotic  
 725 stresses or cancer therapies. It is suggested that sHSP-  
 726 mediated membrane stabilisation precedes the thermal  
 727 adaptation that occurs by adjustment of the lipid composition  
 728 [83]. As we pointed out, the fluidity and microdomain  
 729 organisation of membranes are decisive factors in the  
 730 perception and transduction of stresses into signals that  
 731 trigger the activation of specific heat shock genes [36].  
 732 Conversely, the membrane association of specific HSPs may  
 733 result in the inactivation of membrane-perturbing signal(s),  
 734 and thereby switch off the heat shock response. In that  
 735 context, interactions between certain HSPs and specific lipid  
 736 molecular species might be a previously unrecognised means  
 737 for the compartmentalisation of HSPs to specific signalling  
 738 platforms, where key stress signalling proteins are known to  
 739 be concentrated.

740 Finally, the cancer metabolism can only be perceived as  
 741 a network of pathways with plasticity, feedback loops and  
 742 cross-talk that ensure the ultimate fitness of the tumour  
 743 cells [90]. An understanding of the novel function of lipids  
 744 and chaperones (free, membrane-bound or extracellular  
 745 located) in the modulation of cell death and survival  
 746 signalling, which is of fundamental importance in ATT,  
 747 is just beginning to emerge. As suggested by Gabai and  
 748 Sherman, the role of HSPs in the refolding of damaged  
 749 proteins may not be as essential as earlier believed; instead,  
 750 the role of HSPs is crucial in the regulation of signalling  
 751 pathways [91]. Thus, the acquisition of further knowledge  
 752 will be necessary in order to improve the therapeutic potential  
 753 of hyperthermia.

### 754 **Concluding remarks**

756 Linked either to metabolic reprogramming or to therapy,  
 757 the elevated extent of membrane fluidity and reorganisation  
 758 of lipid rafts must be key determinants in the pleiotropic  
 759 effects of hyperthermia leading ultimately to cellular adap-  
 760 tation or lethality. Comparative studies with heat- and  
 761 membrane-primed melanoma cells reinforce the view that  
 762 ATT involves general as well as stress-specific components.  
 763 It is beyond doubt that, through their molecular chaperone  
 764 activities, the prominent HSP family members can contribute  
 765 to the development of thermotolerance. Further investigation  
 766 of the role of membrane microdomain properties (biophysical  
 767 and biochemical), together with the moonlighting HSPs in  
 768 heat sensing, signalling and adaptation, and understanding  
 769 the way these phenomena act as a network, appears essential  
 770 to explore hyperthermia-induced events.

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## References

1. Pallepati P, Averill-Bates D. Mild thermotolerance induced at 40 °C increases antioxidants and protects HeLa cells against mitochondrial apoptosis induced by hydrogen peroxide: Role of p53. *Arch Biochem Biophys* 2010;495:97–111. 781
2. Issels RD. Hyperthermia adds to chemotherapy. *Eur J Cancer* 2008;44:2546–54. 782
3. Ostberg JR, Repasky EA. Emerging evidence indicates that physiologically relevant thermal stress regulates dendritic cell function. *Cancer Immunol Immunother* 2006;55:292–8. 783
4. Peer AJ, Grimm MJ, Zynda ER, Repasky EA. Diverse immune mechanisms may contribute to the survival benefit seen in cancer patients receiving hyperthermia. *Immunol Res* 2010;46:137–54. 784
5. Mace TA, Zhong L, Kilpatrick C, Zynda E, Lee C-T, Capitano M, et al. Differentiation of CD8+ T cells into effector cells is enhanced by physiological range hyperthermia. *J Leukoc Biol* 2011;90:951–62. 785
6. Takahashi A, Yamakawa N, Mori E, Ohnishi K, Yokota S, Sugo N, et al. Development of thermotolerance requires interaction between polymerase-beta and heat shock proteins. *Cancer Sci* 2008;99:973–8. 786
7. Nivon M, Richet E, Codogno P, Arrigo A-P, Kretz-Remy C. Autophagy activation by NFkappaB is essential for cell survival after heat shock. *Autophagy* 2009;5:766–83. 787
8. Heilbrunn LV. The colloid chemistry of protoplasm. IV. The heat coagulation of protoplasm. *Am J Physiol* 1924;69:190–9. 788
9. Yatvin MB, Clifton KH, Dennis WH. Hyperthermia and local anesthetics: Potentiation of survival of tumor-bearing mice. *Science* 1979;205:195–6. 789
10. Balogh G, Péter M, Liebisch G, Horváth I, Török Z, Nagy E, et al. Lipidomics reveals membrane lipid remodelling and release of potential lipid mediators during early stress responses in a murine melanoma cell line. *Biochim Biophys Acta* 2010;1801:1036–47. 790
11. Péter M, Balogh G, Gombos I, Liebisch G, Horváth I, Török Z, et al. Nutritional lipid supply can control the heat shock response of B16 melanoma cells in culture. *Mol Membr Biol* 2012;29:274–89. 791
12. Baritaki S, Apostolakis S, Kanellou P, Dimanche-Boitrel M-T, Spandidos DA, Bonavida B. Reversal of tumor resistance to apoptotic stimuli by alteration of membrane fluidity: Therapeutic implications. *Adv Cancer Res* 2007;98:149–90. 792
13. Zhang F, Du G. Dysregulated lipid metabolism in cancer. *World J Biol Chem* 2012;3:167–74. 793
14. Nomura DK, Long JZ, Niessen S, Hoover HS, Ng S-W, Cravatt BF. Monoacylglycerol lipase regulates a fatty acid network that promotes cancer pathogenesis. *Cell* 2010;140:49–61. 794
15. Cadenas C, Vosbeck S, Hein E-M, Hellwig B, Langer A, Hayen H, et al. Glycerophospholipid profile in oncogene-induced senescence. *Biochim Biophys Acta* 2012;1821:1256–68. 795
16. Funaki N, Tanaka J, Kono Y, Nonaka A, Yotsumoto F, Lee J-U, et al. Combination of alpha-fetoprotein mRNA-based detection of hematogenously disseminating hepatocellular carcinoma cells and analysis of cancer cell membrane fluidity is more accurate in screening patients at risk of postoperative recurrence. *Oncol Rep* 2004;11:637–9. 796
17. Sok M, Sentjurs M, Schara M, Stare J, Rott T. Cell membrane fluidity and prognosis of lung cancer. *Ann Thorac Surg* 2002;73:1567–71. 797
18. Yukihiro M, Komizu Y, Tanoue O, Matsushita T, Matsumoto Y, Ueoka R. Specific accumulation and antitumor effects of hybrid liposomes on the growth of lung tumor cells. *Yakugaku Zasshi* 2010;130:1581–7. 798
19. Batko J, Plotast-Necas B, Warchol T, Karoń H. The effect of an experimental neoplastic disease on the flux of sodium and potassium ions across red blood cells and on the lipid composition of their membranes. *Acta Biochim Pol* 1992;39:317–26. 799
20. Barker CJ, Bowler K. Lipid composition of the membranes from cells of two rat tumors and its relationship to tumor thermosensitivity. *Radiat Res* 1991;125:48–55. 800
21. Gonda K, Watanabe TM, Ohuchi N, Higuchi H. In vivo nano-imaging of membrane dynamics in metastatic tumor cells using quantum dots. *J Biol Chem* 2010;285:2750–7. 801
22. Zeisig R, Koklic T, Wiesner B, Fichtner I, Sentjurs M. Increase in fluidity in the membrane of MT3 breast cancer cells correlates 802

- with enhanced cell adhesion in vitro and increased lung metastasis in NOD/SCID mice. *Arch Biochem Biophys* 2007;459:98–106.
23. Tarabozetti G, Perin L, Bottazzi B, Mantovani A, Giavazzi R, Salmona M. Membrane fluidity affects tumor-cell motility, invasion and lung-colonizing potential. *Int J Cancer* 1989;44:707–13.
  24. Sherbet G V. Membrane fluidity and cancer metastasis. *Exp Cell Biol* 1989;57:198–205.
  25. Benkő S, Hilkmann H, Vigh L, Van Blitterswijk WJ. Catalytic hydrogenation of fatty acyl chains in plasma membranes: Effect on membrane lipid fluidity and expression of cell surface antigens. *Biochim Biophys Acta* 1987;896:129–35.
  26. Angelucci C, Maulucci G, Lama G, Proietti G, Colabianchi A, Papi M, et al. Epithelial-stromal interactions in human breast cancer: Effects on adhesion, plasma membrane fluidity and migration speed and directness. *PLoS One* 2012;7:e50804.
  27. Segui B, Legembre P. Redistribution of CD95 into the lipid rafts to treat cancer cells? *Recent Pat Anticancer Drug Discov* 2010;5:22–8.
  28. Miller RC, Richards M, Baird C, Martin S, Hall EJ. Interaction of hyperthermia and chemotherapy agents: Cell lethality and oncogenic potential. *Int J Hyperthermia* 1994;10:89–99.
  29. Horváth I, Glatz A, Varvasovszki V, Török Z, Páli T, Balogh G, et al. Membrane physical state controls the signaling mechanism of the heat shock response in *Synechocystis* PCC 6803: Identification of *hsp17* as a ‘fluidity gene’. *Proc Natl Acad Sci USA* 1998;95:3513–18.
  30. Shigapova N, Török Z, Balogh G, Goloubinoff P, Vigh L, Horváth I. Membrane fluidization triggers membrane remodeling which affects the thermotolerance in *Escherichia coli*. *Biochem Biophys Res Commun* 2005;328:1216–23.
  31. Horváth I, Glatz A, Nakamoto H, Mishkind ML, Munnik T, Saidi Y, et al. Heat shock response in photosynthetic organisms: Membrane and lipid connections. *Prog Lipid Res* 2012;51:208–20.
  32. Vigh L, Horváth I, Horváth LI, Dudits D, Farkas T. Protoplast plasmalemma fluidity of hardened wheats correlates with frost resistance. *FEBS Lett* 1979;107:291–4.
  33. Vigh L, Horváth I, Farkas T, Horváth LI, Belea A. Adaptation of membrane fluidity of rye wheat seedlings according to temperature. *Phytochemistry* 1979;18:787–9.
  34. Vigh L, Maresca B, Harwood JL. Does the membrane’s physical state control the expression of heat shock and other genes? *Trends Biochem Sci* 1998;23:369–74.
  35. Vigh L, Escribá P V, Sonnleitner A, Sonnleitner M, Piotto S, Maresca B, et al. The significance of lipid composition for membrane activity: New concepts and ways of assessing function. *Prog Lipid Res* 2005;44:303–44.
  36. Vigh L, Horváth I, Maresca B, Harwood JL. Can the stress protein response be controlled by ‘membrane-lipid therapy’? *Trends Biochem Sci* 2007;32:357–63.
  37. Vigh L, Los DA, Horváth I, Murata N. The primary signal in the biological perception of temperature: Pd-catalyzed hydrogenation of membrane lipids stimulated the expression of the *desA* gene in *Synechocystis* PCC6803. *Proc Natl Acad Sci USA* 1993;90:9090–4.
  38. Simons K, Sampaio JL. Membrane organization and lipid rafts. *Cold Spring Harb Perspect Biol* 2011;3(10):a004697.
  39. Olivera-Couto A, Aguilar PS. Eisosomes and plasma membrane organization. *Mol Genet Genomics* 2012;287:607–20.
  40. Balogh G, Péter M, Glatz A, Gombos I, Török Z, Horváth I, et al. Key role of lipids in heat stress management. *FEBS Lett* 2013;587:1970–80.
  41. Brameshuber M, Weghuber J, Ruprecht V, Gombos I, Horváth I, Vigh L, et al. Imaging of mobile long-lived nanoplateforms in the live cell plasma membrane. *J Biol Chem* 2010;285:41765–71.
  42. Balogh G, Horváth I, Nagy E, Hoyk Z, Benkő S, Bensaude O, et al. The hyperfluidization of mammalian cell membranes acts as a signal to initiate the heat shock protein response. *FEBS J* 2005;272:6077–86.
  43. Nagy E, Balogi Z, Gombos I, Akerfelt M, Björkbohm A, Balogh G, et al. Hyperfluidization-coupled membrane microdomain reorganization is linked to activation of the heat shock response in a murine melanoma cell line. *Proc Natl Acad Sci USA* 2007;104:7945–50.
  44. Giocondi M-C, Besson F, Dosset P, Milhiet P-E, Le Grimellec C. Temperature-dependent localization of GPI-anchored intestinal alkaline phosphatase in model rafts. *J Mol Recognit* 2007;20:531–7.
  45. Chung J, Nguyen A-K, Henstridge DC, Holmes AG, Chan MHS, Mesa JL, et al. HSP72 protects against obesity-induced insulin resistance. *Proc Natl Acad Sci USA* 2008;105:1739–44.
  46. Gombos I, Crul T, Piotto S, Güngör B, Török Z, Balogh G, et al. Membrane-lipid therapy in operation: The HSP co-inducer BGP-15 activates stress signal transduction pathways by remodeling plasma membrane rafts. *PLoS One* 2011;6(12):e28818.
  47. Crul T, Toth N, Piotto S, Literati-Nagy P, Tory K, Haldimann P, et al. Hydroxamic acid derivatives: Pleiotropic HSP co-inducers restoring homeostasis and robustness. *Curr Pharm Des* 2013;19:309–46.
  48. Mukherjee S, Maxfield FR. Membrane domains. *Annu Rev Cell Dev Biol* 2004;20:839–66.
  49. Grassme H, Riethmüller J, Gulbins E, Grassmé H, Riethmüller J. Biological aspects of ceramide-enriched membrane domains. *Prog Lipid Res* 2007;46:161–70.
  50. Patra SK. Dissecting lipid raft facilitated cell signaling pathways in cancer. *Biochim Biophys Acta* 2008;1785:182–206.
  51. Vigh L, Nakamoto H, Landry J, Gomez-Munoz A, Harwood JL, Horvath I. Membrane regulation of the stress response from prokaryotic models to mammalian cells. *Ann N Y Acad Sci* 2007;1113:40–51.
  52. Balboa MA, Balsinde J. Oxidative stress and arachidonic acid mobilization. *Biochim Biophys Acta* 2006;1761:385–91.
  53. Balsinde J, Winstead M V, Dennis EA. Phospholipase A(2) regulation of arachidonic acid mobilization. *FEBS Lett* 2002;531:2–6.
  54. Hirabayashi T, Murayama T, Shimizu T. Regulatory mechanism and physiological role of cytosolic phospholipase A2. *Biol Pharm Bull* 2004;27:1168–73.
  55. Hønger T, Jørgensen K, Biltonen RL, Mouritsen OG. Systematic relationship between phospholipase A2 activity and dynamic lipid bilayer microheterogeneity. *Biochemistry* 1996;35:9003–6.
  56. Ahyayauch H, Villar A V, Alonso A, Goñi FM. Modulation of PI-specific phospholipase C by membrane curvature and molecular order. *Biochemistry* 2005;44:11592–600.
  57. Calderwood SK, Stevenson MA, Price BD. Activation of phospholipase C by heat shock requires GTP analogs and is resistant to pertussis toxin. *J Cell Physiol* 1993;156:153–9.
  58. Samples BL, Pool GL, Lumb RH. Polyunsaturated fatty acids enhance the heat induced stress response in rainbow trout (*Oncorhynchus mykiss*) leukocytes. *Comp Biochem Physiol B Biochem Mol Biol* 1999;123:389–97.
  59. Corda D, Zizza P, Varone A, Filippi BM, Mariggio S. The glycerophosphoinositols: Cellular metabolism and biological functions. *Cell Mol Life Sci* 2009;66:3449–67.
  60. Wymann MP, Schneider R. Lipid signalling in disease. *Nat Rev Mol Cell Biol* 2008;9:162–76.
  61. Calderwood SK, Stevenson MA, Hahn GM. Heat stress stimulates inositol trisphosphate release and phosphorylation of phosphoinositides in CHO and Balb C 3T3 cells. *J Cell Physiol* 1987;130:369–76.
  62. Kiang JG, Tsokos GC. Heat shock protein 70kDa: Molecular biology, biochemistry, and physiology. *Pharmacol Ther* 1998;80:183–201.
  63. Wakelam MJ. Diacylglycerol – When is it an intracellular messenger? *Biochim Biophys Acta* 1998;1436:117–26.
  64. Escribá P V, González-Ros JM, Goñi FM, Kinnunen PKJ, Vigh L, Sánchez-Magraner L, et al. Membranes: A meeting point for lipids, proteins and therapies. *J Cell Mol Med* 2008;12:829–75.
  65. Griner EM, Kazanietz MG. Protein kinase C and other diacylglycerol effectors in cancer. *Nat Rev Cancer* 2007;7:281–94.
  66. Shimizu T, Yokotani K. Bidirectional roles of the brain 2-arachidonoyl-sn-glycerol in the centrally administered vasopressin-induced adrenomedullary outflow in rats. *Eur J Pharmacol* 2008;582:62–9.
  67. Tang X, Edwards EM, Holmes BB, Falck JR, Campbell WB. Role of phospholipase C and diacylglyceride lipase pathway in arachidonic acid release and acetylcholine-induced vascular relaxation in rabbit aorta. *Am J Physiol Heart Circ Physiol* 2006;290:H37–45.
  68. Chau LY, Tai HH. Release of arachidonate from diglyceride in human platelets requires the sequential action of a diglyceride



- lipase and a monoglyceride lipase. *Biochem Biophys Res Commun* 1981;100:1688–95.
69. Jurivich DA, Pachetti C, Qiu L, Welk JF. Salicylate triggers heat shock factor differently than heat. *J Biol Chem* 1995;270:24489–95.
70. Noonan EJ, Place RF, Rasoulopour RJ, Giardina C, Hightower LE. Cell number-dependent regulation of Hsp70B' expression: Evidence of an extracellular regulator. *J Cell Physiol* 2007;210:201–11.
71. Koklic T, Pirs M, Zeisig R, Abramović Z, Sentjurc M. Membrane switch hypothesis. I. Cell density influences lateral domain structure of tumor cell membranes. *J Chem Inf Model* 2005;45:1701–7.
72. Menendez JA, Lupu R. Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. *Nat Rev Cancer* 2007;7:763–77.
73. Hønger T, Jørgensen K, Biltonen RL, Mouritsen OG. Systematic relationship between phospholipase A2 activity and dynamic lipid bilayer microheterogeneity. *Biochemistry* 1996;35:9003–6.
74. Fernandis AZ, Wenk MR. Lipid-based biomarkers for cancer. *J Chromatogr B Analyt Technol Biomed Life Sci* 2009;877:2830–5.
75. Klose C, Surma MA, Simons K. Organellar lipidomics-background and perspectives. *Curr Opin Cell Biol* 2013. doi: 10.1016/j.ceb.2013.03.005.
76. Anderson RL, Tao TW, Betten DA, Hahn GM. Heat shock protein levels are not elevated in heat-resistant B16 melanoma cells. *Radiat Res* 1986;105:240–6.
77. Swan TM, Watson K. Stress tolerance in a yeast lipid mutant: membrane lipids influence tolerance to heat and ethanol independently of heat shock proteins and trehalose. *Can J Microbiol* 1999;45:472–9.
78. Redman CA, Kennington S, Spathopoulou T, Kusel JR. Interconversion of sphingomyelin and ceramide in adult *Schistosoma mansoni*. *Mol Biochem Parasitol* 1997;90:145–53.
79. Nakamoto H, Vigh L. The small heat shock proteins and their clients. *Cell Mol Life Sci* 2007;64:294–306.
80. Horváth I, Multhoff G, Sonnleitner A, Vigh L. Membrane-associated stress proteins: More than simply chaperones. *Biochim Biophys Acta* 2008;1778:1653–64.
81. Horváth I, Vigh L. Cell biology: Stability in times of stress. *Nature* 2010;463:436–8.
82. Török Z, Horváth I, Goloubinoff P, Kovács E, Glatz A, Balogh G, et al. Evidence for a lipochaperonin: Association of active protein-folding GroESL oligomers with lipids can stabilize membranes under heat shock conditions. *Proc Natl Acad Sci USA* 1997;94:2192–7.
83. Török Z, Goloubinoff P, Horváth I, Tsvetkova NM, Glatz A, Balogh G, et al. *Synechocystis* Hsp17 is an amphitropic protein that stabilizes heat-stressed membranes and binds denatured proteins for subsequent chaperone-mediated refolding. *Proc Natl Acad Sci USA* 2001;98:3098–103.
84. Balogi Z, Török Z, Balogh G, Jósvey K, Shigapova N, Vierling E, et al. Heat shock lipid in cyanobacteria during heat/light-acclimation. *Arch Biochem Biophys* 2005;436:346–54.
85. Balogi Z, Cheregi O, Giese KC, Juhász K, Vierling E, Vass I, et al. A mutant small heat shock protein with increased thylakoid association provides an elevated resistance against UV-B damage in *Synechocystis* 6803. *J Biol Chem* 2008;283:22983–91.
86. Tsvetkova NM, Horváth I, Török Z, Wolkers WF, Balogi Z, Shigapova N, et al. Small heat-shock proteins regulate membrane lipid polymorphism. *Proc Natl Acad Sci USA* 2002;99:13504–9.
87. Török Z, Pilbat AM, Gombos I, Hocsák E, Sümegi B, Horváth I, et al. Evidence on cholesterol-controlled lipid raft interaction of the small heat shock protein HSPB11. In: Henderson B, Pockley AG, editors. *Cellular Trafficking of Cell Stress Proteins in Health and Disease*. Dordrecht: Springer; 2012.
88. Kirkegaard T, Roth AG, Petersen NHT, Mahalka AK, Olsen OD, Moilanen I, et al. Hsp70 stabilizes lysosomes and reverts Niemann-Pick disease-associated lysosomal pathology. *Nature* 2010;463:549–53.
89. Juhász K, Thuenauer R, Spachinger A, Duda E, Horváth I, Vigh L, et al. Lysosomal rerouting of Hsp70 trafficking as a potential immune activating tool for targeting melanoma. *Curr Pharm Des* 2013;19:430–40.
90. Carracedo A, Cantley LC, Pandolfi PP. Cancer metabolism: Fatty acid oxidation in the limelight. *Nat Rev Cancer* 2013;13:227–32.
91. Gabai VL, Sherman MY. Invited review: Interplay between molecular chaperones and signaling pathways in survival of heat shock. *J Appl Physiol* 2002;92:1743–8.