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The role of non-canonical Hsp70s (Hsp110/Grp170) in cancer

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Abstract: Although cancers account for over 16% of all global deaths annually, at present, no reliable therapies exist for most types of the disease. As protein folding facilitators, heat shock proteins (Hsps) play an important role in cancer development. Not surprisingly, Hsps are among leading anticancer drug targets. Generally, Hsp70s are divided into two main subtypes: canonical Hsp70 (*E. coli* Hsp70/DnaK homologues) and the non-canonical (Hsp110 and Grp170) members. These two main Hsp70 groups are delineated from each other by distinct structural and functional specifications. Non-canonical Hsp70s are considered as holdase chaperones, while canonical Hsp70s are refoldases. This distinct characteristic feature is mirrored by the distinct structural features of these two groups of chaperones. Hsp110/Grp170 members are larger as they possess an extended acidic insertion in their substrate binding domains. While the role of canonical Hsp70s in cancer has received a fair share of attention, the roles of non-canonical Hsp70s in cancer development has received less attention in comparison. In the current review, we discuss the structure-function features of non-canonical Hsp70s members and how these features impact on their role in cancer development. We further mapped out their interactome and discussed the prospects of targeting these proteins in cancer therapy.

Keywords: Hsp110, Grp170, non-canonical Hsp70, chaperone, cancer (296 words)

1. Introduction

Cancer accounts for approximately one sixth of total global annual deaths [1]. It is estimated that approximately 9.6 million cancer-related deaths were recorded in 2018, and the majority of these were attributed to lung cancer, hepatocellular carcinoma, breast cancer and colorectal cancer [1]. Approximately 70% of all cancer-related deaths occur in low to middle income countries (LMICs) where cancer causing infections such as hepatitis and human papilloma viruses are prevalent [1]. The most widely used interventions against cancer, such as chemotherapy and radiotherapy, are not always effective due to treatment-induced cellular, genetic and biochemical changes that often confer treatment resistance [2]. This, therefore, urgently necessitates the need to identify novel anticancer targets. Apart from their role as molecular chaperones, heat shock proteins (Hsps) play an important role in various cancer

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signalling pathways such as tumourigenesis, carcinogenesis and apoptosis [3-4]. As such, the role of Hsps as cancer biomarkers is increasingly becoming apparent.

Key hallmarks of tumourigenesis include: (i) unregulated proliferative signalling; (ii) escape from apoptosis; (iii) evasion of antigrowth signals; (iv) avoidance of cell senescence; (v) *de novo* angiogenesis; and (vi) cell invasion and metastasis [5]. Several proteins play crucial roles in facilitating each of these tumourigenesis stages (Figure 1). In addition, genome instability and inflammation can also trigger tumourigenesis [6]. It is therefore not surprising that upset of cellular proteostasis is one of the several factors that could drive tumour cell proliferation and metastasis. Deregulation of growth facilitates cell proliferation which occurs under the control of growth factors [7]. Growth factors bind onto extracellular receptor proteins of tumour cells with high affinity to facilitate signal transduction into the interior of the cell through a series of relay proteins, ultimately resulting in cell proliferation [8]. Cancer cell proliferation thus depends on the role of receptors and proteins which constitute an oncogenic cascade pathway. Further to this, cancer cells deploy several proteins towards evading apoptosis which would be prompted by cytotoxicity (e.g. induced by therapeutic interventions) as well as by the accumulation of oncogenic proteins ([9], Figure 1). As signalling molecules, Hsps are intimately linked to cancer progression. For example, a small Hsp (sHsp), Hsp27, and Hsp70 inhibit release of cytochrome c, caspase 3 and 9 from the mitochondria, thus causing cells to evade apoptosis [10-11]

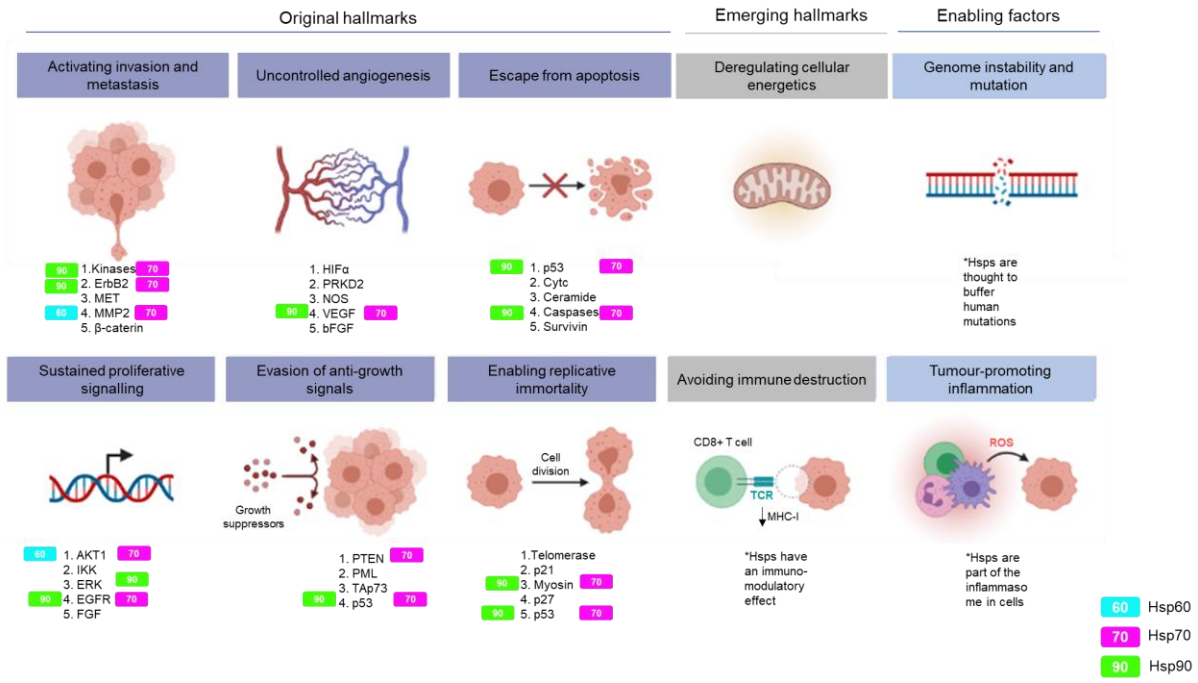

Figure 1. The proteomic landscape of the hallmarks of cancer

The processes of metastasis, uncontrolled angiogenesis, evasion of anti-growth signals, escape from apoptosis, cell proliferation and evasion of senescence are all crucial to tumour cell growth. Each of these processes is

regulated by several proteins that play important roles in the respective signalling pathways. As chaperones, Hsp70, Hsp90 and Hsp60 associate with several proteins that regulate cancer signalling pathways.

Another important characteristic feature of cancer cells is their ability to evade senescence by deploying telomerase, whose role is to shorten the ends of telomeres [12]. Several other proteins, such as p53 are also implicated in senescence. p53 promotes senescence by transcriptionally upregulating the cell cycle protein p21, leading to arrest of cell proliferation [13] . Thus, by ensuring sustained high levels of telomerase coupled to low levels of p53, cancer cells evade senescence leading to tumourigenesis. Kinases such as ErbB2 and MET also play an important role in metastasis of cancer cells [14]. Tumour cells therefore rely on a robust protein folding machinery to provide functional proteins to meet their physiological requirements.

Since they are constantly in a state of proteotoxic stress, cancer cells largely exploit Hsps to protect themselves against the toxic effects of aberrant oncoproteins, genomic instability, hypoxia, and acidosis [15-16]. High Hsp expression levels are associated with poor prognosis and treatment resistance in cancer patients, since Hsps protect tumour cells from therapeutic stressors such as radiation and cytotoxic chemotherapy [17]. Indeed, overexpression of Hsps has been observed in a wide range of cancers, including breast, endometrial, ovarian, gastric, colon, lung and prostate cancer [17-20]Due to their ability to oversee proteostasis, Hsps facilitate the folding and maturation of proteins involved in cancer signalling pathways (Figure 1). Therefore, elevated Hsp levels are associated with tumour progression [21].

It has been demonstrated that, inhibition of Hsp90, induces degradation of oncogenic proteins [22-23]. Additionally, the expression of some oncogenes in the absence of Hsp70 may result in cell inactivation [24]. This underscores the importance of Hsps in modulating oncogenic processes. Remarkably, Hsp70 and Hsp27 have both been shown to interact directly with protein intermediates of the apoptosis pathway [25-26]. Since it is highly expressed in malignant tumours and on the surface of tumour cells, Hsp70 typically serves as a biomarker of poor prognosis in cancer patients. Notably, the roles of the Hsp70 and Hsp90 in cancer development are well established [27-28]. Consequently, most Hsp-targeted anti-cancer treatment efforts have primarily focused on Hsp70 and its ER homologue, Grp78 as well as Hsp90 [29-31]. The kinase inhibitor, Sorafenib, used in the treatment of renal cell carcinoma and hepatocellular carcinoma, is an example of an Hsp70 targeting anti-cancer drug which functions to reduce the expression of Grp78 in cancer cells [32]. Thus, small molecules that modulate Hsp expression as well as those that inhibit their activity constitute possible anticancer agents.

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2. Hsp110/Grp170

The human genome encodes a total of 17 Hsp70s; 4 of which are Hsp110 or Grp170protein homologues ([33]; Table 1). Grp170 proteins are closely related to the Hsp110 family of proteins which occur in the endoplasmic reticulum (ER) and are primarily induced by glucose deprivation [34]. Both Grp170 and Hsp110 proteins constitute non-canonical clade of the Hsp70 family. Hence, in our narrative, except where it distinguishes the function of these two proteins within their distinct cellular localisation, we use the terms Hsp110 and Grp170 interchangeably as the two chaperone are generally similar in structure and function.

Hsp70s are typically characterised by an N-terminal nucleotide binding domain (NBD) and a C-terminal substrate binding domain (SBD), connected by a linker (Figure 2). Although the NBDs of canonical and non-canonical Hsp70s exhibit relatively high sequence conservation, their SBDs are more divergent [35]. In spite of their high conservation, members of the Hsp70 family are characterised by unique signature motifs that define their functional specialization within cells. Notably, Hsp110s are marked by extended acidic insertions located within their substrate binding domain, SDB-β and SBD-α subunits (Figure 2 A; [36]). Additionally, Hsp110s possess linker segments that are distinct from canonical Hsp70s [37-38].

Table 1. Hsp110/Grp170 proteins of human origin

Protein (Accession number)	Size (kDa)	Localization	Stress Inducible (Yes/No)	Cellular functions	References
1. HspH1 (Q92598)	97	Cytosol, nucleus, endocytic vesicle	Yes	Apoptosis suppression, aggregation suppression, NEF	[39]
2. HspH3 (O95757)	95	Cytosol, nucleus	Yes	Ellicits humoral immune responses in leukemia patients	[40]
3. HspH2 (P34932)	95	Cytosol, extracellular exosome	N.D	Implicated in spermatogenesis	[41]
4. Grp170 (Q9Y4L1)	111	E.R	Yes	Aggregation suppression, NEF	[42]

ND: not determined

Hsp110s possess seven β strands in the SBD, while canonical Hsp70s possess eight β strands [36]. Using three-dimensional models, we also observed structural variations within

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the loop regions $L_{1,2}$ and $L_{4,5}$ of a human canonical Hsp70 (HspA1A) and Hsp110 (HspH1) (Figure 2). Structural variations arising within these SBD sections potentially account for the functional delineations of Hsp110s in comparison to canonical Hsp70s. Generally, the SBD of Hsp110s preferentially binds peptide substrates harbouring aromatic residues in contrast to canonical Hsp70s which preferentially bind substrates enriched with aliphatic residues [43]. Furthermore, *P. falciparum* Hsp110 was also previously shown to exhibit unique substrate binding preferences in comparison to its canonical form [44]. Structural variations in loops of Hsp70 are important for the function of the chaperone. For example, loops $L_{1,2}$ and $L_{3,4}$ located in SBDβ and are thought to regulate substrate binding specificity [45]. It was recently reported that most variations in the SBD segments of Hsp70s not only occur within the loop regions of the substrate binding cleft but also in the helical lid (SBDα) sections [35]. Indeed, the SBDα segment of Hsp110 is endowed with acidic insertions that are absent in the canonical isoform (Figure 2). This suggests that the lid regulates functional specificity of Hsp70 [44]. Hsp110 is reported to possess significantly higher substrate binding efficiency than canonical Hsp70 [44, 46]and this can be attributed to its longer SBDα lid segment Furthermore, the yeast Hsp110 homologue, Sse1 was shown to exhibit unique peptide-binding preferences from the canonical Hsp70 homologue (Ssa1), suggesting that Hsp70 and Hsp110 substrates are do not serve entirely overlapping functions [46].

Using three-dimensional structural modelling tools (Biova Discovery Studio 4.5), we noted some structural differences between the four human Hsp110 isoforms (HspH1, HspH2, HspH3, Grp170). Generally, the NBD segments of the 4 non-canonical Hsp70s exhibit high conservation (Figure 2C). Conservation of the NBD of these non-canonical Hsp70s is important in light of the role of this motif in regulating nucleotide exchange of their canonical Hsp70 counterparts. However, notable variations in their substrate binding domains [45]. It is therefore conceivable that, these variations observed within the individual loop segments may account for differences in the preferred substrate clientomes. As such, each of the Hsp110s may play distinct roles in chaperoning proteins involved in cancer signalling pathways. In comparison to Hsp110, Grp170 is exhibits a unique alpha helical section within $L_{4,5}$ and $L_{5,6}$ (Figure 2C). Thus, as an endoplasmic reticulum based chaperone, Grp170 is possibly functionally adapted for its role in the ER. Consequently, it is also conceivable that Grp170 chaperones a specialized set of oncogenic proteins located within the ER.

Functionally, Hsp110/Grp170 subfamily members bind misfolding polypeptides, to prevent their aggregation[37, 47-48]). This way they maintain denatured protein substrates in a soluble, folding-competent state before handing them over to canonical Hsp70 for folding into native state [49-50]. In addition, canonical Hsp70 releases its substrate in the presence of ATP; and stably bind substrate in the ADP-bound state [51]. On the other hand, the chaperone

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function of Hsp110/Grp170 chaperones is not regulated by nucleotides [43]. Thus, Hsp110/Grp170 are more effective holdase chaperones than their canonical Hsp70 counterparts [37]. Hence canonical Hsp70s serve as a refoldase while Hsp110/Grp170 members are buffers against proteostatic stress [37].Hsp110/Grp170 also function as NEFs of canonical Hsp70 [52-53].

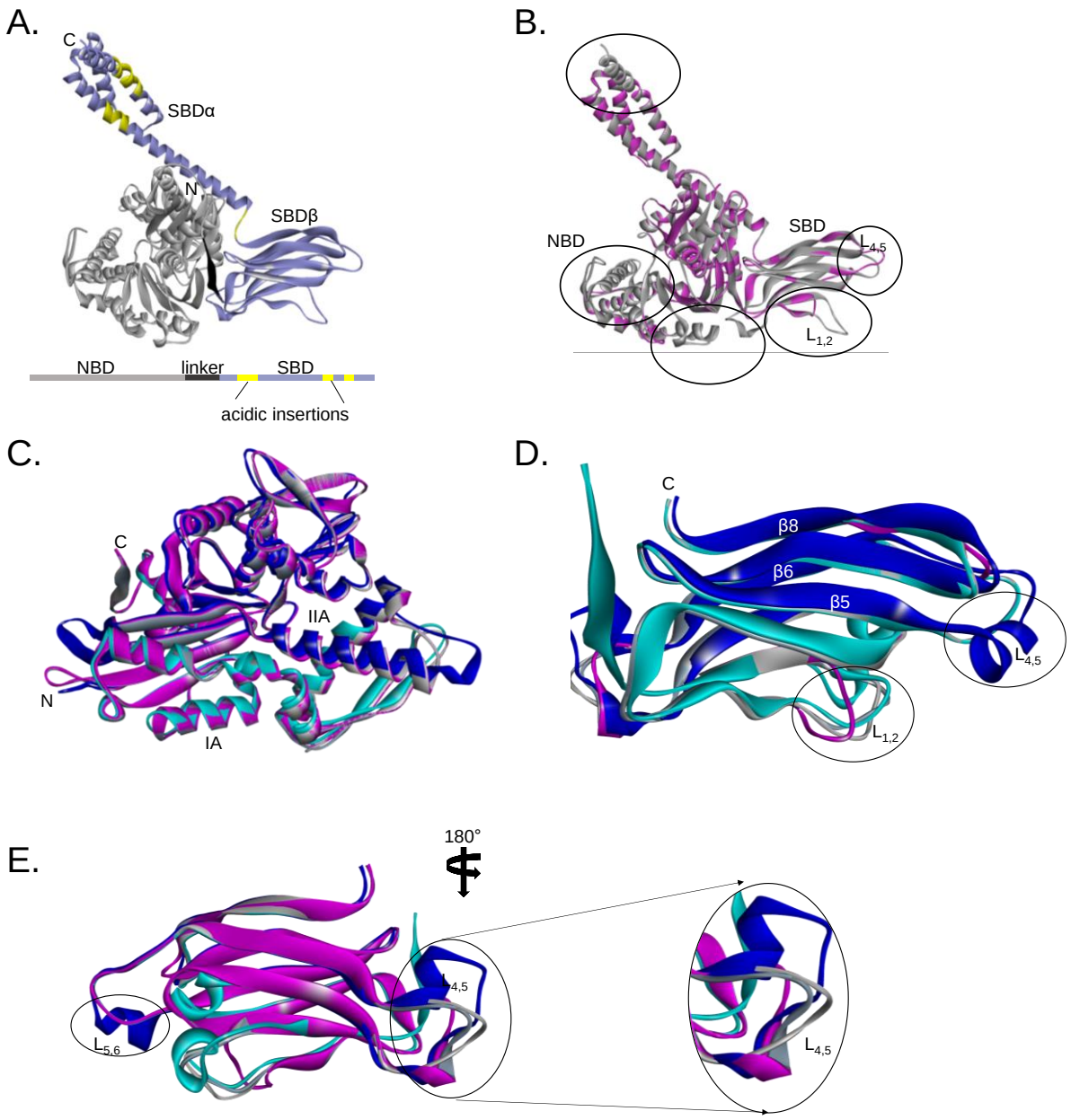

Figure 2. General structural features of human Hsp110s

(A) Structure of Hsp110 showing major features including the unique acidic insertions in the SBDα region. (B) Comparative structural analyses of a canonical Hsp70 (HspA1, purple) and an Hsp110 (HspH1, grey). Variations are predicted to occur within $L_{1,2}$ and $L_{4,5}$. (C) The NBDs of human HspH1 (cyan), HspH2 (purple), HspH3 (grey) and Grp170 (blue) show high conservation. (D) The SBDs of human HspH1 (cyan), HspH2 (purple), HspH3 (grey) and Grp170 (blue) show variation within the SBDβ segments. (E) Major variations are predicted to occur at loops L4,5 and L5,6 of the SBDβ segments of human HspH1 (cyan), HspH2 (purple), HspH3 (grey) and Grp170 (blue).

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3. Hsp110 roles in cancer pathogenesis

Several factors that upset proteostasis such as drug pressure, pH and temperature changes threaten the survival of malignant cells. In response to physiological stress, cancer cells activate cyto-protective adaptive pathways in which Hsp110 expression is upregulated. Indeed, Hsp110 expression is upregulated in various cancers including melanoma, prolactinoma, pituitary adenoma, breast cancer, colorectal cancer, pancreatic cancer, thyroid cancer, oesophageal cancer, lung cancer, bladder cancer, islet cell tumour, gastric cancer, lymphoma, seminoma, and hepatocellular carcinomas [54-59]. Furthermore, high Hsp110 expression is a poor prognostic factor for patients with melanoma, oesophageal cancer, gastric cancer, tongue squamous cell carcinoma, colorectal cancer, non-Hodgkin lymphoma, MDS, or AML [54, 60-61].

In cancer cells, Hsp110 may possibly facilitate protein stability and function by preventing aggregation of misfolded proteins as well as in maintaining protein conformation to enable ligand binding. Recent evidence has implicated the involvement of, Hsp110 (HspH1) in the aggregation suppression of alpha-synuclein [62]. The upregulation of α-synuclein is thought to contribute to aggressive phenotypes of meningiomas via the Akt/mTOR pathway, thus highlighting a key role for HspH1 in the development of malignant meningiomas [63]. It is thus possible that interception of the function of HspH1 could possibly present intervention against malignant meningiomas.

Hsp110 is a crucial component of the primary protection/repair pathway for denatured proteins and thermotolerance in mammalian cells [36]. Furthermore, Hsp110 is involved in STAT3 phosphorylation in the cytosol, thereby promoting cell proliferation (Figure 3; [59]). It has been proposed that STAT3 is constitutively activated in many cancer types and plays a crucial role in tumour growth and metastasis [64-67]STAT3 also regulates several signalling pathways such as cellular proliferation, invasion and angiogenesis which are all critical for metastasis [68-69]. Hsp110s may play a major role in cancer development since they are implicated in apoptosis regulation. Indeed, Hsp110 has been shown to protect cells from stress-induced apoptosis [58, 70]. RNA interference targeting Hsp110 was demonstrated to induce apoptosis in cancer cells thus further pointing to an indirect role of this chaperone in the inhibition of apoptosis [71].

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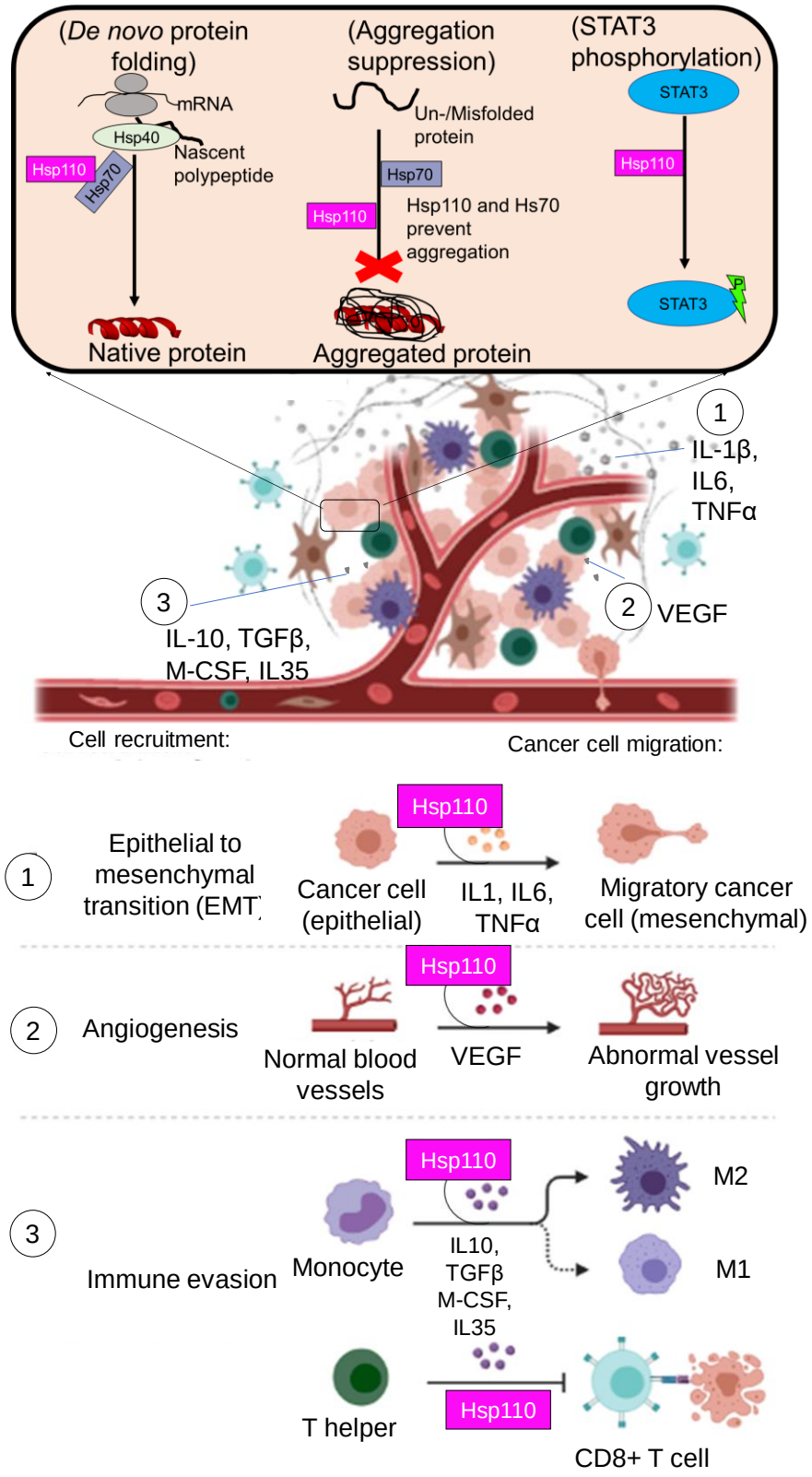

Figure 3. Roles of Hsp110 in cancer cells.

Hsp110 family members co-operate with Hsp70 and Hsp40 to facilitate folding of nascent polypeptides in tumour cells. They also suppress aggregation of oncogenic proteins and are also involved in STAT3 phosphorylation thereby promoting tumour cell proliferation.

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Aberrant cell migration is a major determinant of metastasis leading to the development of malignant tumours [72]. As such, metastasis is the leading cause of cancer related deaths [73]. Cancer cell migration and invasion into surrounding vasculature is a crucial initial step in metastasis [74]. Cell migration is a complex process characterised by several steps, which include, epithelial mesenchymal transition (EMT), abnormal angiogenesis and immune evasion (Figure 3, [75]). During metastasis, cancer cells break free from the primary tumour to join the circulatory system, thus enabling colonization of distant organs. Interestingly, several protein molecules within the tumour microenvironment are associated with metastasis (Figure 3). Notably, Hsp110/Grp170 proteins play important roles in regulating protein activity of some proteins involved in these signalling pathways. Hsp110 association with the pro-inflammatory cytokines IL-6 and TNF-*α* that are also involved in EMT has previously been demonstrated [76]. Upon EMT activation, tumour epithelial cells lose their cell polarity and adhesion properties to gain migratory and invasive properties, becoming mesenchymal cells [77-78]. Interestingly, the role of EMT in different cancers including prostate, lung, liver, pancreatic, and breast cancers has been established [79-80]. Since Hsp110 is implicated in modulating proteins involved in EMT, its potential role in the development of metastasis could be inferred.

Angiogenesis is important in metastasis as the growth and spread of neoplasms largely depends on the establishment of an adequate blood supply. Notably, Hsp110 potentially modulates angiogenesis. It has been established that Hsp110 co-operates with sHsps to suppress protein aggregation under stress conditions [81]. sHsp family members are known to modulate activity of the pro-angiogenic factor, VEGF, which induces structurally and functionally abnormal vasculature formation [82]. Hsp110 may therefore indirectly play a central role in angiogenesis, and may be a rational target for novel anticancer therapy. T cells, monocytes and other immune cells are known to exert anti-metastatic functions [83]. During the metastasic cascade, crosstalk between tumour cells and immune cells triggers in immune evasion. This pathway is modulated by several anti-inflammatory cytokines such as transforming growth factor β (TGFβ), IL10, and IL35 [84-85]. Although the direct association of these cytokines wit Hsp110 is yet to be experimentally validated, Hsp110 likely plays a key role in the folding of these proteins by canonical Hsp70s. Indeed, it has previously been reported that Hsp70s, associates with and modulate these anti-inflammatory cytokines [86].

Hsp110 generally confers cytoprotection by functioning as a stress buffer which prevents stress-induced apoptosis. Previous studies have suggested that Hsp110's anti-apoptotic and chaperone roles are crucial for survival of tumour cells against the action of anticancer drugs or hypoxia [71]. Furthermore, Hsp110 upregulation suppresses cancer cell apoptosis by inhibiting the activation of caspase 9 and caspase 3 by blocking cytochrome c release from

mitochondria [71, 87-88]. Interestingly, the role of Hsp110 in activated B-cell diffuses large B-cell lymphoma (ABC-DLBCL) survival mechanisms has been also been established [89]. Hsp110 overexpression in ABC-DLBCL cell lines induces increased NF-kB signalling thereby suggesting a tight interplay between Hsp110 and the NF-kB pathway [89]. This is particularly important since ABC-DLBCL tumours rely on sustained NF-kB activation for survival. At the intracellular level, Hsp110 possesses anti-aggregation properties and also participates in the folding of nascent polypeptides or misfolded proteins in cells (Figure 3, [37]). Further studies to elucidate the roles for Hsp110/Grp170 in cancer development are thus urgently required.

3. The role of the ER resident, Grp170 chaperone in cancer pathogenesis

Since the ER is a critical organelle that facilitates several aspects of protein synthesis including post-translational modification and proper folding of client proteins, Grp170 plays a particularly significant role in cellular proteostasis. Like other Hsp110s, Grp170 generally exhibits dual functions as an NEF for Grp78 (the ER Hsp70) and in aggregation suppression of secretory or transmembrane proteins in the ER [29]. The cytoprotective activity of intracellular Grp170 provides a survival benefit in cancer cells during tumour progression or metastasis [29]. Accumulating evidence demonstrates that Grp170 can directly bind to a variety of incompletely folded protein substrates *in vivo* in a nucleotide-independent fashion [90-91]. As such Grp170 remains tightly bound to peptide substrates in both the ATP and ADP states, making it an efficient buffer against cellular stress [91]

Stress factors including glucose and oxygen deprivation within the tumour microenvironment are known to activate a Grp170-mediated unfolded protein response (UPR) to promote tumour cell survival [92]. Grp170 is thought to be a potential prognostic factor of breast cancer, since altered Grp170 levels correlate with different stages of tumour invasiveness [93-94]. Due to its ability to chaperone several proteins associated in cancer signalling pathways, Grp170 appears to possess pro-tumour activity (Figure 3; [95-96]). In addition, Grp170 involvement in angiogenesis of tumours has been described through its ability to chaperone the major pro-angiogenic factor vascular endothelial growth factor (VEGF) [97-98]Similarly, an antisense approach was used to demonstrate Grp170's ability to reduce tumourigenicty in a prostate cancer model by blocking secretion of matured VEGF [98]. Grp170 has also been shown to associate with matrix metalloproteinase-2 (MMP-2) thereby promoting tumour invasion [99].

In as much as additional studies are necessary to glean a better understanding of the precise mechanistic contribution of the non-canonical Hsp70s in tumourigenesis, their chaperoning property appears to be a major underlying mechanism involved in their pro-tumour activity.

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Complete proteomic studies on the involvement of Hsp110/rp170 in cancer pathophysiology are worth exploring. Notably Hsp110/Grp170 are predicted to interact with a large complement of proteins that are implicated in cancer development (Figure 3; Table S1).

4. The unique proteomic signatures of Hsp110/Grp170

We predicted the interactome of Hsp110/GRP170 homologues established using the STRING 10.5 database (http://string-db.org/,[100]). The predicted interactomes of the proteins revealed possible associations of these chaperones with several proteins implicated in tumourigenesis (Table S1). Generally, while there were overlapping interaction partners between the various Hsp110 forms, we noted that the chaperones are also marked with unique interactomes (Figure 4). For instance, HspH2 and Grp170 are predicted to interact with a large complement of protein modifying enzymes as opposed to HspH1 and HsapH3 (Figure 4). Additionally, Grp170 also seems to interact with a large complement of proteins that are involved in several other roles including protein translocation. The observed variations in interactomes may possibly arise from the structural variations and ER-localization of Grp170 which makes it functionally specialized for binding ER proteins. Seemingly, the different Hsp110 isoforms play unique roles in chaperoning proteins involves in the different cancer signalling pathways as described below.

Notably, all the Hsp110 isoforms (HspH1, HspH2, HspH3 and Grp170) are predicted to associated with the cyclin G dependent kinase, GAK (Table S1). Cyclin dependent kinases are key regulatory enzymes that are involved in cell proliferation which is an important hallmark of tumorigenesis. Previously, it has been established that GAK enhances the androgen receptor (AR) transcriptional response in androgen-independent prostate cancer [101]. Furthermore, GAK has been proposed as a druggable anticancer candidate that has broad therapeutic applications across numerous tumour types including breast and colorectal cancers [102]. Given its important role in maintaining GAK in a functional state, it is therefore conceivable that Hsp110s have a crucial role in promoting tumourigenesis.

Intriguingly, Hsp110 isoforms, HspH3 and HspH2, are predicted to interact with a large complement of nucleoporins as opposed to Grp170 and HspH1. Nucleoporins are components of nuclear pore complexes (NPCs) which are huge macromolecular assemblies in the nuclear envelope, through which bidirectional cargo movement between the nucleus and cytoplasm occurs [103]. Several nucleoporins are linked to cancer, mostly in the context of chromosomal translocations, which encode nucleoporin chimeras [104]. Tumour cells are thought to exploit specific properties of nucleoporins to deregulate transcription, chromatin

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boundaries, and essential transport-dependent regulatory circuits [104]. The nucleoporin POM121, which is predicted to interact with HspH3 and Hsp2, has reportedly been linked with prostate cancer [105]. POM121 has also been reported as a novel prognostic marker of oral squamous cell carcinoma [106]. It is therefore plausible that, HspH3 and HspH2 play crucial roles in chaperoning POM121 in cancer progression. Several other nucleoporins including translocated promoter region (TPR), Nup98 and Nup214 are also predicted to interact with HspH3 and HspH2 (Table S1). These proteins have previously been described as 'promiscuous nucleoporins' due to their unique ability to associate with various partners to produce a variety of oncogenic fusion proteins [107]. Thus, HspH3- and HspH2-directed therapies may also hold prospects in prostate cancer intervention.

It is also predicted that HspH3 and Grp170 interact with EDEM3, whose upregulation is linked to thyroid cancer (Table S1). It remains to be established if the enhanced expression of EDEM3 associated with thyroid cancer is accompanied with a concomitant increase in HspH3 levels. The possibility of HspH3 as a biomarker for thyroid cancer is therefore worth exploring. Previous evidence suggests that, SUMOylation is implicated in cancer cell signalling and gene networks that regulate carcinogenesis, proliferation, metastasis and apoptosis [108]. Interestingly, HspH3 is predicted to interact with the SUMO protein, RANBP2 (Figure 4, Table S1). This implies that the chaperone potentially modulates SUMOylation in cells, possibly resulting in tumourigenesis.

Sec proteins form part of the heterotrimeric Sec61 and the dimeric Sec62/Sec63 complexes located in the ER membrane [109]. These complexes are thought to play a central role in the translocation of nascent and newly synthesized precursor polypeptides into the ER. Notably, Sec overexpression has been linked to cancer. Interestingly, several Sec proteins are predicted to interact with Hsp110 and Grp170 chaperones (Table S1). In a study conducted by Diwadkar et al [110], interbreeding of Sec tRNA transgenic mice with a model of prostate cancer resulted in accelerated development of prostatic intraepithelial neoplasia (PIN) and more aggressive high-grade lesions.

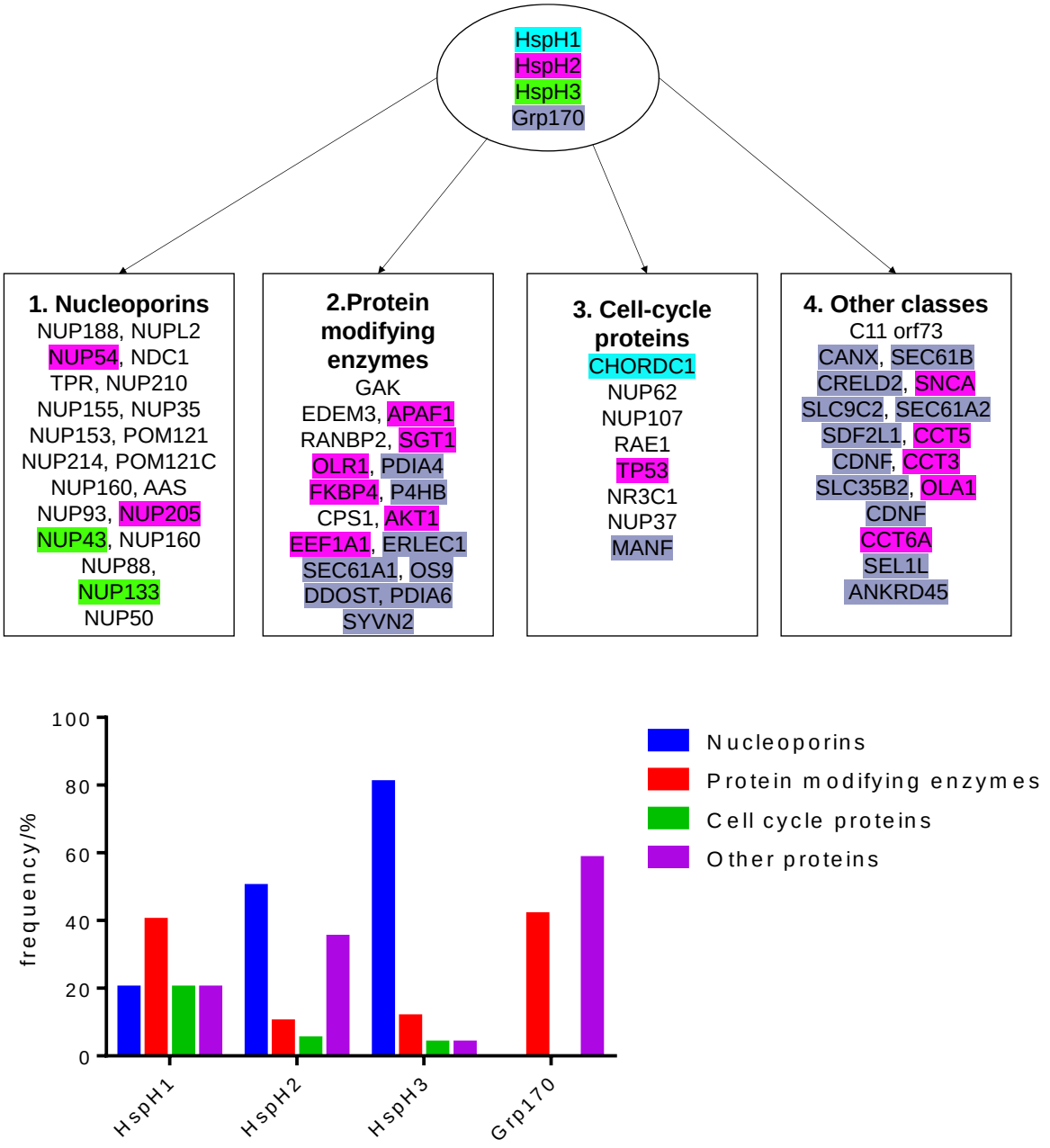

Figure 4. Predicted clientome of Hsp110 and Grp170 members

The predicted clientele of HSP110 and Grp170 members is largely comprised of nucleoporins and protein modifying enzymes. Client proteins that are unique to each Hsp110/Grp170 type are shown highlighted in blue (HspH1), purple (HspH2), green (HspH3) and green (Grp170). Client proteins that are shared by more than 1 Hsp110/Grp170 type are not highlighted. Predictions were conducted using STRING database at a cut off score of 0.75. The complete data table listing all the proteins is shown in Table S1.

4. Heroes or villains: the role of heat shock proteins in preventing cancer progression

Primarily, as chaperones, the role of Hsps is to assess the folding status of a protein, towards assisting it refold or to channel it towards degradation. In this way, Hsps may either hide

metabolic consequences of mutations or they may also expose them. More intriguingly, Hsps are thought to serve as buffers of protein mutations, i.e. they enable otherwise mutated proteins to fold into their functional conformations [111]. In this way, depending on the pathway that their respective substrates are implicated in, Hsps may promote or obstruct cancer development. However, on the balance it appears that malfunctioning of chaperones leads to general deregulation of several metabolic pathways, including those involved in signal transduction [111].

Hsp110s are involved in the proliferation and survival of tumour cells [59]. Hsp110s likely play multiple roles in cancer cells since their chaperone function involves the stabilization of oncogenic proteins and those involved in cancer signalling pathways. The emerging role of Hsp110 in immunomodulation has been described [59, 76] and may trigger inflammatory responses that propagate tumour growth. It therefore suggests that Hsp110 may play key roles in chaperoning oncogenic proteins that are critical in tumourigenesis. Recent evidence demonstrates that ER-stressed tumours propagate stress signals to the neighbouring cells through secretion of soluble mediators triggering an exaggerated inflammatory response that facilitates tumour progression [112]. Although it is not clear as to whether Grp170 directly contributes to this inflammatory response induced pro-tumour effect, it's involvement in this pathway is highly probable.

Previous studies have shown that Hsp110s and Grp170 are immunogenic chaperones([29, 113]). As such, these chaperones pose as promising cancer vaccine candidates. In a murine cervical cancer model, Hsp110 was demonstrated to not only improve the antitumour efficacy of the cytotoxic T-lymphocyte epitope E7 but also significantly inhibit tumour growth [114]. In addition, Hsp110 and carbonic anhydrase IX, of which the latter is the renal cell carcinoma specific tumour protein, were shown to inhibit growth of renal cell carcinoma in mice [115] . This may imply that Hsp110 plays important roles in ensuring the functionality of carbonic anhydrase IX. Hsp110-HER2 complex based vaccines also induce immune protection against spontaneous breast tumours in a transgenic mice model [116]. Studies have also been conducted on the development of Grp170-based anticancer immunotherapy. It has been demonstrated that a complex of Grp170 and tumour protein antigens activated the immune response leading to inhibition of tumour growth in a melanoma mouse model [117]. Furthermore, mouse prostate cancer cells engineered to effectively secrete Grp170 exhibited enhanced tumour immunogenicity and cytolytic activity of distant tumours [118]. These studies provide evidence of the immunomodulatory roles of Grp170 possibly presenting them as potential vaccine candidates.

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Hsp110 has the capability to inhibit immune activation of dendritic cells through scavenger receptor binding [95]. Notably, Hsp110 has been described as the main chaperone involved in colorectal tumorigenesis and the presence of an Hsp110 inactivating mutation is directly associated with a good prognosis [119]. Interestingly, increased Hsp110 expression has been linked to tumour immunosurveillance [59]. A study by Berthenet et al [59] demonstrated that Hsp110 overexpression in colorectal cancer cells induces the formation of macrophages with an anti-inflammatory profile. Although the precise mechanisms underlying extracellular release of Hsps (active vs. passive) remain speculative, increased Hsp levels are generally observed in the tumour microenvironment [120]. Indeed, several studies have indeed demonstrated the anti-inflammatory role of extracellular Hsp, and depending on their levels, Hsps could either promote inflammation or suppress it [121]). In this regard, Hsp110/Grp170 may thus play a signal transducer role. It would therefore be interesting to conduct proteomic analyses to monitor variations in extracellular Hsp110/Grp170 levels towards using it as a biomarker of specific cancers.

5. Could Hsp110/Grp170 be targeted in cancer therapy?

Owing to their prominent role as chaperones, Hsp110 and Grp170 could serve as novel chemotherapeutic targets against cancer. Small molecule inhibitors such as polymyxin B (PMB) and epigallocatechin gallate (EGCG) possess great potential in this regard, as they have been successfully used to inhibit the activity of the *Plasmodium falciparum* Hsp110 protein (PfHsp70-z) *in vitro* [37]. These two compounds bind to the NBD, thus abrogating the basal ATPase activity of the Hsp110. However, since the holdase chaperone function of Hsp110/Grp170 is not modulated by nucleotides, targeting the Hsp110 NBD using nucleotide mimetics might not interfere with their direct chaperone role. However, NBD-targeted drugs, may disrupt the NEF function of these chaperones which in turn would adversely impact on fold and function of several proteins implicated in cancer development. Selectively targeting the SBD of Hsp110/Grp170 using peptide substrate mimetics may be an alternative approach Two drugs targeting the SCD of Hsp70, targeted drug 2-phenylethynesulfonamide (PES) and the TKD-motif directed peptide inhibitor, cmHsp70.1 have entered clinical trials stage [122, 123, 124]. The design of domain specific inhibitory compounds may prove useful in Hsp110 targeted anticancer therapy. Since Hsp110/Grp170 functions in co-operation with several other Hsps, their inhibition may impact on the folding fate of the protein compliment that drives cancer development and progression. In a recent study, Gozzi and colleagues (2019) [125] designed a novel NBD-binding small molecule inhibitor which compromises Hsp110 chaperone function, thereby inhibiting STAT3 phosphorylation and colorectal cancer cell

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proliferation. There is therefore an urgent need for further screening of novel compounds that target Hsp110/Grp170 chaperones in the fight against cancer. Apart from chemical compounds, antibodies and aptamers could alternatively be designed towards abrogating Hsp110/Grp170 functions. In a colon cancer murine model, an Hsp70 monoclonal antibody based inhibitor, cmHsp70.1, which binds the TKD motif was shown to significantly reduce tumour weight and improve survival rate [126]. One of the main challenge in the design of anti-cancer agents is to come up with compounds that are safe. The varied proteomic composition of cancer cells compared to normal cells make the inhibition of Hsps promising in light of their role as custodians of proteostasis.

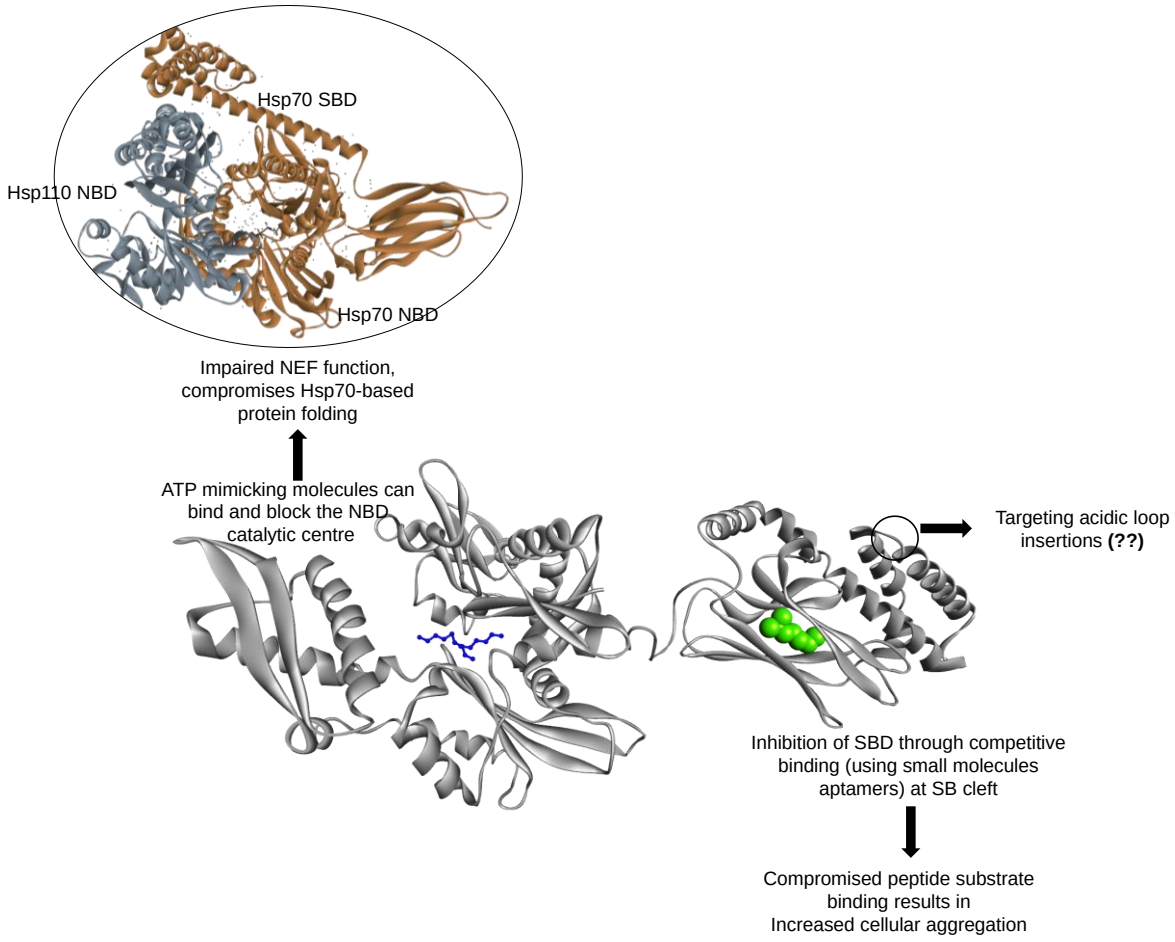

Figure 5. Proposed strategies for targeting Hsp110

Small molecule inhibitors that possess the ability to bind and block Hsp110 NBD and SBD segments possess potential in Hsp110-directed therapy.

6. Conclusion:

Recently, Hsp110/Grp170 chaperones have emerged to a focal point in prospecting for novel chemotherapeutic targets, mainly due to their central roles in both proteostasis and signalling pathways. As nucleotide-independent holdase chaperones, Hsp110/Grp170 are regarded as cellular buffers against proteostatic stress. It is thus not surprising that their role in the cytoprotection of tumour cells, particularly in response to both drug- and hypoxic-stress is becoming apparent. This review explored the possible interactome of these proteins and established that molecules involved in cancer development, are amongst some of their most distinct clientele. This, coupled to their correlated expression with cancer prognosis, suggest a crucial role for these chaperones in cancer development. It is thus envisaged that targeting these group of chaperones has potential as an intervention tool against cancer.

Conflict of Interest

The authors have no conflicting interests to declare

Author Contributions

GC and AS conceived and wrote the manuscript. Both authors approved the final version of the manuscript.

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Supplementary section

Table S1. Predicted interaction of Hsp110/Grp170 with cancer-associated proteins

HspH1 interaction partners		
Protein	**Function**	**Score**
1.GAK-cyclin G (kinase)	Associates with cyclin G and CDK5 and is involved in the uncoating of clathrin- coated vesicles by Hsc70	0.879
2.CPS1(Carbamoyl-phosphate synthase)	Involved in the urea cycle and plays an important role in removing excess ammonia from the cell	0.874
3.EDEM3 (ER degradation-enhancing alpha-mannosidase-like protein 3	Accelerates ER-associated degradation (ERAD) of glycoproteins by proteasomes	0.757
4.CHORDC1 (Cysteine and histidine-rich domain-containing protein 1)	Regulates centrosome duplication, probably by inhibiting the kinase activity of ROCK2. Proposed to act as co-chaperone for HSP90. Prevents tumourigenesis.	0.747
HspH3 interaction partners		
1.NUP188 (Nucleoporin)	May function as a component of the nuclear pore complex (NPC)	0.937
2.C11 orf73	Acts as a specific nuclear import carrier for HSP70	0.931
3.NUP37 (Nucleoporin)	Component of the Nup107-160 subcomplex of the nuclear pore complex (NPC) required for normal kinetochore microtubule attachment, mitotic progression and chromosome segregation	0.926
4.RANBP2 (E3 SUMO-protein ligase)	Facilitates SUMO1 and SUMO2 conjugation, (Ran-GTP, karyopherin)-mediated protein import. Component of the nuclear export pathway.	0.924
5.TPR (Nucleoprotein TPR)	Essential for normal nucleocytoplasmic transport of proteins and mRNAs, plays a role in the establishment of nuclear-peripheral chromatin compartmentalization in interphase, and in the mitotic spindle checkpoint signalling during mitosis.	0.917
7.RAE1 (mRNA export factor)	Plays a role in mitotic bipolar spindle formation. May function in nucleocytoplasmic transport	0.908
8.NUP155 (Nuclear pore complex protein)	May be essential for embryogenesis. Nucleoporins may be involved both in binding and translocating proteins during nucleocytoplasmic transport	0.907

9.NUP153 (Nuclear pore complex protein)	Essential for normal nucleocytoplasmic transport of proteins and mRNAs. Involved in the quality control and retention of unspliced mRNAs in the nucleus	0.904
10.NUP214 (Nuclear pore complex protein)	May serve as a docking site in the receptor-mediated import of substrates across the nuclear pore complex	0.904
11.NUP62 (Nuclear pore glycoprotein)	Plays a role in mitotic cell cycle progression by regulating centrosome segregation, centriole maturation and spindle orientation. It might be involved in protein recruitment to the centrosome after nuclear breakdown	0.904
12.NUP93 (Nuclear pore complex protein)	During renal development, regulates podocyte migration and proliferation through SMAD4 signalling	0.904
13.NUP43 (Nucleoporin)	Component of the Nup107-160 subcomplex of the nuclear pore complex (NPC) required for normal kinetochore microtubule attachment, mitotic progression and chromosome segregation	0.903
14.NUP88 (Nuclear pore complex protein)	Essential component of nuclear pore complex	0.903
15.NUP133 (Nuclear pore complex protein)	Involved in poly(A)+ RNA transport	0.903
16.NUP50 (Nuclear pore complex protein)	Interacts with regulatory proteins of cell cycle progression including CDKN1B.	0.902
17.NUP107 (Nuclear pore complex protein)	Required for the assembly of peripheral proteins into the NPC.	0.902
18.NDC1 (Nucleoporin)	Plays a key role in *de novo* assembly and insertion of NPC in the nuclear envelope. Required for NPC and nuclear envelope assembly, possibly by forming a link between the nuclear envelope membrane and soluble nucleoporins, thereby anchoring the NPC in the membrane	0.902
19.NUP210 (Nuclear pore membrane glycoprotein)	Essential for nuclear pore assembly and fusion, as well as structural integrity	0.901
20.NUP35 (Nucleoporin)	Can play the role of both NPC structural components and of docking or interaction partners for transiently associated nuclear transport factors.	0.901
21.POM121 (Nuclear envelope pore membrane protein)	Essential component of the nuclear pore complex (NPC). May be involved in anchoring components of the pore complex to the pore membrane.	0.900
22.POM121C (Nuclear envelope pore membrane protein)	Essential component of the nuclear pore complex (NPC). May be involved in anchoring components of the pore complex to the pore membrane.	0.900
23.NUP160 (Nucleoporins)	Involved in poly(A)+ RNA transport	0.900
24.NUPL2 (Nucleoporin-like protein)	Required for the export of mRNAs containing poly(A) tails from the nucleus into the cytoplasm.	0.900
25.AAS (Nucleoporin)	Plays a role in the normal development of the peripheral and central nervous system	0.900
26.GAK (Cyclin-G-associated kinase)	Involved in the uncoating of clathrin- coated vesicles by Hsc70 in non-neuronal cells.	0.874
27.STIP1 (Stress-induced-phosphoprotein)	Mediates the association of the molecular chaperones HSPA8/HSC70 and HSP90	0857
28.EDEM3 (ER degradation-enhancing alpha-mannosidase-like protein 3)	Involved in endoplasmic reticulum-associated degradation (ERAD) of glycoproteins by proteasomes, by catalyzing mannose	0.792
HspH2 interaction partners		
4.SNCA (Alpha-synuclein)	Induces fibrillization of microtubule-associated protein tau. Reduces neuronal responsiveness to various apoptotic stimuli, leading to a decreased caspase-3 activation	0.965
5.C11 orf73	Acts as a specific nuclear import carrier for HSP70	0.964
6.NUP62 (Nuclear pore glycoprotein)	Plays a role in mitotic cell cycle progression by regulating centrosome segregation, centriole maturation and spindle orientation. It might be involved in protein recruitment to the centrosome after nuclear breakdown	0.944
7.RANBP2 (E3 SUMO-protein ligase)	Facilitates SUMO1 and SUMO2 conjugation, transport factor (Ran-GTP, karyopherin)-mediated protein import via the F-G repeat-containing domain which acts as a docking site for substrates. Component of the nuclear export pathway	0.940
8.TPR (Nucleoprotein)	Essential for normal nucleocytoplasmic transport of proteins and mRNAs, plays a role in the establishment of nuclear-peripheral	0.935

	chromatin compartmentalization in interphase, and in the mitotic spindle checkpoint signalling during mitosis.	
9.NUP37 (Nucleoporin)	Component of the Nup107-160 subcomplex of the nuclear pore complex (NPC) required for normal kinetochore microtubule attachment, mitotic progression and chromosome segregation	0.929
10.OLR1 (Oxidized low-density lipoprotein receptor)	Mediates the recognition, internalization and degradation of oxidatively modified low density lipoprotein (oxLDL) by vascular endothelial cells.	0.927
11.NUP155 (Nuclear pore complex protein)	Essential for embryogenesis. Nucleoporins may be involved both in binding and translocating proteins during nucleocytoplasmic transport.	0.925
12.NUP54 (Nucleoporin p54)	Component of the nuclear pore complex, a complex required for the trafficking across the nuclear membrane	0.921
13.CCT2 (T-complex protein 1 subunit beta)	Molecular chaperone; assists the folding of proteins upon ATP hydrolysis. Known to play a role, in vitro, in the folding of actin and tubulin	0.917
14.RAE1 (mRNA export factor)	Plays a role in mitotic bipolar spindle formation. Binds mRNA. May function in nucleocytoplasmic transport	0.916
15.NUP107 (Nuclear pore complex protein)	Required for the assembly of peripheral proteins into the NPC. May anchor NUP62 to the NPC	0.916
16.NUP214 (Nuclear pore complex protein)	May serve as a docking site in the receptor-mediated import of substrates across the nuclear pore complex	0.915
17.NUP88 (Nucleoporins)	Essential component of nuclear pore complex	0.915
18.NUP93 (Nuclear pore complex protein)	During renal development, regulates podocyte migration and proliferation through SMAD4 signalling	0.914
19.AHSA1 (Activator of Hsp90 ATPase)	Activates the ATPase activity of HSP90AA1 leading to increase in its chaperone activity.	0.913
20.NUP153 (Nuclear pore complex protein)	Essential for normal nucleocytoplasmic transport of proteins and mRNAs. Involved in the quality control and retention of unspliced mRNAs in the nucleus	0.912
21.NDC1 (Nucleoporin)	Plays a key role in *de novo* assembly and insertion of NPC in the nuclear envelope.	0.907
23.NUP205 (Nuclear pore complex protein)	Plays a role in the nuclear pore complex (NPC) assembly and/or maintenance.	0.907
24.NUP160 (Nuclear pore complex protein)	Involved in poly(A)+ RNA transport	0.907
25.NUP50 (Nuclear pore complex protein)	Interacts with regulatory proteins of cell cycle progression	0.906
26.NUP35 (Nucleoporin)	Can play the role of both NPC structural components and of docking or interaction partners for transiently associated nuclear transport factors.	0.903
27.AAAS (Nucleoporins)	Plays a role in the normal development of the peripheral and central nervous system	0.903
28.FKBP4 (Peptidyl-prolyl cis-trans isomerase)	Immunophilin protein with PPIase. Plays a role in the intracellular trafficking of heterooligomeric forms of steroid hormone receptors between cytoplasm and nuclear compartments. Acts also as a regulator of microtubule dynamics by inhibiting MAPT/TAU ability to promote microtubule assembly.	0.903
29.NUPL2 (Nucleoporin-like protein)	Required for the export of mRNAs containing poly(A) tails from the nucleus into the cytoplasm.	0.903
30.NUP85 (Nuclear pore complex protein)	Required for spindle assembly during mitosis.	0.903
31.NUP188 (Nucleoporin)	May function as a component of the NPC	0.903
32.NUP210 (Nuclear pore membrane glycoprotein)	Nucleoporin essential for nuclear pore assembly and fusion, nuclear pore spacing, as well as structural integrity	0.902
33.POM121C (Nuclear envelope pore membrane protein)	Essential component of the nuclear pore complex (NPC).	0.902
34.POM121 (Nuclear envelope pore membrane protein)	Essential component of the nuclear pore complex (NPC).	0.902
35.GAK (Cyclin-G-associated kinase)	Involved in the uncoating of clathrin- coated vesicles by Hsc70 in non-neuronal cells.	0.893

36.CPS1 (Carbamoyl-phosphate synthase)	Involved in the urea cycle; plays an important role in removing excess ammonia from the cell	0.891
37.CLPB (Caseinolytic peptidase B protein)	May function as a regulatory ATPase and be related to secretion/protein trafficking process	0.889
38.AKT1 (RAC-alpha serine/threonine-protein kinase)	Regulates many processes including metabolism, proliferation, cell survival, growth and angiogenesis. AKT is responsible of the regulation of glucose uptake	0.883
39.CCT5 (T-complex protein 1 subunit epsilon)	Known to play a role, in vitro, in the folding of actin and tubulin	0.881
40.TP53 (Cellular tumour antigen p53)	Acts as a tumour suppressor in many tumour types; induces growth arrest or apoptosis depending on the physiological circumstances and cell type. Involved in cell cycle regulation as a trans-activator that acts to negatively regulate cell division by controlling a set of genes required for this process.	0.881
41.CCT3 (T-complex protein 1 subunit gamma)	Known to play a role, in vitro, in the folding of actin and tubulin	0.856
42.EEF1A1 (Elongation factor 1-alpha 1)	Promotes the GTP-dependent binding of aminoacyl-tRNA to the A-site of ribosomes during protein biosynthesis. Forms a complex that acts as a T helper 1 (Th1) cell-specific transcription factor and binds the promoter of IFN-gamma to directly regulate its transcription, and is thus involved importantly in Th1 cytokine production	0.853
43.CCT4 (T-complex protein 1 subunit delta)	Known to play a role, in vitro, in the folding of actin and tubulin	0.851
44.CCT6A (T-complex protein 1 subunit zeta)	Known to play a role, in vitro, in the folding of actin and tubulin	0.848
45.NR3C1 (Glucocorticoid receptor)	Has transcriptional repression activity	0.845
46.APAF1 (Apoptotic protease-activating factor 1)	Mediates the cytochrome c-dependent autocatalytic activation of pro-caspase-9 (Apaf-3), leading to the activation of caspase-3 and apoptosis.	0.841
47.CFTR (Cystic fibrosis transmembrane conductance regulator)	Regulation of epithelial ion and water transport and fluid homeostasis. Mediates the transport of chloride ions across the cell membrane.	0.836
48.SGT1	May play a role in ubiquitination and subsequent proteasomal degradation of target proteins	0.820
49.OLA1 (Obg-like ATPase)	Hydrolyzes ATP, and can also hydrolyze GTP with lower efficiency.	0.820
Grp170 interaction partners		
1.PDIA4 (Protein disulphide-isomerase)	Belongs to the protein disulphide isomerase family	0.993
2.SIL1 (Nucleotide exchange factor)	Functions as a nucleotide exchange factor for the ER lumenal chaperone HSPA5	0.989
3.SEC63 (Translocation protein)	Required for integral membrane and secreted preprotein translocation across the endoplasmic reticulum membrane	0.962
4.P4HB (Protein disulphide-isomerase)	May therefore cause structural modifications of exofacial proteins. Inside the cell, seems to form/rearrange disulphide bonds of nascent proteins.	0.954
5.CALR (Calcium-binding chaperone)	Promotes folding, oligomeric assembly and quality control in the endoplasmic reticulum (ER) via the calreticulin/calnexin cycle. Interacts transiently with almost all of the monoglucosylated glycoproteins that are synthesized in the ER.	0.954
6.PDIA6 (Protein disulphide-isomerase A6)	Negatively regulates the unfolded protein response (UPR) through binding to UPR sensors such as ERN1, which in turn inactivates ERN1 signalling.	0.950
7.MANF (Mesencephalic astrocyte-derived neurotrophic factor)	Inhibits cell proliferation and endoplasmic reticulum (ER) stress-induced cell death (182 aa)	0.946
8.CANX (Calcium-binding protein)	May act in assisting protein assembly and/or in the retention within the ER of unassembled protein subunits. It seems to play a major role in the quality control apparatus of the ER by the retention of incorrectly folded proteins	0.934
9.PDIA3 (Protein disulphide-isomerase A3)	Belongs to the protein disulphide isomerase family	0.924
10.SEC61A1 (Protein transport protein)	Plays a crucial role in the insertion of secretory and membrane polypeptides into the ER. Required for assembly of membrane and secretory proteins.	0.906

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11.CRELD2	Cysteine rich with EGF like domains	0.901
12.SLC9C2	Sodium/hydrogen exchange; Involved in pH regulation	0.876
13.ANKRD45	Ankyrin repeat domain-containing protein	0.876
14.ERLEC1 (Endoplasmic reticulum lectin 1)	May function in endoplasmic reticulum quality control and endoplasmic reticulum-associated degradation (ERAD) of both non-glycosylated proteins and glycoproteins	0.876
16.CLGN (Calmegin)	Functions during spermatogenesis as a chaperone for a range of client proteins that are important for sperm adhesion onto the egg zona pellucida and for subsequent penetration of the zona pellucida	0.868
17.DDOST (Dolichyl-diphosphooligosaccharide-protein glycosyltransferase)	Essential subunit of the N-oligosaccharyl transferase (OST) complex which catalyzes the transfer of a high mannose oligosaccharide from a lipid-linked oligosaccharide donor to an asparagine residue Required for efficient N-glycosylation	0.866
18.SEC61A2 (Protein transport protein)	Plays a crucial role in the insertion of secretory and membrane polypeptides into the ER. It is required for assembly of membrane and secretory proteins.	0.826
19.OS9	Functions in endoplasmic reticulum (ER) quality control and ER-associated degradation (ERAD).	0.803
20.SYVN2 (E3 ubiquitin-protein ligase synoviolin)	Component of the endoplasmic reticulum quality control (ERQC) system. Protects cells from ER stress-induced apoptosis.	0.786
21.SDF2L1	Stromal cell derived factor 2 like 1	0.783
22.SEL1L	Plays a role in the endoplasmic reticulum quality control (ERQC) system. Plays a role in LPL maturation and secretion. Required for normal differentiation and survival of pancreatic cells.	0.783
23.CDNF (Cerebral dopamine neurotrophic factor)	Prevents the 6- hydroxydopamine (6-OHDA)-induced degeneration of dopaminergic neurons. Also prevents the degeneration of dopaminergic neurons.	0.777
24.EDEM3 (ER degradation-enhancing alpha-mannosidase-like protein 3)	Involved in endoplasmic reticulum-associated degradation (ERAD). Accelerates the glycoprotein ERAD by proteasomes.	0.773
25.SLC35B2	May indirectly participate in activation of the NF- kappa-B and MAPK pathways.	0.768
26.GAK (Cyclin-G-associated kinase)	Is involved in the uncoating of clathrin- coated vesicles by Hsc70 in non-neuronal cells.	0.752

***Cut off score 0.75**