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Heat shock proteins in main pediatric malignancies. A clinical overview

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1. ABSTRACT

Heat shock proteins belong to a group of molecular chaperones responsible for the regulation of many intracellular processes. HSPs play a pivotal role in the survival of cells under stressful conditions. Over-expression of these proteins have been found in both healthy and a great number of cancer cells. HSPs may be involved in numerous carcinogenic and chemoresistant processes. Due to that fact, they may be referred to as diagnostic biomarkers of oncogenesis and potential targets for anticancer drugs. Thus, we decided to review the involvement of major HSPs in the most malignant childhood cancers.

2. INTRODUCTION

Heat shock proteins (HSPs) belong to a group of molecular chaperones classified according to their molecular mass into six families: HSP100, HSP90, HSP70, HSP60, HSP40 and small HSPs (ranging from 15 kDa to 30 kDa) including HSP27. The amino acid sequence of HSPs is strongly conserved (1). The function of HSPs depends mainly on their cellular localization. Intracellular HSPs take part in protein folding and their transport to appropriate organelles. They participate in the repair of damaged proteins or direct them to proteasomal degradation. Extracellular HSPs induce innate immune responses through antigen presentation, activation of lymphocytes and macrophages, or maturation of dendritic cells (2-6).

HSPs are constitutively produced, though their expression increases when cells are exposed to various stress factors (e.g. elevated temperature, toxins, UV, radiation, hypoxia). One of the most abundant HSPs is HSP90. It is an antiapoptotic protein associated with a number of signaling pathways. Two isoforms of HSP90 have been distinguished - HSP90 alpha, constitutively expressed in the cytoplasm of most mammalian cells, and HSP90 beta, with its expression induced by stress factors (7). Interestingly, elevated expression of HSP90 has been observed in ovarian, breast, pancreatic and gastric cancer cells (8). HSP27 and HSP70 are induced by numerous physical and chemical factors, while their expression under physiological conditions is low (9). Increased concentrations of HSPs have been found in the cells of cancer patients. Thus, some HSPs may be useful as biomarkers of carcinogenesis, differentiation, and tumor aggressiveness in addition to being used as new anticancer targets. As a result, a great number of molecules inhibiting HSPs have

Table 1. Major HSPs inhibitors in ongoing clinical trials of cancer therapy

HSP inhibitor	Cancer	Phase of clinical trial
Tanespimycin with trastuzumab	Her2 positive metastatic breast cancer	II
Tansespimycin with bortezomib	Relapsed-refractory multiple myeloma	II/III
Tanespimycin with bortezomib	Advanced solid tumors of lymphomas	I
Retaspimycin with trastuzumab	Her2 positive metastatic breast cancer	II
BIIB021 with exemestane (aromasin)	Hormone receptor- positive metastatic breast cancer	II
BIIB021	GIST refractory to imatinib and sunitinib	II
Luminespib (NVP-AUY922) with trastuzumab	Her2 positive advanced breast cancer	I/II

been synthetized (8-10). Among the best known HSPs inhibitors evaluated in ongoing clinical trials, the most distinguishable are: tanespimycin (17-N-allylamino-17-demethoxygeldanamycin, 17-AAG) for multiple myeloma and breast cancer, IPI-504 (retaspimycin) for Her2-positive metastatic breast cancer, BIIB021 for breast cancer and GIST, or AUY922 for breast cancer therapies (10) (Table 1). Although the involvement of HSPs in adult patients' tumors is fairly well described, less is known about their role in malignancies of childhood and adolescence. Herein, we will try to review the impact and expression of major HSPs in pediatric cancers.

3. ENGAGEMENT OF HSPS IN MAJOR PEDIATRIC MALIGNANCIES

3.1. Leukemia

The involvement of HSPs in leukemia mainly refer to adult patients. Only a few studies concerning the association of HSPs with acute leukemia in children have been published (11-13). These data have been obtained from bone marrow samples of patients with acute lymphoblastic and myeloblastic leukemia, or performed *in vitro*.

3.1.1. Involvement of HSP27

HSP27 is one of the most important factors involved in the pathogenesis and chemoresistance of acute leukemia (especially myeloid leukemia, subtype M4 and M5) (13). HSP27 is overexpressed in bone marrow of patients with newly diagnosed acute myeloid leukemia (AML) M4/M5 (13-15). The increased level of HSP27 seems to be correlated to the clinical status of pediatric M4/M5 subtypes. Interestingly, the incidence of relapse and refractory leukemia have been more frequently observed in patients overexpressing HSP27. Chemosensitivity of leukemia cells and the effectiveness of anticancer drugs increased with decreased expression of HSP27. Yang and co-workers have examined the level of HSP27 in a few leukemia cell lines, including pediatric AML M5 (THP-1) and T-cell acute lymphoblastic leukemia (ALL, Jurkat) (15). HSP27 levels were elevated in THP-1 leukemia cells as opposed to noticeably lower levels in Jurkat cell lines (14,15). Madsen and co-workers found increased expressions of the M2 isoform in B-cell ALL and in non-leukemic ALL precursors in comparison to the normal B-cell precursors (12).

3.1.2. Involvement of HSP60 and HSP10

Significantly increased HSP60 gene expression has been reported in all leukemia cell lines in comparison to the healthy control samples. The highest level of HSP60 mRNA has been observed in Jurkat and CCRF-CEM cell lines (13). The main function of HSP60 depends on HSP10 - a co-chaperone that participates in the folding of client proteins (16). Cappello and co-workers have evidenced the positive expression of HSP10 characteristics for precursors of myeloid and megakaryocytic cells, while mature cells remained consistently negative (17). Thus, further investigations should be carried out to determine the role of HSP10 in hematological diseases including leukemia.

3.1.3. Involvement of HSP70 isoforms

HSP72, a member of the HSP70 chaperone family, has been detected on the cell surface of tumor cells and not in normal cells (18). However, the increased expression of Hsp70 on the cell surface of leukemia cells probably depends on biological sample processing methods (13). Any manipulation, chemical reagents, freezing/thawing may induce Hsp70 expression on the cell surface (13). Interestingly, high Hsp70 membrane positivity has been reported mainly in leukemia cell lines (19,20). This is consistent with the data of Gehrmann *et al*., who observed intense expression of HSP70 in leukemia cells after thermal stress, radiation, and chemotherapy (21). Furthermore, a new parameter, plasma-circulating HSP70 (cHSP70), may be an interesting and useful tool in clinical practice. The advantage of cHSP70 is its independency on any physical or chemical manipulation. cHSP70 levels have been associated with the severity of the cancer. Thus, cHSP70 may be an important biomarker of poor prognosis (22).

3.1.4. Involvement of HSP90 isoforms

HSP90 gene expression has been reported to play an important role in the proliferation of leukemia cells. Yufu *et al*. have found increased levels of HSP90 alpha

mRNA in acute leukemia and mononuclear cells, derived from peripheral blood patients with acute leukemia, in comparison to the normal peripheral blood mononuclear cells (23). Moreover, Xiao *et al*. have found elevated HSP90 alpha mRNA levels in patients with acute non-lymphoid leukemia and acute lymphoid leukemia (24). Intracellular over-expression of HSP90 alpha/beta has been also observed by Flandrin *et al*. in a poor-prognosis AML (25). Due to that, it has been suggested that Hsp90 plays an important role in the regulation of cell survival and resistance to chemotherapy (25). AML cell lines have been evidenced with increased HSP90 alpha gene expression. Moreover, K562 (chronic myeloid leukemia (CML)), Jurkat (ALL), and CCRF-CEM (ALL) cells are characterized by high levels of HSP90 alpha mRNA (13).

3.2. Central nervous system (CNS) tumors

CNS tumors are the second most common cancers in children. About 21% of childhood cancers are CNS tumors. Many brain tumors have been distinguished with medulloblastoma (MB) being the most common malignant brain tumor. MB, according to the WHO classification published in 2007, is divided into the four histological subtypes: classic; desmoplastic/nodular; extensive nodularity; and large-cell/anaplastic. Classic and nodular/desmoplastic MBs are the 2 major histological subtypes. The extensive nodular subtype and large-cell/anaplastic MBs are the least frequent and mainly associated with poor prognosis.

3.2.1. Involvement of major HSPs in MB tumors

HSPs expression and activity have been often highly upregulated in brain tumors. Hauser *et al*. have evidenced the high expression of HSP27, HSP70, and HSP90 in MB cells (26). Thus, these proteins may be used as potential therapeutic targets for novel anticancer drugs. Correlating HSPs expression directly with the MB histological subtype and the patients' prognoses have been suggested (26-28). Expression of almost each group of HSPs (HSP40, HSP60, HSP70, HSP90 alpha, HSP27 phosphorylated in position of Serine 82and Serine 15) have been examined in MB cells (26-28). HSP27 (pSer15), HSP70 and HSP60 may have prognostic implications in patients with MBs. MB with extensive nodularity presents definitely lower expression of HSP27 (pSer15) but higher HSP60 expression than classic MB (28-33). Significantly elevated expression of HSP70 has been found in a large-cell MBs (28-33).

Over-expression of HSP90 has been related to the prognosis of many tumors (34,35). HSP90 inhibitors have been reported to reduce the survival of tumor cells derived from a variety of pediatric neoplasms including MB (34,35). One of the HSP90 inhibitors is a derivative of geldanamycin, 17-demethoxy-17-N,N-dimethylaminoethylaminogeldanamycin (17-DMAG) (7). Calabrese *et al*. have reported that MB cells are sensitive to another derivative of geldanamycin, 17-allylamino-demethoxygeldanamycin (17-AAG) (35). Interestingly, the recombinant HSP70 administered into the cavity after resection of a brain tumor can be a novel strategy for the treatment of malignant brain tumors in children (36).

3.3. Osteosarcoma (OS)

OS is one of the most aggressive malignant bone neoplasms of childhood and adolescence (37-40).

According to the WHO classification, OS is divided into several subtypes with distinct biological behaviors and clinical outcomes: conventional, telangiectatic, small cell, low-grade central, secondary, parosteal, periosteal, and high-grade surface (37). The most common pathologic subtype is a conventional central osteosarcoma. The other subtypes are much less common (38). Remarkable progress has been made in terms of improving surgical techniques combined with neoadjuvant and adjuvant chemotherapy; however, the 5-year survival rate of OS patients has plateaued at 50–70% (39,40).

3.3.1. Involvement of major HSPs in OS

The expression and potential role of HSP27, HSP60, and HSP70 have been reported as conventional and low-grade central OSs (41). A low-grade tumor is usually noninvasive, small, and associated with an excellent outcome (41). The conventional subtype of OS demands more aggressive actions including neoadjuvant chemotherapy followed by limb-salvage procedures and postoperative-multidrug treatment (42). Significantly higher expressions of HSP27 and HSP70 have been observed in the conventional subtype in comparison to the low-grade central OS (42) while HSP60 has been over-expressed in both types of OS. Higher expression of HSP27 has been significantly related to the metastases in conventional OS (43). HSP27, together with HSP60 and HSP70, have been shown as a negative factors of OS patients' survival (41,42). Furthermore, HSP47, a collagen-specific HSP, is more highly expressed in OS than other HSPs. Expression of HSP90 alpha decreased in patients after biopsy and surgery (44). Trieb *et al*. have suggested that the expression of HSP90 seems not to be a predictive value of cancer but more likely the humoral immune response (45). The presence of anti-HSP90 antibodies in patients' serum is consistent with a good response to neoadjuvant chemotherapy with the absence of the anti-HSP90 antibodies being connected to the occurrence of metastases (45). The mechanism of the protective effect HSP90 has not been yet clarified. It might be accompanied by the immune response or direct protective effects of anti-HSP90 antibodies (45). The serum antibodies against HSP60 have been definitely increased in patients with OS (47), though the level of the anti-HSP60 antibodies has not been correlated to such clinical parameters as: response to preoperative chemotherapy, duration of symptoms, age, gender, tumor size, serum alkaline-phosphatase levels or metastases (47).

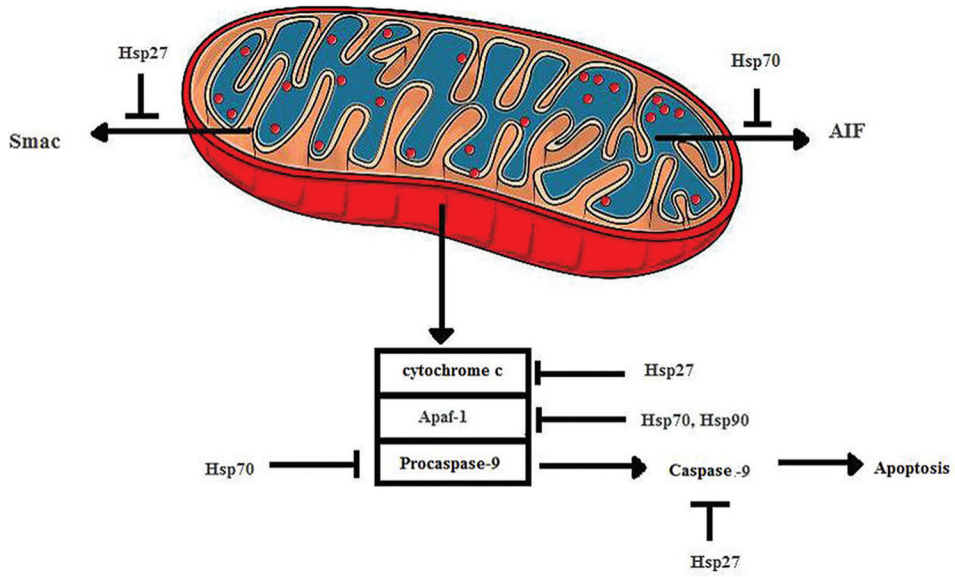

Figure 1. The mechanisms of inhibition of apoptosis by major HSPs. HSP27 blocks the release of Smac and cytochrome c from mitochondria and activation of caspase 9. HSP70 and HSP90 inhibits apoptotic protease activating factor 1 (Apaf-1), in addition HSP70 blocks activation of procaspase-9.

3.4. Neuroblastoma (NB)

NB is the most common extracranial solid cancer in childhood and the most common cancer in infancy. Prognosis and treatment of NB depend on clinical and biological risk factors (48).

3.4.1. Involvement of major HSPs in NB

Little is known about the functions of HSPs in NB cells (49). Glucose-regulated protein 75 (GRP75), a member of the HSP70 family of chaperones, is up-regulated in NB tumor tissues. Positive GRP75 expression has been strongly correlated to the differentiation of NB and early clinical stages. The expression of GRP75 seems to be a favorable prognostic factor in NB (50), with the expression of HSP27 associated with an increase in the aggressiveness of the histological type of NB. It has been suggested that the protein may be used as a differentiation and prognostic marker for NBs (51). 17-DMAG inhibits NB cell growth by triggering NB cell apoptosis (52). Downregulation of HSP70 signaling in NB has resulted in apoptotic cell death with effects similar to that of triptolide, the new HSP70 inhibitor. Administration of triptolide induced NB cell death by multiple signaling pathways (53,54). Among these pathways, triptolide decreased HSP70 mRNA and protein levels in a dose-dependent manner. These findings support the hypothesis that inhibition of HSP70 signaling plays a significant role in NB cell death (53,54).

4. POST-TRANSLATIONAL MODIFICATIONS OF HSPS

Post-translational modifications may affect the chemical and physical properties of the proteins, including their stability, activity, and function. Post-translational modifications such as hyper-phosphorylation, hyper-acetylation, S-nitrosylation, oxidation, and ubiquitination regulated the chaperones' function (55). Phosphorylation of HSPs is one of the most investigated topic and the most important post-translational modification. The phosphorylation of HSP90 resulted in changes of its interaction with other proteins. Tyrosine phosphorylation of HSP90, induced by 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins), increased its association with endothelial nitric oxide and potentiated nitric-oxide-dependent angiogenic processes (56). Moreover, suppression of constitutive phosphorylation of HSP90 beta at positions Ser-226 and Ser-255 may have contributed to chemoresistance (57). Acetylation and hyper-acetylation of HSPs are other very important post-translational modifications, where acetyl groups are usually attached to lysine residues. The acetylation of HSP90, mediated by histone deacetylase inhibitors (HDACi), resulted in the destabilization of HSP90 complexes with its client proteins (ErbB2, Raf-1, mutant p53)(58). Additionally, we have demonstrated that hyperacetylation of HSP60 is associated with anticancer activity of geldanamycin and leads to induction of apoptosis in OS cells (46) (Figure 1).

5. SUMMARY AND PERSPECTIVE

Due to the complex mechanisms of action of HSPs and their expression in both healthy cells and cancer cells, HSPs may be considered as novel prognostic biomarkers of increased cell proliferation, differentiation, metastasis or poor therapeutic outcome. Despite numerous studies, the involvement of HSPs in the

processes of carcinogenesis and chemoresistance needs to be further elucidated. Undoubtedly, the combination of HSP inhibitors with conventional chemotherapy may constitute the future of effective anticancer strategies.

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Abbreviations: ALL: acute lymphoblastic leukemia, AML: acute myeloid leukemia, Apaf-1: apoptotic protease activating factor 1, cHSP70: circulating HSP70, CML: chronic myeloid leukemia, CNS: central nervous system, HMG-CoA: 3-hydroxy-3-methylglutaryl-coenzyme A, HSP: heat shock protein, MB: Medulloblastoma, NB: neuroblastoma, OS: osteosarcoma 17-AAG: 17-allylamino-demothoxy geldanamycin,17-DMAG:17-demethoxy-17-N,N dimethylaminoethylaminogeldanamycin

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