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Expression of Heat Shock Protein 70 (Hsp-70) in Children's Acute Lymphoblastic Leukemia

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ABSTRACT

Heat shock protein (Hsp) 70 is one of the most common Hsp proteins found in humans. Like other types of Hsp, Hsp-70 acts as a chaperone protein in the process of protein synthesis. Its important role in protein synthesis means that these proteins are supposed to be inside the cell and cannot be examined outside the cell. In its development, Hsp-70 turned out to be examined outside of cells due to an increase in Hsp-70 caused by stress on cells which were caused by environmental stress conditions (heat shock, cold shock, heavy metal, oxygen-free radicals), inflammatory and infectious processes, ischemia, tissue damage, aging, and oncogenesis. Cancer studies have found high expression of Hsp-70 in solid tumors and hematological malignancies including ALL. High expression of Hsp-70 is said to have adverse implications on the outcome of ALL and therefore Hsp-70 is auspicious as a therapeutic target in the pediatric treatment of ALL. This review aims to elucidate the role of Hsp-70 expression in pediatric ALL patients.

Keywords: Heat shock Protein; Hsp-70; ALL

INTRODUCTION

Heat shock protein (Hsp) was first discovered by Ritossa in 1960 in the description of chromosomal changes in the salivary glands of Drosophila melanogaster after being exposed to high temperatures. In its development, the protein was also found in the cells of other creatures including humans as a response of a cell to extreme temperature changes and was named Hsp [1]. The classification of Hsp is based on its molecular weight, so far there are 6 categories of Hsp, namely small Hsp, Hsp-40, Hsp-60, Hsp-70, Hsp-90, and Hsp-100. The Hsp-70-membered proteins (Hsp-70, Hsp-72, GRP75, GRP78) are mainly located in the mitochondria, cytosol, and endoplasmic reticulum [1,2]. The Hsp-70 protein plays a role in the protein folding process by cooperating with the Hsp-70 interacting protein (Hip) so that the protein is folded correctly and does not undergo denaturation. This protein also helps Hsp-40 along with binding immunoglobulin protein (Bip) for protein folding in the endoplasmic reticulum. In addition, Hsp-70 also plays a role in the process of destroying damaged proteins into lysosomes with the help of Hsp-40, Hip, Hsp-70/Hsp-90 organizing protein (Hop), and Bag-1 protein [1,3,4].

A hallmark of cancer in humans is the evasion of apoptosis in response to stress stimuli, which contributes to tumorigenesis and treatment resistance. Cell death pathways can in principle be blocked at various levels of the signaling cascade by upregulating anti-apoptotic proteins and/or by downregulating or dysfunctioning proapoptotic molecules [5].

The expression of Hsp-70 which was initially useful for homeostasis of body cells, but in cancer cells is used as a means of defending themselves from death so that cancer cells do not die and become more virulent [3,6,7]. Hsp-70 overexpression in cancer has been widely reported, especially in solid tumors [7,8]. In development, several studies have also reported high Hsp-70 in hematological malignancies including ALL in vitro studies [9-11]. A study in pediatric ALL found that high Hsp-70 expression was associated with chemotherapy failure and had a poor outcome. This protein is said to inhibit apoptosis both intrinsically and extrinsically. High levels of hsp-70 in leukemia are said to be one of the causes of the lower survival rate of leukemia [12,13]. The great role of Hsp-70 against cancer makes Hsp-70 utilized as a potential cancer treatment target [14-16]. In this review, we summarize the expression of Hsp-70 in ALL, its implications for chemotherapy outcomes, and the possibility of becoming a more effective target for ALL treatment.

Apoptosis and Hsp-70

Chemotherapy as a treatment for pediatric ALL consists of a variety of anti-cancer drugs. One mechanism of chemotherapy in killing cancer cells is by triggering apoptosis. Apoptosis will increase by 20-30% in the first 24 hours after chemotherapy [17,18]. Apoptosis can be activated through 2 pathways, namely the intrinsic pathway and the extrinsic pathway. In the intrinsic pathway, B cell lymphoma 2 (Bcl-2) will respond to cell death signals by inducing the opening of the mitochondrial

membrane pores, thereby releasing cytochrome c into the cytosol. Cytochrome c cooperates with apoptosis protease activating factor-1 (Apaf-1) to activate procaspase-9 to form the apoptosome, which then activates and releases caspase-3 which facilitates the degradation of damaged cells [19,20]. Chemotherapy drugs that play a role in this pathway are glucocorticoids and vincristine drugs [21-23]. Apoptosis in the extrinsic pathway is characterized by interactions between the Fas receptor, Tumor Necrosis Factor Receptor -1 (TNRF1), Death Receptor 3 (DR3), TNF-Related Apoptosis-Inducing Ligand (TRAIL), Fas-ligand (FasL), and Tumor Necrosis Factor (TNF) on the surface of lymphoblasts which will activate the Fas Associated Death Domain (FADD) then will form a Death Inducing Signaling Complex (DISC) so that it will activate Caspase-8. Caspase-8 will activate caspase-3 which will degrade damaged cells [20,24]. Chemotherapeutic drugs that play a role in this pathway are methotrexate, daunorubicin, and doxorubicin [23,25-27].

One way for cancer cells to defend themselves is to increase the expression of Hsp-70 to prevent cell death by avoiding apoptosis. In the intrinsic pathway, Hsp-70 inhibits the activation of Bcl-2 thereby inhibiting the release of cytochrome c which results in inhibition of the release of caspase 9 so that caspase 3 will not be active to destroy cells. Hsp-70 can also directly inhibit Apaf-1 so that cytochrome c cannot activate caspase-9 and caspase-3. In the extrinsic pathway, Hsp-70 inhibits the interaction between fasL, TRAIL, and TNF so that it will not form DISC which results in the inactivation of caspase-8 so that caspase-3 will also be inactive [5,20,24,28].

Hsp-70 and ALL

It is said that Hsp protein can only be seen at the cellular level, but in its development, it turns out that Hsp is also released from the extracellular compartment to the bloodstream so that it can be detected. In normal body conditions, it is said that Hsp will be very difficult to detect in the body because the amount is very small [29]. It is known that Hsp is increased in malignant diseases as a response of cancer cells to fight apoptosis. An in vitro study in 2007 by culturing Myc proto-oncogene U-937 leukemia cells after exposure to heat shock protein was compared to culturing Myc proto-oncogene U-937 cells with chemotherapy drugs camptothecin, etoposide, adriamycin, and sodium butyrate. It was found that the expression of Hsp-70 increased 8-10 times after 3 hours of incubation [30].

The high expression of Hsp-70 at the beginning of diagnosis is said to be evidence of the avoidance of cancer cells against apoptosis through intrinsic and extrinsic mechanisms. High Hsp-70 expression will contribute to poor outcomes in children with leukemia. Expression of Hsp-70 in leukemia tends to be higher, especially in patients with a poor prognosis [12,13,31]. A 2019 study in China compared a sample of 40 ALL patients (20 adults and 20 children) who had not received chemotherapy with 40 healthy control samples (20 adults and 20 children), resulting in higher Hsp-70 expression in ALL patients compared to healthy patients with $p < 0.01$ [32].

Similar results were also obtained in a study in Ukraine in 2020, which examined Hsp-70 in 46 ALL children before chemotherapy, 29 ALL children with complications, and with a control group. It was found that the Hsp-70 level in peripheral blood at the beginning of diagnosis had a median of 5.51 ng/mL (3.69-8.93 ng/mL), in patients with complications it had a median Hsp-70 value of 4.79 ng/mL (2.89-7.45ng/ mL) while in the control group the levels of Hsp-70 were 0.45 ng/mL (0.38-0.68ng/mL) [33]. High expression of Hsp-70 is also associated with poor outcomes after chemotherapy in pediatric ALL.

A 2005 study stated that Hsp-70 was expressed in 3-82% (median 39%) in ALL cases, and in 72% of cases expressed Hsp-70 more than 20%. A higher Hsp-70 expression was found in patients who were not in remission compared to those who were in complete remission [12]. A 2010 study that assessed Hsp-70 in 263 subjects (AML, ALL, MDS, and controls) found circulating heat shock protein 70 (cHsp-70) levels in peripheral blood samples of AML and ALL subjects were higher than others with a p-value < 0.001. ALL samples showed the highest levels of cHsp-70. High levels of cHsp-70 are associated with low survival rate, in this study, the survival rate for ALL with high levels of cHsp-70 (>55ng/ml) was 123 weeks while at lower levels of cHsp-70 the survival rate was 350 weeks [13].

Among the studies that support the theory that overexpression of Hsp-70 is associated with poor outcomes in pediatric ALL, there are also several studies that yield opposite results. Research in China in 1996 examined the expression of Hsp-90, Hsp-70, and Hsp-27 using samples from 22 leukemia patients compared with blood samples from non-leukemic patients. Increased expression of Hsp-27 in 4 samples of ALL, 7 samples of LMA, and 2 samples of MDS compared to normal blood samples. Expression of Hsp-70 was lower in all ALL, LMA samples except for 1 ALL sample and 1 MDS sample compared to normal blood samples. High expression of Hsp-90 is found in all samples (11 LMA, 5 ALL, and 1 MDS sample) [34]. A 2003 study in Denmark was conducted on a sample of 13 bone marrow aspirates of ALL children patients (7 new ALL, and 6 relapsed ALL) and 8 healthy children. There was no significant difference in Hsp-70 levels among ALL patients either at diagnosis or at relapse. However, compared to normal mature cells, an increase in Hsp-70 levels was found but not significant [35]. The cohort study by Sedlakova (2011) evaluated 6 ALL and 9 adult AML patients and 1 person with biphenotype leukemia and 21 healthy controls to calculate the levels of Hsp-70, Hsp-90, Hsp-60, Hsp-27 by flow cytometry. Expression of Hsp-70 on the cell surface did not differ between bone marrow and peripheral blood samples. Hsp-70 expression was significantly lower in the bone marrow and peripheral blood compared to healthy controls in peripheral blood. No statistical differences were found for Hsp-70 expression in peripheral blood or bone marrow between patients with leukemia-onset and healthy peripheral blood controls. There was a significant increase in the expression levels of Hsp-60, Hsp-90, and Hsp-27 when compared to a healthy control cohort ($p < 0.001$) [36].

TABLE 1: Hsp-70 expression in ALL

Author (year)	Methods	Participant	Results
Xiao *et al.*, 1996	Case-control study	• 7 ALL patients • 11 acute non-lymphoid leukemia • 2 CML patients • 2 MDS patients • 6 healthy donors' group • Sample from bone marrow aspiration	• Hsp-70 was expressed lower in the ALL group and acute non-lymphoid leukemia group compared to the healthy donors' group
Wehner *et al.*, 2003	Case-control study	• 7 pediatrics ALL patients (newly diagnosed) • 6 pediatrics ALL patients (relapse) • 8 healthy kids • Sample from bone marrow aspiration	• Expression of Hsp-70 was not significantly different in the newly diagnosed ALL group compared to the relapse group • There was no significant difference in Hsp-70 expression in the newly diagnosed group and relapse group compared to the healthy control group
Thomas et *al.*, 2005	Case-control study	• 18 ALL patients • Normal B cell precursor	• Hsp-70 expression was 3-82% (median 39%) in ALL • 72% of total sample expressed Hsp-70 > 20% • Hsp-70 was expressed higher in the no remission group compared to the complete remission group
Afanasyeva *et al.*, 2007	*In vitro*	• Leukemia cell myc protooncogene U-937 bred after heat shock compared to U-937 bred with camptothecin, etoposide, adriamycin	• Hsp-70 increased 8-10x in both samples
Yeh *et al.*, 2010	Case-control study	• 96 adult AML patients • 40 adult ALL patients • 28 adult MDS patients • 99 adult healthy control group • Sample peripheral blood	• ALL patients expressed the highest cHsp-70 compared to others • cHsp-70 > 55ng/ml had 123 weeks of sustainable life • Lower cHsp-70 had 350 weeks of sustainable life
Sedlakova *et al.*, 2011	Case-control study	• 6 adult ALL patients • 9 adult AML patients • 1 adult bifenotipe • 21 healthy control group • Sample from peripheral blood and bone marrow aspiration	• There was no significant difference in Hsp-70 expression in leukemia patients compared to healthy control • Hsp-70 expression in the bone marrow and peripheral blood of ALL patients were lower compared to Hsp-70 in the healthy control group
Guo *et al.*, 2019	Case-control study	• 20 adult ALL patients • 20 pediatric ALL patients • Healthy control group	• Hsp-70 expression was higher in the LLA patients group compared to the healthy sample group ($p<0.01$)
Kondratiuk et al., 2020	Case-control study	• 46 pediatric ALL patients before chemotherapy • 29 pediatric ALL patients at complications • Healthy control group • Sample from peripheral blood	• Hsp-70 was higher in the pediatric ALL patients group on both samples (before chemotherapy and at complications) compared to the healthy control group • There was no significant difference in Hsp-70 before chemotherapy and in complications

Hsp-70 Inhibitor as a leukemia therapy

The large role of Hsp-70 in inhibiting apoptosis makes this protein a target for further ALL treatment [14]. Until now, several studies have been carried out to obtain drugs that focus on Hsp-70 inhibitors [8,14]. There is 1 candidate Hsp-70 inhibitor that is currently still in the in vitro research stage that can be used in ALL, namely 2-phenylethyne-sulfonamide (PFT-μ). PFT-μ inhibits Hsp-70 by interacting with the carboxyterminal peptide-binding domain (PBD) of Hsp-70.

At the in vitro level, administration of this drug induces apoptosis in ALL cells at certain doses and directly increases caspase-3 activation [37]. None of the other candidates are specifically designated for the treatment of ALL. Until now, there is 1 candidate Hsp-70 inhibitor that is already in the clinical trial stage, namely AG-858, but that candidate is designated for Chronic myeloid leukemia (CML) [38]. Another candidate is VER-155008 which is in the in vitro research stage used for AML and methylene blue which is also still in the in vitro research stage and is used for CML [39,40].

TABLE 2: Hsp-70 inhibitors

Author, years/NCT	Hsp-70 inhibitors	Research status	Disease
Kirszberg *et al*., 2005	Methylene blue	*In vitro*	K562 (CML)
Kaiser *et al*., 2011	2-phenylethyne-sulfonamide (PFT-μ)	*In vitro*	Human cell line KG-1a (AML), NALM-6 (B-precursor ALL), TOM-1 (B-precursor ALL; BCR-ABL +), Jurkat, BE-13 (T-cell Leukemia), and K562 (CML, blast Crisis)
NCT00058747	AG-858	Clinical Trial	AML
Reikvam *et al*., 2013	VER-155008	In vivo	AML

CONCLUSION

Hsp-70 is one of the proteins released by lymphoblast cells to inhibit apoptosis to avoid death. High Hsp-70 expression is thought to be one of the causes of chemotherapy failure due to its inhibitory effect on apoptosis. Hsp-70 inhibitors candidate can be an opportunity to develop therapies centered on inhibiting apoptotic death signals. Further research is needed on the expression of Hsp-70 in children's ALL that can be used as a marker for diagnosis, prognosis, and therapy.

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