REVIEW

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The distinctive role of small heat shock proteins in oncogenesis

Krzysztof Laudanski[1] and Dorota Wyczechowska[2]

[1] Department of Medicine, Griffin Hospital, Derby, CT 06418, USA
[2] Department of Medicinal Chemistry, Medical University of Łódź, Poland

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Abstract

Recently, the role of small heat shock proteins (HSPs) has been widely recognized in cancer research. Small HSPs are tumor-protective via numerous, independent mechanisms such as: oxidative stress, protection preventing protein denaturation, anti-apoptotic activity, and likely direct suppression of the immune system. However, it is unclear whether they play any role in the initial steps in carcinogenesis. This article seeks to familiarize the reader with general characteristics of small HSPs (especially HSP-27, αA/B-crystallins), their tissue distribution with special regard on expression in malignant specimens, and biological properties of intracellular HSP-27 and αA/B-crystallins promoting tumor genesis and growth. A separate chapter describes immunomodulatory characteristics of extracellular HSP-27 with special emphasis on their plausible effect on the neoplasm development.

Key words: small heat shock proteins, αA-crystallin, αB-crystallin, HSP-27, carcinogenesis, neoplasm, immune system.

Corresponding author: Krzysztof Laudanski, Department of Medicine, Griffin Hospital, 130 Division St., Derby, CT 06418, USA, e-mail: klaudanski@sbcglobal.net

SMALL HEAT SHOCK PROTEINS – GENERAL CHARACTERISTICS

Small heat shock proteins (sHSPs) belong to a class of abundant molecules sharing several common properties and whose monomeric molecular mass is between 12–43 kDa [19, 27, 64, 78, 88, 102, 106, 107]. 1) In contrast to other proteins, their expression is induced by elevated temperature [4, 15, 19, 27, 34, 105], 2) and regulated by heat shock transcription factors (HSFs) [17, 44, 47, 71, 77, 85, 86, 95]. 3) Amino acid sequences of sHSPs are conservative and consist of distinctive, pivotal for their function, conservative domain of 80–100 amino acids, called "α-crystallin domain" [5, 23, 27, 30, 32, 76, 78, 100]. 4) sHSPs form large oligomeric complexes mediating folding of other polypeptides, but they are not components of their final functional structures (Fig. 1) [12, 31, 32, 35, 61, 64, 78, 88, 100]. Based on molecular structure, 10 mammalian sHSPs are traditionally identified: HSPB1/HSP-27, HSPB2/MKBP, HSPB3/ /HSPL27, HSPB4/αA-crystallin, HSPB5/αB-crystallin, HSPB6/HSP-20/p20, HSPB7/cvHsp, HSPB8/HSP-22, HSPB9, HSPB10/ODF1 [5, 10, 19, 30, 40, 54–56, 65, 78, 100, 101]. Recently, new HSPB1-10 has been characterized as structurally related to sHSPs [55]. Additionally, some authors include mitochondrial Hsp10, cytoplasmic HO-1/HSP-32, and clusterin as members of sHSP family based on the molecular structure and/or chaperone-like activities [1, 13, 45, 48, 83]. However, these proteins do not have the characteristic "α-crystallin domain" [13].

Our review aims at familiarizing the reader with the tissue distribution and biological activities of sHSPs with emphasis on the features, which may play a role in tumor development (Fig. 1). After a short description of the sHSPs' expression in healthy and cancerous tissues, the mechanisms regulating expression and biological activity of HSP-27 and αA/B-crystallins are depicted. Then, sHSPs' biological properties are described in terms of importance for promotion of oncogensis. Finally, we briefly reviewed immunomodulatory properties of extracellular HSP-27 suggesting that immunosuppressive properties of this molecule may facilitate neoplasm growth.

EXPRESSION AND ROLE OF sHSPs IN HEALTHY, DYSPLASTIC, AND NEOPLASTIC TISSUES

Small HSPs are abundant proteins. HSP-27 is expressed in cytoplasm and nucleus of many tissues, espe-

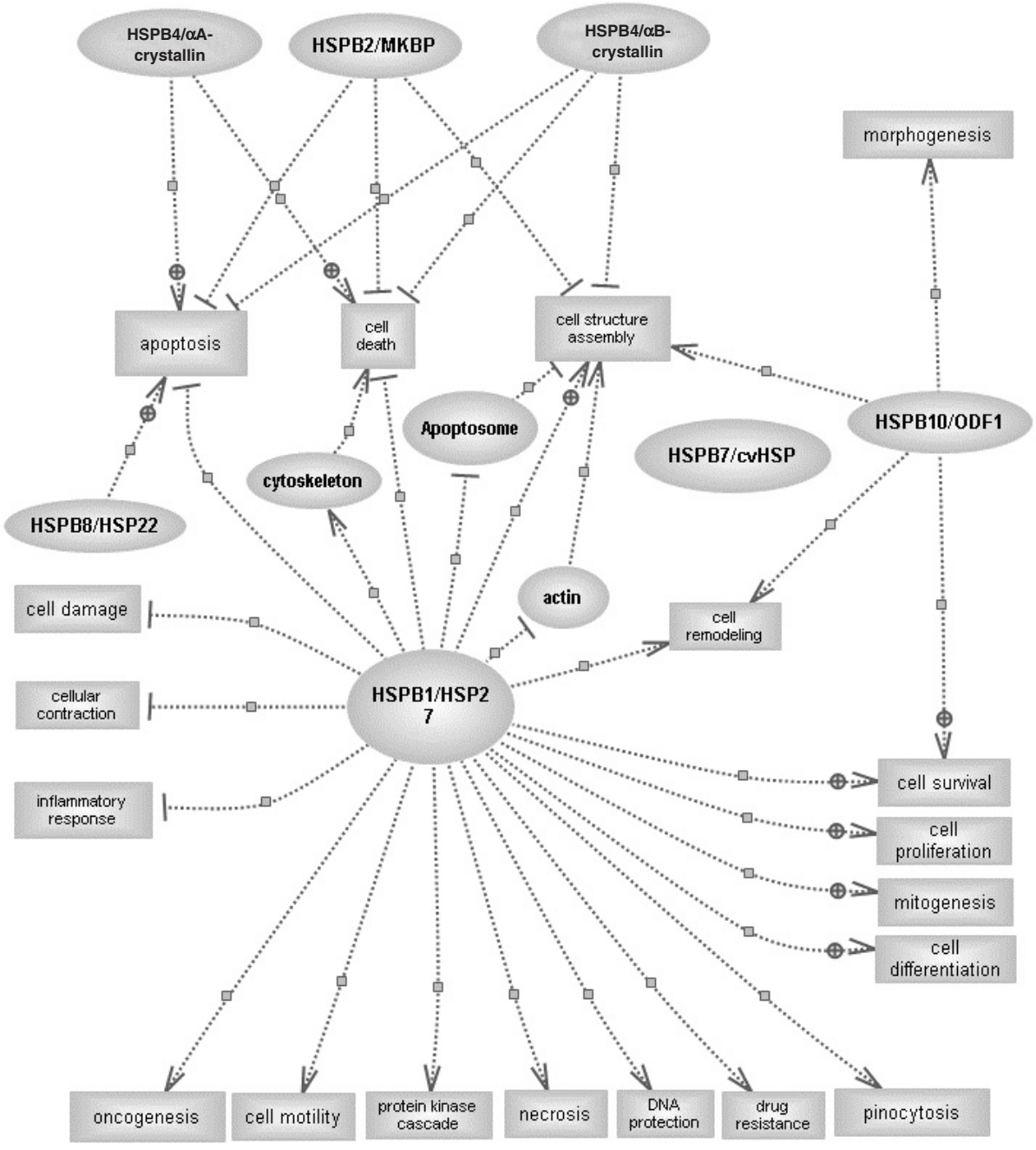

Fig. 1. Schematic presentation of the sHSPs involvement in critical cellular processes extracted from ResNet database (Iobion, Inc) using language processing tool. Different sHSPs are shown to simple interactions (single arrows), positively affect (arrows with plus), or inhibition (blunt ended arrows) several cellular processes (square shapes) by different sHSPs (oval boxes).

cially those sensitive to estrogen stimulation [16, 19, 111]. HSPB2/MKBP, HSPB3/HspL27, HspB5/αB-crystallin, HspB6/Hsp20/p20/, HspB7/cvHsp, HspB8/Hsp22 are highly expressed in muscles [40, 56, 65, 99, 100]. Traditionally, the presence of αA/B-crystallins are depicted in eye, but their expression has also been detected in kidney, brain, and others [5, 23, 30, 40]. Finally, HspB9 is testicular protein, whereas HspB10/ODF is specific for the outer surface of the sperm [30, 55, 56].

An increased expression of some sHSPs is noted in numerous neoplasms [12, 19, 28, 29, 39, 43, 57, 68, 69, 81, 94, 103, 107, 109]. Since the presence of sHSPs is detected also in non-malignant specimens, they cannot serve as specific tumor markers. Most prominently, HSP-27 has been reported in estrogen-dependent malignancies such as: ovarian cancer (vs. benign changes), breast and endometrial carcinoma, nasopharyngeal and gastric cancer [3, 26, 39, 52, 57, 63, 68, 69, 92, 103]. The gastric mucosa expresses HSP-27 in a continuous fashion from low levels observed in normal tissue, through modest expression in dysplastic mucosa, to the highest in metaplastic tissues [57, 92]. This is inter-

esting example in which expression of sHSPs parallels an increasingly and/or malignant environment but an importance of this observation remains to be discovered. HSP-27 is highly expressed in cranipharyngomas, anaplastic glioblastomas, anaplastic ependynomas, and metastatic tumors [2, 19, 46, 58]. Numerous reports attempted to characterize the prognostic role of increased HSP-27 expression in malignant tissues. In ovarian or gastric cancer increased expression of HSP-27 is related to higher degree of malignancy, chemoresistance, and an unfavorable outcome of treatment [19, 39]. On the other hand, the presence of HSP-27 in malignant aggressive histiocytoma and sarcoma correlates with improved survival and lower incidence of metastasis suggesting, thus elevated expression of sHSPs should not be considered exclusively as an unfavorable factor [30]. Very conflicting data exist about the relationships between HSP-27 expression and survival, and/or metastasis in breast carcinoma [25, 52, 53, 68, 81, 82, 103]. Such relationship has been excluded in prostate and bladder cancer [98]. Elevated expression of HSP-27 has also been reported in leukemia, skin cancer, and malignant melanoma, but its prognostic significance is unknown [19, 25, 96, 97, 105].

αB-crystallin is predominantly present in astrocytic tumors and chordomas [2, 46, 58]. Multigene profiling identified αB-crystallin as a marker of kidney cancer and some metastatic cell lines [18, 94]. HSP-22 has been detected in melanoma and breast carcinoma but no more data are available on its prognostic role [100]. The remaining sHSPs have not been described in cancerous tissues to date.

REGULATION OF sHSPs' EXPRESSION

Heat shock is the best known sHSP inducer, but other stimuli (cold shock, estrogens, ischemia, phorbol

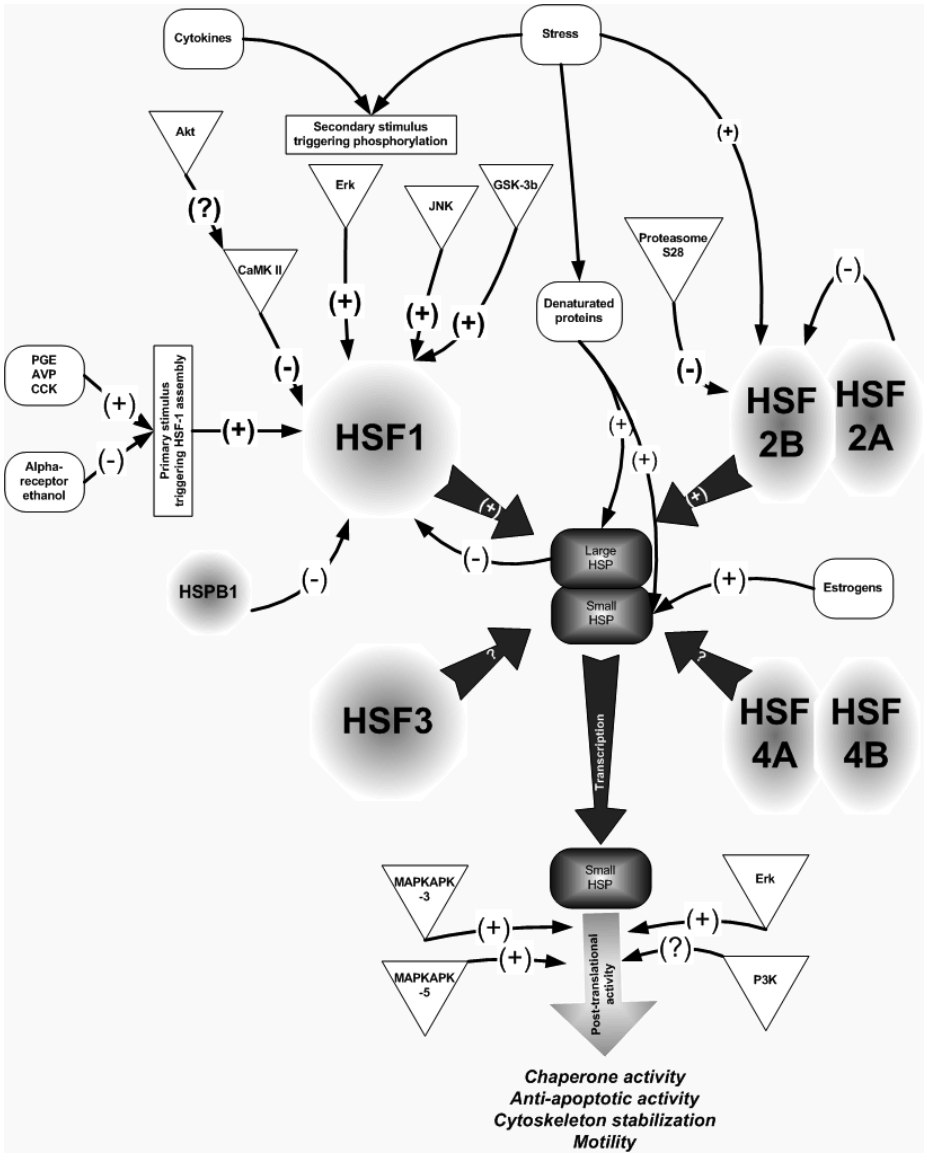

Fig. 2. The expression and biological activity of sHSPs is regulated on transcriptional and translational levels via numerous positive (+) and negative stimuli (–).

ester, hormones, ceramides, prostaglandins, antibiotics, chemotherapeutic drugs) are also potent triggers [14, 25, 43, 45, 49, 51, 58, 62, 89, 90, 97, 111]. Traditionally, sHSPs' expression is regarded as function of transcriptional activity of HSFs (HSF1-4) [17]. HSF1 and HSF2 are ubiquitously expressed but distinctively regulated [17, 44, 59, 71, 77, 86]. Under resting conditions, HSF1 exists predominantly as non-active, transcriptional monomers retained in their dormant state by binding to HSP-70, HSP-90, Hdj1, HSBP1 (HSP-binding protein 1), and/or phosphorylation of HSF-specific proline/serine motifs (Fig. 2) [17, 75, 86, 93, 95]. Small basal activity of HSF1 provides sustained, low-turnover expression of inhibitory HSP-70 [86, 95]. It is compelling to think that this negative feedback provided by large HSPs, underlies oncogensis. Trimerization of HSF1 is pivotal for DNA-binding activity of HSF1 and is affected by several factors (prostaglandin, $PGE_2\uparrow$, vasopressin, AVP↑, CCK↑, α-adrenergic blockade↓, ethanol↓) [17, 51, 62, 74, 86]. Moreover, the second stimuli (free radicals, cytokines) are necessary for full HSF1's transcriptional activity via differential HSF1 phosphorylation with stress-specific kinases like JNK (c-Jun N-terminal kinases), GSK-3β (glycogen synthase kinase 3), CaMKII (calcium/calmodulin-dependent protein kinase), PI3K, and probably Akt (serine-threonine protein kinase) [7, 9, 17, 34, 42, 50, 86] (Fig. 2). Then, the active HSF1 binds to specific DNA promoter region (HSE-heat shock protein element binding HSFs), inducing concomitant expression of both large and sHSPs [7, 59, 75]. This induction can be further enhanced via HSF1-independent, estrogen-sensitive promoter, which is responsible for an increased expression of small HSPs in estrogen-sensitive tissues and tumors [3, 29, 42, 82, 109, 111]. HSF2 was considered as very distinct from HSF1 in respect to activation stimuli and biological activity [17, 44, 71] (Fig. 2). Recent data indicates that HSF2 isoform A is also co-activated with HSF1 during heat shock but certain HSF1-specific stimuli fail to co-activate HSF2 [86]. Although several authors quote HSFs as critical factors for sHSPs expression, many more studies are necessary to establish the role of HSF and other inducers in regulating sHSPs' expression as the steps in oncogenesis and tumor growth [25, 28, 75, 80].

The transcriptional control of small HSPs' expression is important, but the posttranslational modifications are essential for the activity of sHSPs [7, 8, 12, 49, 54, 61, 74, 76, 107, 110]. In general, HSP-27 and αA/B-crystallins are phosphorylated on three different sites by MAPKAPK-2 (mitogen activated protein kinase activated kinase 2)/SAPK2 (stress-activated protein kinase 2)/p-38α(stress activated kinase p38), MAPKAPK-5, Erk (extracellular signal regulated kinases), G-proteins and protein kinase C [7, 39, 50, 54, 78, 79, 107, 110] (Fig. 2). To date, the majority of evidence showed that MAPKAPK-2/SAPK2/p-38α, and MAPKAPK-5 are the major kinases for HSP-27 phosphorylation [50, 107, 110]. The activation of sHSPs by p38/MAPKAPK-2 pathway is essential for anti-apoptotic features and chemoresistance induced by sHSPs whereas MAPKAPK-5 involvement is critical in response to stress and proinflammatory cytokines [7, 27, 79, 102].

Considering the complex mechanism of HSFs activation and induction of sHSPs, one may wonder whether their abnormal activation can be responsible for increased expression of sHSPs in tumors. Alternatively, can the abnormal expression of sHSP be the first step in oncogensis? Increased expression of HSF1 has been reported in prostate carcinoma. Interestingly, HSF1 in this type of neoplasm induces preferentially HSP-27 vs. HSP-70 or HSP-90 thus eluding negative signal provided by HSP-70. Under physiological conditions, HSF1 induces expression of both large and sHSPs providing a reciprocal negative feedback for HSF1 activity via HSP-70 [75, 86]. If HSF1 induces HSP-70 not to usual extend, excessive amounts of HSP-27 are being produced providing an additional protection to the malignant specimens [72, 85]. Aberrancies in signal transduction kinases affecting sHSPs' expression may also play a role in early event in carcinogenesis. It has been reported that inappropriate activation of Erk vs. p38 results in distinct phosphorylation of HSP-27 with adverse effect on the cell survival (cell blebbing) [24, 49]. The role of these aberrancies in cancer promotion remains to be described.

MECHANISMS OF NEOPLASM PROMOTION AND INTRACELLULAR sHSPs

Intracellular sHSPs are involved in several activities that can be characterized as protective to any homeostasis imbalance (Fig. 1). These interactions are very diverse, but we focus our review on the role of sHSPs in chaperoning denaturated proteins, apoptosis inhibition, and cytoskeleton formation as the best described in literature. Remarkably, the increased expression of the sHSPs in malignant tissues provides them with additional protection against hostile environment.

In the main mechanism of action, sHSPs prevent aggregation and precipitation of proteins under stressful conditions [13, 64, 89]. Such denaturating conditions results from low tissue oxygen tension and immune system activity present in tumor environment. Consequently, the chaperone sHSPs' action is especially beneficial for survival of the ATP-depleted, stressed tumor cells [12, 70, 80, 108, 110]. In main mechanism of action, phosphorylation of sHSPs results in rearrangement of their oligomers followed by formation of stable complexes with endangered proteins preventing irreversible protein aggregation and desolubilization [6, 31, 32, 35, 61, 89]. After ATP supply is replenished, sHSP-protected proteins are released from chaperone complexes and their biological activity is further resorted by large HSPs [32, 35, 88, 108].

Since several chemotherapeutic agents act through inducing oxidative stress on the neoplastic cells, protective effects of sHSPs are clearly disadvantageous for the outcome of treatment [19, 33, 37, 70, 90]. The activity of the immune system can also be significantly hampered

via this mechanism as well [74, 89]. In fact, most of reports showed that increased expression of HSP-27 enhances tolerance of the tumor cells to oxidative stress after chemotherapeutic agent exposure [3, 20, 25, 28, 37, 38, 49, 74, 90, 105]. Expression of HSP-27 and/or αA/B-crystallins correlates also with resistance of the neoplasm to tumor necrosis factor α-mediated oxidative stress and activities of cytotoxic T cells, which are the cornerstones of anti-cancer defense [49, 50, 74, 89, 105]. These sHSP-mediated adaptive mechanisms are proportionally activated against unfavorable local conditions, anti-tumor activity of the immune system, and/or chemotherapeutic drugs [5, 32, 51, 74, 78, 89, 101, 110].

Close to the concept of anti-oxidative protection of small HSPs are their anti-apoptotic features [20, 27, 102]. For example, cytochrome c is normally present inside mitochondria and its release initiates activation of pro-apoptotic caspase 3 and 9 [36]. Some authors showed that HSP-27 binds directly to cytochrome c, or indirectly prevents cytochrome c release due to cytoskeleton depolimerization, prevents subsequent activation of apoptosis [11, 12, 36, 84]. Similarly, dimmers of HSP-27 prevent translocation of Daxx, thus inhibiting this apoptotic pathway [15]. One report suggested that HSP-27 can also interact with Akt, which is an upstream kinase involved in the inactivation of pro-apoptotic Bcl-2, Bad, FKHR, capscase 9, and FasR-dependent pathway [60, 73, 87, 110]. Additionally, αA/B-crystallines protect procapscase 3 from being processed, further enhancing the anti-apoptotic action of HSP-27 [34, 54, 87]. It is unknown whether similar mechanism is active in tumor cells.

In yet another mechanism, sHSPs protect and stabilize cytoskeleton [27, 30, 41, 49, 67, 84, 102]. HSP-27 can bind to F-actin whereas αA/B-crystallins have affinity to intermediate filaments [5, 35, 49, 67, 78, 84, 110]. Together they prevent cytoskeleton disruption induced by heat, oxidative stress, or cytochalasin B [5, 35, 41, 49, 50, 84]. Since cytoskeleton disorganization is one of the critical events in apoptosis resulting in the disorganization of mitochondria, lysosomes, and cellular trafficking, leading ultimately to cell death, this protective mechanism overlaps with sHSPs' anti-apoptotic features. Moreover, stabilization of the cytoskeleton is also an example of protection against completely different classes of chemotherapeutic-like vincrystine or vinblastine but so far no study directly addressed this question. Several authors suggested that small HSPs' involvement in cytoskeleton function is also related to the adhesion of the malignant cell and their metastasis [18, 25, 68, 91, 104].

IMMUNOLOGICAL PROPERTIES OF EXTRACELLULAR sHSPs (HSP-27) PROMOTING CARCINOGENESIS

Most of research has focused on the role of intracellular sHSPs, but extracellular HSP-27 has been detected also in serum of the breast cancer victims. The question arises whether serum HSP-27 has any effect on the response of the immune system to the tumor growth. To date, only immunomodulatory activities of HSP-27 have been described. The immunological features of HSP-27 are rather immunoinhibitory [66, 106]. Immunosuppression is very important at the level of the local tumor environment, where large quantities of HSP-27 are released from apoptotic cancerous tissue. This released HSP-27 acts locally, or leaks into serum, in each case modulating immune system response. αHSP-27 IgA antibody has been found in serum along with increased levels of immunizing HSP-27 suggesting that HSP-27 triggers immune response [26, 63]. The level of this αHSP-27 antibody correlates with the survival of breast cancer patients suggesting that inactivation of extracellular HSP-27 is beneficial for a long-term outcome, possible due to diminished immunosuppression [63]. However, there are also suggestions that HSP-27 can increase tumorgenecity of cancer cells by inhibit apoptosis of some immune system cells, indirectly boosting immune response [12, 37, 87]. The question arises what are the plausible mechanisms responsible for unfavorable effect of serum HSP-27? De et al. [21] have reported that HSP-27 can predominantly activate production of large quantities of interleukin-10, which is one of the most potent immunosuppressive agents. Also induction of PGE_2 and M-CSF, immunoinhibitory agents, by HSP-27 may further potentate immunoparalysis [66]. All these cytokines quench cytotoxic activity of T cells and NK cells, downregulate MØ activation, and hamper the emergence of dendritic cells from precursory MØ [21, 22, 66]. This HSP-27-mediated immunosuppression has a synergistic effect with previously described intracellular mechanisms protecting tumors mediated by sHSPs. At this step, further data are necessary to characterize immunomodulatory properties of HSP-27 in neoplasm development as well as studies investigating whether similar immunomodulatory properties are exhibit by other extracellular sHSPs.

CONCLUSION

Small HSPs are ubiquitous proteins, with increased expression in stressed tissues, including tumors. They provide a protection against several noxious stimuli thus promoting tumor progress via intracellular and possible extracellular actions. Further insight into aberrant transcriptional activity, posttranslational modification of sHSPs, and their immunomodulatory properties are necessary to describe in more detail how sHSPs affect tumorgenesis and development. No experiment has been carried out to show a direct linkage between cancer pathology, treatment, survival in oncology patients, and modulation of sHSPs function. Theoretically, such an in-depth description of sHSPs protective function could provide new modalities for treatment.

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