Heat-shock proteins in inflammation and cancer

REVIEW

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ABSTRACT

Heat shock protein in inflammation and cancer. Recent data have expanded the concept that inflammation is a critical component of tumor progression. Many cancer types have their origin in infection places, chronic irritation and inflammation. It is now becoming clear that the tumor microenvironment, which is largely orchestrated by inflammatory cells, is an indispensable participant in the neoplasic processes. The tumor cells have co-opted some of the molecules of signaling of the innate immune system to be able to carry out the invasion processes, migration and metastasis. At the present time, the chaperones or heat shock protein (HSPs) emerge as stimulating of the immune system and they are key pieces in the modulation of the inflammation. In this review we integrated the functional relationships between inflammation and cancer, as well as the role of the chaperones in the interface of both pathologies.

Keywords: inflammation, cancer, heat shock proteins, cytokines, chaperones, HSPs inhibitors, apoptosis

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RESUMEN

Las proteínas de estrés térmico en la inflamación y el cáncer. Recientemente se ha difundido el concepto de que la inflamación es un componente crítico de la progresión del tumor. Muchos tipos de cáncer tienen su origen en sitios de infección, irritación crónica e inflamación. Es un hecho evidente que el microambiente del tumor, grandemente conformado por células inflamatorias, es un participante indispensable en los procesos de neoplasia. Las células tumorales cooptan algunas de las moléculas de señalización del sistema inmune innato para poder llevar a cabo los procesos de invasión, migración y metástasis, con la producción de chemokinas y citocinas así como una activación mantenida del factor transcripcional NF-kB, por ejemplo. En la actualidad, las chaperonas o proteínas de estrés térmico emergen como estimuladoras del sistema inmune y son piezas clave en la modulación de la inflamación en dos modos alternativos: inflamación estéril, la cual resulta de estímulos endógenos y se hace necesaria para mantener la homeostasis, e inflamación séptica, la cual protege de la invasión de elementos patógenos. En el presente trabajo se exponen las relaciones funcionales entre inflamación y cáncer así como el papel de las chaperonas en la interrelación de ambas situaciones patológicas.

Palabras clave: inflamación, cáncer, proteínas de estrés térmico, citocinas, chaperonas, inhibidores de HSPs, apoptosis

*I*ntroduction

The functional link between inflammation and cancer was formerly postulated by Virchow in 1863, when he stated the hypothesis that cancer may preferentially originate at chronic inflammation site. He based on the fact that some irritants, together with its derived tissue damage, increase the cellular proliferation [1]. Today, it has been clearly established that cellular proliferation *per se* does not generate cancer. However, sustained cellular proliferation in an environment enriched of inflammatory cells, growth factors, stromal activation and DNA damage promoting agents, certainly increases the risk for neoplasia development. Interestingly, Dvorak has proposed that tumors are non-healing wounds [2]. During the healing process of a wound-associated tissue, cells tend to proliferate while tissue regeneration proceeds; proliferation and inflammation decrease once the damage-triggering agent is controlled, or the damage is completely repaired. By the contrary, proliferating cells, which carry sustained damage on their DNA, able to generate mutations or genetic changes (*e.g.*, initiated cells), continue to proliferate in the microenvironment rich on inflammatory cells and growth factors, follo-wed by the appearance of oncogenes and the inhibition of tumor suppressor genes. All these stimulate the proliferation of initiated cells and the development of a tumor [3].

There are several cellular and molecular factors involved in inflammation and cancer. Among them, chaperones (also known as heat shock proteins, HSPs), play a key role in the pathogenesis of these two processes.

HSPs were formerly identified as a group of proteins induced during heat shock, but they were further shown to be induced by other stimuli, such as growth factors, inflammation and infections [4]. Their expression in several types of cancer cells and their association to programmed cell death has been well documented [5]. Nevertheless, the cellular and molecular bases governing the interaction of HSPs with inflammation and cancer remain unraveled, in spite of current knowledge on the causal relationship between HSPs and both pathological conditions. For these reasons, this review focuses on molecular aspects relating inflammation with cancer, and the

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role of HSPs as regulatory elements in these two processes.

Inflammation and neoplasia progression

To understand the role of inflammation in the development of cancer, first we need to know what inflammation is, and how it contributes to physiological and pathological processes of tissue damage repair and infection.

In response to tissue injury, a multifactorial signaling cellular network is activated, to provide the host with a "repair" or "cure" response in the damaged tissue. This involves the activation and homing of leukocytes (neutrophils, monocytes and eosinophils) from the vascular system toward the damaged area. Recruitment of these inflammatory cells to the damage zone provides a temporary extracellular matrix niche whereto fibroblasts and endothelial cells can migrate and proliferate, to reconstitute the normal microenvironment of the tissue. These stages involve the action of cytokines/chemokines which dictate the natural evolution of the inflammatory response [6].

The resident inflammatory cytokine/chemokine profile determines the development of chronic disease. The tumor necrosis factor alpha (TNF-α) controls the population of inflammatory cells and modulates most of the factors involved in inflammation, such as the release of pro- or anti-inflammatory cytoki nes and growth factors of the vascular endothelium, and the activation of the NF-kB transcription factor. The transforming growth factor beta (TGF-β) is also essential to modulate inflammation; both cytokines, TNF-α and TGF-β, exerting positive and negative effects on the inflammation and repair processes [6].

A key concept is that a normal inflammation process, associated to tissue repair or to solve an infection, is self-controlled. However, when any of the convergent factors become deregulated, abnormalities can arise and a pathogenic state can be established, an effect observed during the progression of malignant neoplasia [6]. Following this line of thinking, Peyton Rous was the first to recognize that cancer develops from a "primary carcinogenesis state" caused by chemical or viral carcinogens, which induce somatic changes [7]. These stages known as "initiation" involve irreversible alterations in the DNA, which can persist in the normal tissue until the occurrence of a second type of stimulation known as "promotion". Promotion results from the exposure of initiated cells to chemical irritants such as phorbol esters, factors release to the site of tissular damage, the partial excision of an organ, the release of hormones, chronic irritation and inflammation. Functionally, many "promoters" directly or indirectly induce cellular proliferation, recruitment of inflammatory cells or increased production of reactive oxygen species, all of them inducing a misbalanced oxidative state and promoting irreversible damage of the DNA. The abrogation of programmed cell death or repair pathways occurs in chronically inflamed tissues, transcending in the replication of the DNA and the proliferation of cells devoid of capacity to control its own cell cycle [6].

It has been also observed in chronic inflammation that the pattern of cytokines and their receptors are altered in neoplasic cells. These cytokines induce differential effects on stromal and neoplasic cells, besides their function to regulate leukocyte recruitment. Therefore, tumor cells not only regulate the expression of its own cytokines neither promote the recruitment of inflammatory cells, but also use these factors to increase tumor growth and progression. This is an essential difference from physiological inflammation, in which the production of pro- and anti-inflammatory cytokines is highly coordinated and self-limited in the coordinated production (Figure 1) [6].

In any tumor, the balance of cytokines is essential to regulate the type and intensity of inflammatory responses. Tumors producing low or null anti-inflammatory cytokine levels induce limited inflammation and a vascular response which restrains

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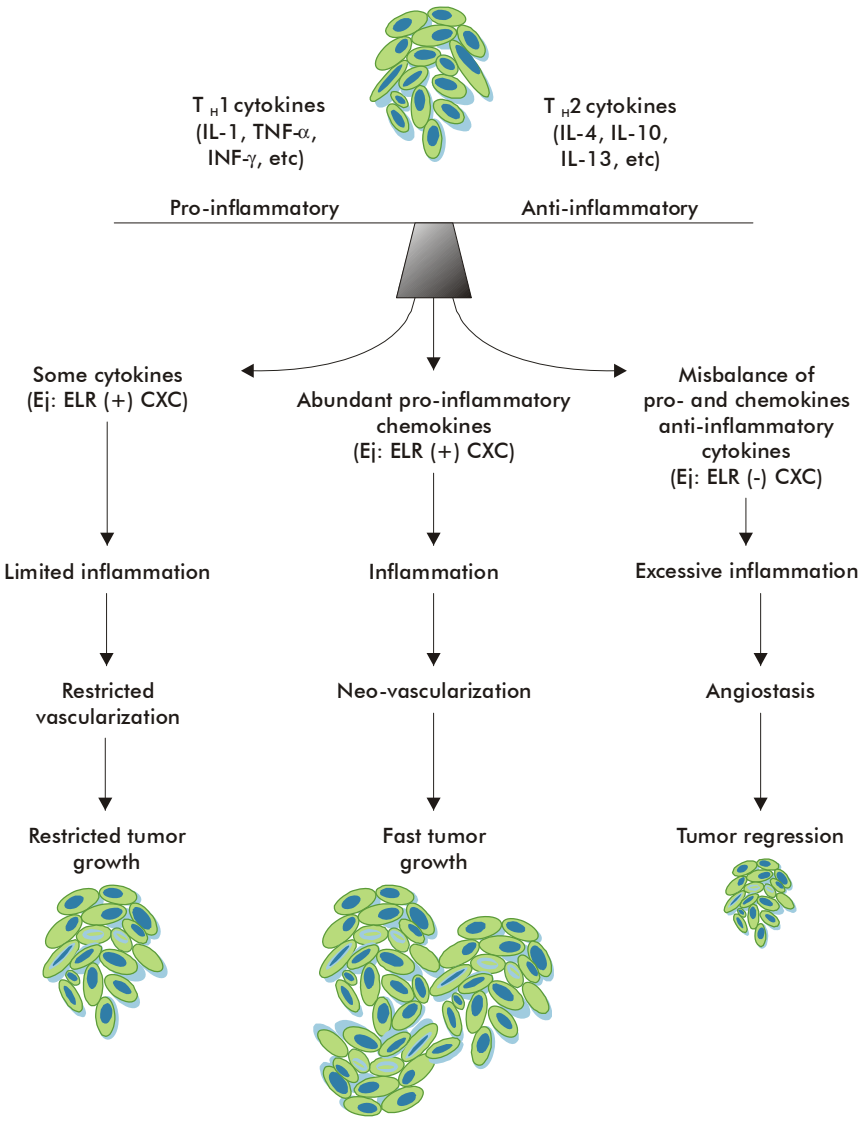

Figure 1. Balance between pro- and anti-inflammatory cytokines and chemokines that regulate the appearance of neoplasias. ELR-elastin-laminin receptor, CXC- chemotactic cytokines (adapted from reference 6).

their growth. In contrast, the production of high cytokine levels could lead to inflammation, enhancing angiogenesis and favoring tumor development. Alternatively, higher levels of monocytes or monocyte infiltration in response to a misbalance between pro- and anti-inflammatory cytokines can be associated to cytotoxicity, angiostasis and tumor regression.

As previously mentioned, the acute inflammatory response is very useful for the host to eliminate pathogens. Nevertheless, there are certain situations on which this complex system becomes deregulated, establishing chronic inflammation states that could be harmful for the organism. Therefore, the interconnection between "initiators" and the failure of mechanisms controlling the chronic inflammatory response provides to the damaged (tumor) cell a niche suitable for its development and progression.

Cancer as a chronic inflammation-associated condition

A plausible hypothesis on how inflammatory cells can promote neoplasic processes is based on the fact that several neoplasias arise in inflamed and infected tissues, just as part of the normal response of the host. Actually, an increasing number of evidences support the assumption that neoplasias are initiated by infections (Table 1) [1, 6, 8-13]. Nearly 15% of the tumors detected worldwide every year can be attributed to infections, corresponding to 1.2 million of cases [14]. It has been also observed that a persistent infection of the host can lead to chronic inflammation, because leukocytes and other phagocytic cells induce DNA damage in the proliferating cell. This is caused by reactive oxygen and nitrogen species released by these cells as normal components of the immune response. These species react to form peroxinitrites, a type of mutagenic agents which promote permanent genomic alterations such as deletions, point mutations and rearrangements in the DNA of epithelial proliferating cells [15]. In fact, the frequency of mutations observed in the tumor suppressor protein p53 in different neoplasias is similar to that found in other chronic inflammation diseases, such as rheumatoid arthritis and the inflammatory bowel disease [9].

The strongest relationship between chronic inflammation and malignancies occurs in the colon carcinogenesis, in individuals suffering from inflammatory bowel disease, chronic ulcerative colitis and Crohn's disease [9]. The infection with the hepatitis C virus also predisposes to hepatic carcinoma, and schistosomiasis increases the risk of suffering from colon and bladder carcinoma [6]. The chronic infection with *Helicobacter pylori* has also been associated to stomach cancer, this bacteria recognized as the inducer carcinogen for gastric cancer, a condition ranked in the second place of cancer mortality worldwide [10, 11]. Its proposed mechanism of action is consistent with the DNA damage induced during chronic inflammation [10]. On the other hand, the macrophage migration inhibitory factor is a potent cytokine produced at high levels by infiltrating macrophages and T lymphocytes in inflammation sites, which overrides the function of p53 by suppressing its transcriptional activity. This chronic diversion in the p53 regulation in infiltrated tissues could increase proliferation, simultaneously promoting a microenvironment which favors a deficient DNA damage repair response and amplifying the potential of oncogenic mutations [16].

Malignancies can also be caused by viral infections. That is the case of the Rous' sarcoma virus infection, which promotes an inflammation essential for the development of the tumor. The inflammation is mediated by factors such as the TGF-β and other inflammatory cytokines [17]. In the case of the Epstein-Barr virus infection, the sustained proliferation of B lymphocytes, which coexists with a secondary mutation, can progress to neoplasia and malignant conversion, resulting in Burkitt's lymphoma [6].

Nonetheless, cancer patients present deficiencies in their inflammatory response, and some of the emergent therapies are directed to stimulate the host immune system, favoring a cytotoxic T helper lymphocyte-like response (Th1) and the production of their pro-inflammatory cytokines [18]. This apparent paradox could be surpassed when trying to manage cancer as a whole, keeping in mind that therapy should act locally. The microenvironment of the tumor (*e.g.*, immune inflammatory cells, tumor and stromal cells and the extracellular matrix) is the main "battlefield" during neoplasia and supports proliferation, survival and

Table 1. Inflammatory chronic conditions associated to neoplasias

Pathological condition	Associates neoplasia(s)	Etiological agent
Asbesto-silicosis	Mesothelioma, lung carcinoma	Asbestos fibers, silica particles
Bronchitis	Lung carcinoma	Silica particles, asbestos, cigarettes (nitrosamines, peroxides)
Cystitis, bladder inflammation	Bladder carcinoma	Chronic microbial residents, urinary catheters
Gingivitis, lichen planus	Squamous cells oral carcinoma	Lichen planus
Inflammatory bowel disease, Crohn´s disease, chronic ulcerative colitis	Colorectal carcinoma	*Helicobacter pylori*
Lichen sclerosus	Squamous cell vulvar carcinoma	Sclerotic and atrophic lichen
Chronic and hereditary pancreatitis	Pancreas carcinoma	Alcoholism, mutation in chromosome 7 in the trypsinogen gene.
Esophageal reflux, Barrett's metaplasia	Esophagus carcinoma	Unknown
Sialadenitis	Salivar gland carcinoma	Unknown
Sjögren syndrome, Hashimoto's thyroiditis	Malt lymphoma	Autoimmune
Skin inflammation	Melanoma	Ultraviolet light
Cancer associated to infectious agents		
Opistorchis, Cholangitis	Cholangiosarcoma, cholangiocarcinoma	Intestinal worms (*Opistorchis viverrini*), bile acids
Chronic cholecystitis	Bile duct carcinoma	Bacteria, bilestones
Gastric ulcer/ gastritis	Gastric and GALT carcinoma	*Helicobacter pylori*
Hepatitis	Hepatocellular carcinoma	Hepatis B and C viruses
Mononucleosis	Non-Hodgkin B lymphocyte lymphoma, Burkitt's lymphoma	Epstein-Barr virus
Acquired immunodeficiency syndrome	Non-Hodgkin lymphoma, Squamous cell carcinoma, Kaposi sarcoma	Human immunodeficiency virus, Human herpes virus 8
Osteomyelitis	Skin carcinoma in draining sinuses	Bacterial infection
Pelvic inflammatory disease	Ovary carcinoma, cervical/anal carcinoma	*Neisseria gonorrhoeae*, *Chlamydia*, human papillomavirus
Chronic cystitis	Bladder, liver and rectal carcinoma	Schistosomiasis

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migration of tumor cells. But the tumor not only proliferates and disseminates. Tumor cells also mimic some signaling pathways of the immune system, propagating those conditions which favor immune tolerance to the tumor and evasion of immune surveillance. Events occurring in the microenvironment of the tumor will influence the global immune response of the host. Therefore, the challenge of future therapies will be to attack the tumor in a concerted way to: a) suppress pro-tumoral factors of infiltrating cells, b) supply the patient with immune capacities to revert the tolerance to the tumor and c) suppress angiogenesis and tumor progression [19].

In summary, when the initiating cause (either an infectious agent or any other damage inducer) of the cellular transformation is not properly eliminated and persist in the tissue, they stimulate a perdurable inflammatory response that exacerbates a chronic damage of the tissue and the malfunctioning of the affected organ. Indeed, neoplasias require cancer cells becoming autonomous (*i.e.*, secreting their own growth factors) and extrinsic (controlling the immune response) to progress and develop. Only when they control the immune system, they can progress and kill the host. Chronic inflammation can induce immune suppression, generating an unresponsive immune state against the transformed cell and promoting uncontrolled cellular growth, ultimately leading to tumor growth and development. Therefore, chronic inflammation operates at different levels to guarantee tumor development.

We recommend the readers to consult other publications for further information about these topics [19, 20], beyond the scope of this article.

Table 2. Main mammalian heat-shock proteins and their functions [24]

Main protein families and members	Cellular location	Cellular function
αβ–crystalin	Cytoplasm	Cytoskeleton stabilization
HSP27	Cytoplasm/nucleus	Actin dynamics
Hemoxygenase, HSP32	Cytoplasm	Hemocatabolism, anti-oxidant properties
HSP60 or chaperonines		
HSP60	Mitochondria	Binding to partially folded peptides for proper folding and assembly of multimeric complexes
TCP-1	Cytoplasm	
HSP70		
HSP70 (inducible)	Cytoplasm/nucleus	All of then bind to extended polypeptides, prevent the aggregation of unfolded peptides, dissociate some oligomers, bind ATP and bear ATPase activity. HSP70 is also involved in the regulation of HSF-1 and repression of HSP genes
HSC70 constitutive (cognate)	Cytoplasm/peroxisome	
Grp78/BiP	Endoplasmic reticulum	
mtHSP70/ Grp75	Mitochondria	
HSP90		
HSP90 (α and β)	Cytoplasm	Bind to and regulate other proteins, prevent peptide aggregation and re-folding, promote the proper assembly and folding of *de novo* synthesized proteins.
Grp94/gp96/HSP100	Endoplasmic reticulum	HSP90 appears to be involved in maintaining the monomeric form of HSF-1 at non-stress conditions; represents 1-2% of total proteins
HSP110		
HSP110 (human)	Nucleus / cytoplasm	Thermal tolerance
Apg-1 (mouse)	Cytoplasm	Protein refolding
HSP105	Cytoplasm	
Other		
HSP40/HDJ2	Cytoplasm	Prevents folding, forming complexes with HSP70
HSP27	Cytoplasm	Prevent protein aggregation, participate in cellular growth and differentiation

Inflammation and heat-shock proteins

In 1962, Ferrucci Ritossa discovered that *Drosophila melanogaster* larva subjected to high temperatures expressed a specific set of genes [21]. However, it was not until 1974 that the first products of these genes were identified and named as heat shock proteins [22]. Further experiments showed that the name was not completely appropriate, because these proteins could also be induced by other stimuli, such as growth factors, viral infections, oxidative stress, inflammation and infections [23]. HSPs are classified in different families and named according to their molecular weight (Table 2) [24], appearing in all the species and considered among the most phylogenetically-conserved proteins. In spite of this diversity, they share chaperone activity, contributing to cell survival under stress conditions and facilitating the proper assembly and folding of denatured proteins [25].

HSPs are immunodominant molecules, a significant element of the immune response against pathogens being directed to HSPs-derived peptides. This phenomenon, together with the ubiquitous nature of the human chaperones and the high grade of sequence homology among mammalian and bacterial HSPs (~50-60% of identical residues), has led to the debate on how HSPs are recognized either as dominant microbial antigens or as harmful autoantigens [25]. Some authors have suggested that HSPs would provide a link between infection and autoimmunity [26], both by cross-reactivity/mimicry or through conserved epitopes. There are evidences pointing to a link between HSPs activity and the pathogenesis of diseases such as autoimmunity, vascular diseases and organ allograft rejection. The direct link between host chaperones and bacteria during infection is extensively reviewed by Henderson *et al.* [27].

The role of HSPs in inflammation was evidenced by Srivastava *et al.* [28], who demonstrated that these proteins are endogenous adjuvants that can be used to induce a strong immune response, specific for tumors or pathogens. Additionally, Cohen and Quintana [29] referred the production of HSP60 and HSP70 proteins in a eukaryotic cell-free system and devoid of bacterial contaminants, demonstrating that these pure HSPs were able to activate primary macrophages.

Several experiments have related the effects of HSPs on macrophages and dendritic cells [30, 31], T lymphocytes [32-35], B lymphocytes [36, 37] and NK cells, to non-clonal receptors, such as the Toll-like receptors (TLRs) [6].

Based on the functions of HSPs, alone or as part of molecular complexes, we can consider some of their physiological effects on the regulation of the immune and inflammatory responses (Figure 2). As can be observed, inflammation is intertwined in processes as diverse as angiogenesis, wound healing, tissue regeneration and the recovery of central nervous system functions following trauma. They also maintain a fine control on invading pathogens. Therefore, inflammation provides both support and defense

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mechanisms. In this sense, it can be classified in sterile and septic inflammation; the former relates to organism homeostasis and results from endogenous stimuli, and the latter is triggered by and activated against an invading pathogen [6].

The autologous HSPs play an important role in both types of inflammations, activating and controlling the immune response.

In septic inflammation, microbial molecules form complexes with microbial or host HSPs, facilitating the induction of an effective immune response. Conversely, microbial products could interfere with sterile inflammatory mechanisms needed for the homeostasis of the organism, stimulating the development of inflammatory diseases. HSPs promote the production of pro-inflammatory cytokines, being involved in antigen recognition and processing and the activation of antigen presenting cells (APCs). Nevertheless, their mechanisms of action remain to be completely elucidated. Additionally, it has been observed that the HSP70 can bind viruses, bacteria and tumor peptides, making them more immunogenic [38], while the HSP60 can stimulate macrophages and dendritic cells. Both HSPs induce the release of inflammation mediators such as TNF-α, interleukin 6 (IL6), and nitric oxide production [39]. Moreover, they induce IL2 and IL15 expression, cytokines related to the Th1 pattern and directly involved in the 'danger' signal for the activation of the innate immune system [35]. Several regulatory mechanisms have been settled during the evolution, due to the deleterious effect for the organism of the uncontrolled inflammatory response. The immune system has to determine the exact location, kinetics and intensity of the response for an adequate biological function. Therefore, HSPs involved in both types of inflammation are key components for a balanced inflammatory response of the host.

The role of hsps in infection

During infection, the endogenous HSPs interact with a myriad of microbial peptides, increasing their immunogenicity. Concertedly, the host recognizes its own chaperones from those bacterial ones. This new paradigm offers several possibilities for molecular chaperones to mediate many still unrecognized biological functions. Currently, the most relevant discovery so far has been the inclusion of two chaperones of the host, HSP70 and HSP90, as part of the main protein complex recognizing the lipopolysaccharide (LPS) which belongs to the family of pattern recognition receptors (PRRs). It has been reported that the intracellular HSP90 is involved in signal transduction through TLR9, which is activated by unmethylated CpG motifs present in the DNA of certain pathogens. This finding is supported by experiments demonstrating that geldanamicin, a specific inhibitor of the HSP90, blocks the activation of the transcription factor NF-kB, also involved in intracellular signaling pathways mediated by TLRs [40].

Besides, it has been established the relationship of HSPs with the amplification of the immune response to LPS. An study carried out by Tantafilou *et al.*, showed that the LPS receptor is formed by a protein complex including HSP70 and HSP90, combine with

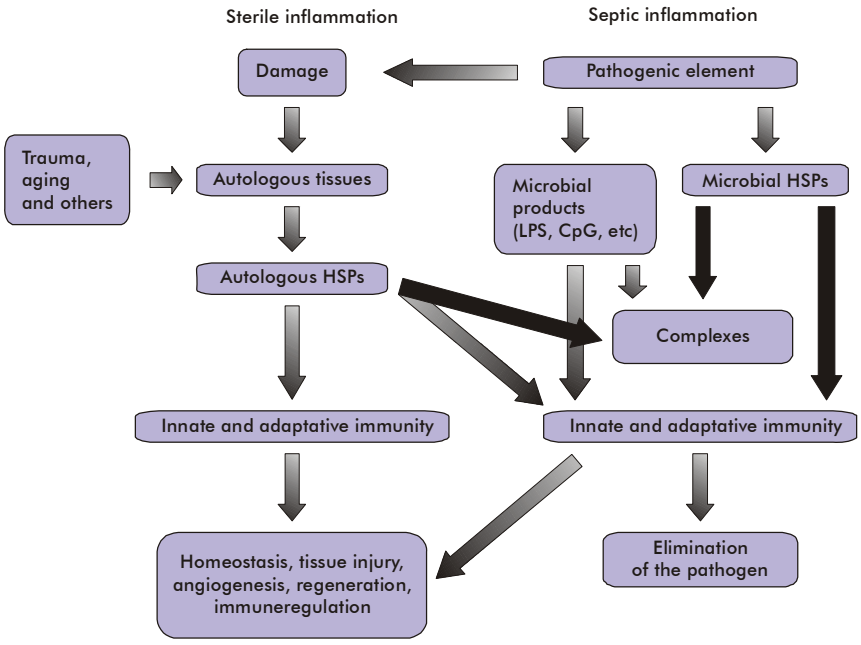

Figure 2. Sterile and septic inflammation.

the MD2 co-receptor and the TLR4 [41]. Following the attachment of LPS to its receptor, HSP70/HSP90 associated to the TLR4 move to the Golgi apparatus, suggesting that these HSPs are involved both in the transference of LPS to the TLR4-MD2 complex and in the transduction pathway for the LPS-activated signal [42].

A recent investigation by Poulaki *et al.* [43] showed that an inhibitor of the HSP90, the 17-(allylamino)-17-demethoxygeldanamycin (17-AAG), bears anti-inflammatory activity in a model of endotoxin-induced uveitis in rats. After the induction of uveitis by administering LPS, rats received a single dose of 17-AAG by intraperitoneal route. After 20 h, different inflammatory response markers were analyzed in the retina of the animals. The treatment with 17-AAG significantly suppressed the inflammatory response induced by LPS, as evidenced by decreased concentrations of TNF-α, IL1b, vascular endothelial growth factor, decreased activity of the transcription factor NF-kB and low leukocyte adhesion [43]. These results suggest that the inhibition of the HSP90 suppresses some cardinal effects of the inflammatory response activated by LPS.

Other results have established HSPs as positive regulators of inflammation during infection, perpetuating it. During infections, HSPs are over-expressed and release, binding to microbial products and increasing their pro-inflammatory activity, subsequently releasing more HSPs, all these reinforcing the inflammation pattern. This positive feedback can indeed generate chaos. Current evidences relate HSPs to septicemia, a process induced by an uncontrolled inflammatory response during infection. It has been recently found high extracellular levels of HSP60 and HSP70 in children suffering from septic shock [44, 45]. Additionally, the inhibition of HSP90 in a murine model of sepsis prolonged animal survival, attenuated

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the inflammation and decreased tissue damage in lungs [46].

HSPs can be potent activators of the innate immune response during infection, as previously mentioned. It was also evidenced by the induction of pro-inflammatory cytokine production by HSP60, HSP70 and HSP90 in the monocyte/macrophage system, and by the activation and maturation of APCs similar to LPS and bacterial lipoproteins effects, mediated by TLRs and signal transduction. Therefore, future studies should unravel the mechanisms used by HSPs to exert these functions and the particular role of each HSP in the production of specific cytokine subsets.

*H*SPs and cancer biology

It is well known that chaperones are critical for an appropriate assembly and folding of intracellular proteins, highly expressed in injured tissues to guarantee the survival of cells exposed to a variety of agents, such as heat, hypoxia and acidosis. Interestingly, these two last conditions are common in tumors, the increase in HSPs possibly reflecting the ability of malignant cells to maintain homeostasis in an unfavorable environment [47-52]. Moreover, HSPs make cells resistant to intracellular alterations such as mutations in molecules involved in critical signal transduction pathways, which could be lethal if not compensated by HSPs.

The increased expression of one or more HSPs at levels significantly higher than those commonly observed in normal tissues seems to be one of the most common findings in human tumors, either solid [47-52] or hematopoietic [53, 54]. In breast cancer, the over-expression of HSP70 and HSP90 correlates with a bad prognosis of the disease [55, 56]. It has been observed that HSPs protect tumor cells from the action of oxygen peroxide (H_2O_2) and oxyradicals generated by anti-cancer drugs. Another interesting phenomenon of wide therapeutic implications is the resistance of neoplasic HSP27-over-expressing cells to anti-neoplasic drugs such as doxorubicin, colchicin and vincristin. The increase in either HSP27 or HSP70 not only contributes to drug resistance, but also induces a poor response to combined therapeutic regimes [57, 58].

The rise in HSPs expression within physiological levels in advanced cancer could simply reflect an adaptation of the cancer cells to stress. These cells have to survive in a hostile microenvironment of hypoxia, acidosis and nutrients starvation. At rather basal levels, HSPs activity could provide cancer cells with apoptosis evasion mechanisms activated by neoplasia-associated alterations in signal transduction pathways [5, 59]. Nevertheless, deterioration of the apoptosis signaling pathways is common in cancer cells. It facilitates their survival and expansion, making tumor cells independent of normal regulation factors and resistant to host defense mechanisms and chemothe-rapeutic drugs. HSP70 and the co-chaperone proteins associated to the *bcl-2* gene are known as anti-apopto-tic factors. Recent studies showed the HSP70 blocks the assembly of a multiprotein complex denominated apoptosome [60, 61]. This complex is essential to activate protease cascades responsible for the apoptotic program. Consistent with this discovery, depletion of HSP70 in different breast cell lines caused massive death only in tumor cell lines, the non-tumorigenic epithelial cell lines remaining unaffected [62]. Recently, Aghdassi *et al*. [63] reported that the expression of the HSP70 increases in cancer *versus* normal pancreas cells, the depletion of HSP70 by the flavonoid quercetin inducing apoptosis specifically in malignant cells. Moreover, a small interference RNA specific for the HSP70 produced similar results, showing the essential role of this chaperone in the resistance to apoptosis in pancreas tumor cells [62].

Although the results mentioned suggest that the anti-apoptotic function of the HSP70 could provide a useful target for cancer therapeutics, there are no publications on the use of small molecules targeting this chaperone in the clinical field.

Other investigations showed that the HSP90 and their co-chaperones also modulate tumor apoptosis, mainly through their effects on the AKT kinase [64], TNF-α receptors [65] and the NF-kB transcriptional factor [66].

However, the role of HSP70 in facilitating the neoplasic transformation of the cell goes beyond inhibiting apoptosis; this chaperone is unique in perpetuating the malignant transformation state, being required to maintain several oncoproteins in a functionally active conformation. In this context, HSP90 regulates signaling pathways needed for growth, survival and unlimited replicating potential of the tumor [67, 68]. The HSP cellular substrates, or "client" proteins, relevant for cancer includes: the family of SRC kinases (SRC, LCK and FYN), tyrosine-kinase receptors (HER2, EGFR, IGF1R and FLT3), serine/threonine kinases (RAF-1, AKT and CDK4), hormone receptors (testosterone, estrogen and progesterone receptors), transcription factors (p53, HSF-1 and HIF-1) and telomerase (hTERT). Nevertheless, the number of proteins interacting with HSP90 expands rapidly. For a better and updated knowledge on this matter we recommend to visit the webpage of Dr. Picard´s laboratory (http://www.picard.ch/downloads).

In normal cells, the HSP90 interacts in a low affinity dynamic with a myriad of protein, helping them to improve their folding and functionality. But in malignant cells, it establishes a tight association with client oncoproteins, supporting their aberrant state and function, essential for malignant transformation. Current research is focused on the impact of HSP90 inhibitors on those aberrant complexes. Although small molecules are therapeutically attractive and have the unique property of simultaneously inhibiting the interaction of HSP90 with multiple oncogenic client proteins , it is very difficult to predict which patients can be actually benefitted by using these drugs, based on the particular combination of genetic and molecular defects present in a given tumor [69, 70]. In this sense, it is well known that HSP90 regulates the malignant phenotype in a tumor cell line-specific pattern. It has been recently reported that HSP90 is the main regulator of the intrinsic apoptosis pathway in small cell lung cancer (SCLC) cell lines [71]. Inhibitors specific for the function of this chaperone promote tumor cell apoptosis by a mechanism involving the regulation of Apaf-1 and controlling the phosphoinositide-3-kinase/AKT survival pathway, activating caspases 3 and 9. These results demonstrate that HSP90 can suppress apoptotic elements, the magnitude and final results of

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their inhibition being dictated by the malignant transformation. In agreement with these results, it has been shown that the main mechanism of malignant transformation in human colon cancer cell lines involves the interaction of HSP90 with IkappaB kinases (IKKs), including IKK-β, IKK-γ, and the sustained activation of the NF-kB transcriptional factor [72].

At this moment, it is evident the main role of HSPs in immune regulation as key component for the appropriate control of the inflammatory response. The strategic use of HSPs to improve therapies for cancer, infection and inflammation will depend on the capacity to fully understand their function in nature.

Inhibitors of HSP90 as cancer immunotherapeutics

For those interested in molecular therapy against cancer, we present arguments about major principles of chaperones' biology, with their correspondent immediate implications for future discoveries and development of therapeutic drugs:

- In spite of their name, heat-shock proteins, most chaperones are produced at considerable levels under basal conditions. The expression of HSP90 even under normal conditions reach 1-2% of total cellular proteins.

Therefore, chaperones are ubiquitously expressed proteins in both normal and tumor cells. A probable therapeutic intervention would rely on the altered functions of their client proteins, stabilized by chaperones, and not simply due to their expression or absolute levels [73].

- Complementation studies have shown that most of the chaperones' functions are highly conserved through long phylogenetic distances.

Works on model organisms (*i.e.*, yeasts, plants and fruit fly) provide valuable hints on the function of chaperones, useful to understand human cancer biology [73].

- Chaperones rarely function alone, working as part of large multiprotein complexes containing co-chaperones and accessory proteins.

The use of drugs affecting the recruitment of specific accessory molecules can modulate the action of chaperones in those multiprotein complexes without halting their global functions [73].

- The use of small molecules to block the ATP binding site could provide a way to block the therapeutically inaccessible protein-protein interactions, because they occur in multiple contact points and very distant to each other [73].

- Chaperones participate in several first-order functions for the cellular development, such as post-transcriptional regulation of cellular signaling molecules, assembly/disassembly of the transcriptional complex and the processing of immunogenic peptides by the immune system.

The potential simultaneous blocking of several oncogenic client proteins is a unique and therapeutically attractive property of HSPs inhibitors. At the same time, the possible pleiotropic effects derived of using chaperones as therapeutic targets, could difficult the identification of specific markers used as predictive markers of the anticancer activity in patients [73].

Although the function of HSP90 provides an attractive target for cancer therapy, drugs inhibiting its activity have reach only in recent years the clinical phase [73]. The most frequent complaint to the use of HSP90 as a target for cancer therapy is based on the observation that drugs affecting an essential chaperone would generate deleterious effects on its cellular functions. The phase I clinical trial carried out with the 17-AAG HSP90 inhibitor revealed that the drug could be administered to patients with tolerable toxicity, and alterations in the levels of client proteins of this chaperone were detected after the therapeutic regimen [74]. These promising results indicate that the use of drugs to inhibit the ATPase activity in the amine region of the HSP90 is not functionally equivalent to the constitutive genetic knockout that is lethal in eukaryotes.

The clinical evaluation of 17-AAG promoted depletion of HSP90 client proteins such as CDK4, C-RAS and MEK-ERK1/2 as evidenced in biopsies of different solid tumors [74]. Noteworthy, these proteins contribute to the distinctive properties of cancer (Figure 3). The inhibition of HSP90 caused degradation of client proteins through the ubiquitin-proteosome pathway, simultaneously depleting several oncoproteins and providing redundant negative regulation of multiple oncogenic signaling pathways that ultimately lead to tumor development and perpetuate the malignant phenotype. The most recent result on the evaluation of 17-AAG was presented at the Gastrointestinal Cancer Symposium of the American Society of Clinical Oncology (ASCO) Congress, held in Orlando, Florida in January of 2007. An analog of the 17-AAG, named IPI-504, was evaluated in a phase I clinical trial with scaled dosage in patients with stromal gastrointestinal tumor (GIST) metastasis and tumor resistant to Gleevec.

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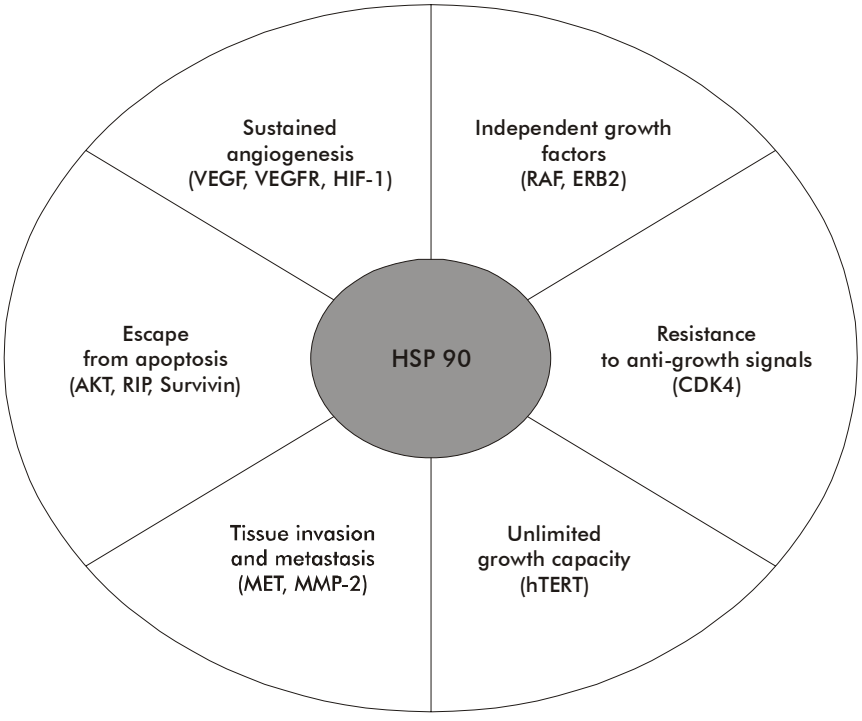

Figure 3. Contribution of HSP90 to cancer evolution processes.

Preliminary data showed that IPI-504 was tolerated and safe, representing a new therapeutic strategy for patients suffering from GIST and tumors resistant to small tyrosine kinase inhibitors [75].

These results broaden the therapeutic potential of HSP90 inhibitors and extend them to different types of solid and hematological tumors, including those resistant to other drugs.

Conclusions

In spite of advances in modern medicine, cancer and infections remain as a challenge for science, considering that cancer is the second cause of death worldwide and that septic shock continues to contribute 50% of deaths in developed countries. It has been evidenced the tight relationship between inflammation and these conditions. Infections can trigger proliferation of anomalous cells and inflammation providing the adequate microenvironment for tumor cell develop-ment and in this complex scenario several heat-shock proteins are involved, undoubtedly related to the previously-mentioned pathological situations. There is an open field of basic, applied and clinical research waiting for unraveling the molecular mechanisms mediating the induction and regulation of HSPs. Additionally, the knowledge on their biology and functions over the immune system, and their implications to the induction and progression of cancer remain to be elucidated. In the upcoming years, studies on the relationship inflammation-HSPs-cancer will be studied due to the relevance of HSPs as therapeutic agents.

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