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Visions & Reflections

Is chlamydial heat shock protein 60 a risk factor for oncogenesis?

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Abstract. Heat shock protein 60 (HSP60) plays an important role in the protein folding of prokaryotic and eukaryotic cells. Most of the papers published on chlamydial HSP60 concern its role in immune response during infection. In the last decade, exposure to *Chlamydia trachomatis* has been consistently associated with the development of cervical and ovarian cancer. Moreover, it has been suggested that chlamydial HSP60 may have an anti-apoptotic effect during persistent infection. We hypothesize that the accumulation of exogenous chlamydial HSP60 in the cytoplasm of actively replicating eukaryotic cells may interfere with the regulation of the apoptotic pathway. The concomitant expression of viral oncoproteins and/or the presence of mutations may lead to the ability to survive apoptotic stimuli, loss of replicative senescence, uncontrolled proliferation and, finally neoplastic transformation.

Key words. Carcinogenesis; cancer biology; *Chlamydia trachomatis*; cancer risk factors.

Heat shock proteins (HSPs) protect cells against different forms of stress, such as hypoxia, ischemia and hyperoxia; in particular, they have been formerly identified as cellular proteins involved in a sudden increase in temperature. The four best-known HSPs are HSP 27, 60, 70 and 90. They are chaperones capable of stabilizing and transporting proteins within the cell and its compartments. They are found in all living cells, from microorganisms to vertebrates, and in different cell compartments [1, 2].

Apart from their role inside the cell, bacterial HSPs also seem to be risk factors in the pathogenesis of asthma, autoimmunity, inflammatory response and cancer. Because of their similarity among different organisms, they may function as both antigens and analogues of the native HSPs [3].

In the last few years, we have evaluated the presence and the expression of HSP60 and HSP10 in a variety of human carcinogenic models, i.e. the 'dysplasia-carcinoma' sequences of uterine exocervix, colorectal mucosa and prostate gland. Our results have suggested that immunohistochemical and biomolecular studies of these proteins in tumoral tissues may represent modern diagnostic and prognostic tools [4]. Indeed, our data demonstrated that these proteins are overexpressed in the pre-neoplastic stages, as well as in the invasive ones; in particular, all these lesions showed an intracytoplasmic accumulation of these molecules, and their expression gradually increased from dysplastic to neoplastic tissues [5]. Finally, in invasive cancers, we also found these molecules in interstitial 'peritumoral' spaces. We hypothesize a role for these HSPs during carcinogenesis [6, 7]; however, the exact nature of this role is far from being understood. Indeed, because of the overexpression of HSP60 and HSP10 in pre-neoplastic lesions, we could postulate that these chaperones have a different role during carcinogenesis.

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In this paper, we propose that interactions between HSP60 and other cellular proteins, outside mitochondria, lead to development of neoplasms.

HSP60 and HSP10 as molecular chaperones

Studies on HSP60 and HSP10 bacterial analogues – GROEL and GROES, respectively – have enabled a better understanding of the complex structure and function of both these molecules. Bacterial HSP60 and HSP10 are localized in the cytoplasm of prokaryotic cells, and they are involved in protein folding as well in eukaryotic cells. HSP60, together with HSP10, forms a 'double-doughnut' structure inside the mitochondria of eukaryotic cells, where it assists mitochondrial protein folding. Both HSP60 and HSP10 can accommodate proteins with a molecular weight of up to 50 kDa; their conformation prevents interactions between the folding proteins and other surrounding molecules [8].

Preferential substrates for HSP60 are intermediates that have not yet acquired their definitive shape. These proteins bind to hydrophobic amino acid residues that are localized inside the opening of the macromolecule. Binding of an ATP molecule to a single subunit determines conformational changes and reduces the overall hydrophobicity of the cavity surface; as a consequence, bound proteins detach from the HSP60/HSP10 complex and undergo new folding attempts [8].

Other molecular roles of HSP60 protein

New functions of mammalian HSP60 have recently been discovered. In particular, Samali et al. [9] have demonstrated that human HSP60 may have a role in triggering apoptosis through caspase cascade activation. They demonstrated an association between HSP60/HSP10 complex and pro-caspase-3 inside mitochondria and subsequent release of the same HSP60 in the cytoplasm, upon stimulation with a pro-apoptotic agent. They also demonstrated, in vitro, that it is possible to induce caspase-3 activation by overexpression of recombinant HSP60 and HSP10.

Kirchhoff et al. [10] have shown that cellular HSP60 may have an anti-apoptotic effect mediated by its ability to form a complex with Bax and Bak in the cytoplasm of normal cardiomyocytes in culture, blocking their ability to induce apoptosis in vitro. In particular, as cytosolic HSP60 decreases, the unbound fraction of Bax decreases, and the amount of Bax associated with mitochondria and cell membranes increases. The addition of Bax to mitochondria has been shown to be sufficient to trigger cytochrome c release and subsequent caspase cascade activation.

The same authors also demonstrated that in hypoxic myocytes, cytosolic HSP60 relocates to the plasma membrane. Concomitantly with the translocation of HSP60 to the plasma membrane, Bax moves to mitochondria, and this is accompanied by the release of cytochrome c [11].

In addition, some scientific papers have postulated that HSP60 homologous proteins from several microorganisms, including common human pathogens such as *Chlamydia* spp., may elicit a strong and dominant immune response in their mammalian hosts [12].

Chlamydia spp. produces large quantities of HSP60

Chlamydia spp., an obligate intracellular bacterium, produces large quantities of HSP60, which is reported to be localized in the cytoplasm and in the outer membrane of the host cell during persistent infection [13]. Clinical persistence is a key concept in chlamydial pathogenesis; in fact, in most cases, the immune system is unable to eliminate the pathogen, which lives in an unusual environment within eukaryotic cell vacuoles (called inclusions) [14].

The abundance of chlamydial HSP60 (CHSP60) during chronic persistent infection could promote the activation of CHSP60-specific immune T and B cells, engaging Toll-like receptors (TLRs) [12]. TLRs are transmembrane proteins of eukaryotic cells with extracellular domains that recognize alkaline components such as bacterial lipopolysaccharides, peptidoglycans and lipoproteins [15]. Moreover, Vabulas et al. [16] and Sasu et al. [17] recently demonstrated that endocytosed CHSP60 binds to TLRs, inducing cellular signalling networks and proliferation in human vascular smooth muscle cells.

Outer-membrane CHSP60 cross-reactivity has also been implicated in the pathogenicity of autoimmune diabetes, arthritis, asthma and atherosclerosis [18–21]. In particular, CHSP60 can stimulate tumor necrosis factor-α (TNF-α) secretion by macrophages [22] and can stimulate endothelial cells, smooth muscle cells and macrophages to produce adhesion factors and pro-inflammatory cytokines [interleukin (IL)-11, IL-8, IL-12, IL-6, granulocyte macrophage colony stimulating factor (GM-CSF)], by activation of the nuclear factor NF-kB [23]. They showed also how a purified CHSP60 may activate pathways similar to the responses induced during *Chlamydial* spp. infection.

In the last decade, exposure to *Chlamydia trachomatis* (CT), a species of the family of *Chlamydiaceae*, has been consistently associated with cervical cancer.

Correlations between *Chlamydia trachomatis* and cancer of female genital tract

Uterine cervix carcinoma is still one of the leading causes of death among women, especially in developing countries, where the role of human papilloma virus (HPV) in cervical cancer development has been confirmed by both experimental and epidemiologic evidence [24].

Sexually transmitted infections (STIs) are usually associated with cervical cancer, and CT infection is considered the most common bacterial STI. Although this infection is asymptomatic, it may cause pelvic inflammatory disease, adverse pregnancy outcome and infertility in women. CT is also a common cause of urethritis, cervicitis, epididymitis, proctitis and reactive arthritis.

To date, only two epidemiologic studies on the correlations between CT infection and female genital cancer have been published. The first is a population-based prospective study on CT infection and cervical carcinoma. In this study, Wallin et al. [25] conducted a population-based invitational cervical cancer-screening program in northern Sweden, where 118 cases were identified with invasive cervical cancer. CT DNA was present in 10 (8%) of the 118 cases that subsequently developed an invasive cervical cancer, but it was present in none of the controls. Most of them developed cancer from 3 to 6 years after CT infection. Nonetheless, the concomitant presence of HPV DNA seemed virtually required for cancer development.

The other epidemiologic study on CT infections was based on a serologic evidence of past infection in relation to ovarian cancer. In this study, Ness et al. [26] measured the levels of antibodies against the three isoforms CHSP60-1, CHSP60-2 and CHSP60-3 in 117 women with ovarian cancer and in 171 age- and ethnicity-matched population-based control subjects from Hawaii. They found that women with ovarian cancer had higher levels of antibodies only against CHSP60-1. These observations suggested that chronic persistent CT infection and the presence of anti-CHSP60-1 antibodies may be risk factors for cancer.

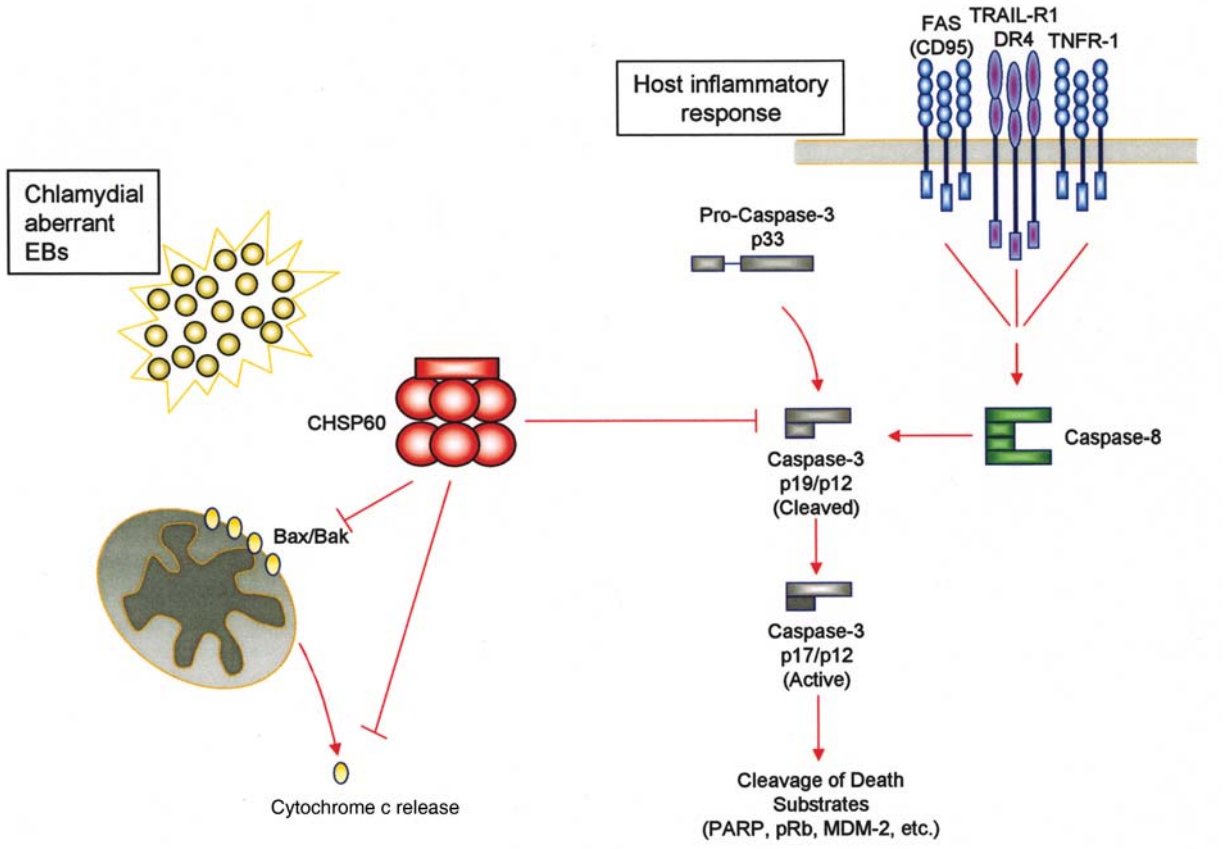

Figure 1. During persistent infection chlamydial HSP60 may interfere with apoptosis induced by host inflammatory response. During *Chlamydia* spp. persistent infection, aberrant elementary bodies (EBs; light green) produce large quantities of CHSP60 (red), which localizes in the cytoplasm and in the outer membrane of the infected cell. The presence of the bacterium induces a strong B and T cell-mediated immune system response. Apoptosis induced by activated lymphocytes is initiated by stimulation of the receptors Fas, TNFR-1 or TRAIL-R1 (blue). Their ligands are in the form of trimers, and when they bind, they induce trimerization of the receptors themselves. These in turn interact through adaptor proteins with caspase-8 (dark green) leading to caspase cascade activation with cleavage of death substrates. To elude the immune system, cytoplasmic CHSP60 (red) may interact with cleaved caspase-3 (gray) in the cytoplasm, blocking caspase cascade activation. Furthermore, CHSP60 may bind to Bax or Bak, inhibiting cytochrome c (yellow) release from mitochondria.

CHSP60 may have an anti-apoptotic effect

Since CHSP60 has an amino acidic sequence similar to its human counterpart, with the exclusion of the mitochondrial localization signal, it may be possible to correlate some cellular responses during *Chlamydia* spp. infections with the accumulation of CHSP60 in the cytoplasm of the host cell and its possible association with cleaved caspase-3 and/or Bax-family proteins, considering this chaperone as the only mediator of bacterial pathogenesis. During chronic persistent infection, aberrant chlamydial forms produce large quantities of CHSP60 which can be transported to the cytosol of the host cell through a type III secretory apparatus, which has been reported to mediate translocation of chlamydial proteins from the elementary bodies to the host cell cytoplasm [27–30]. CHSP60 could form a complex with cleaved caspase-3 and/or Bax and Bak, inhibiting apoptosis before cytochrome c release and caspase-3 activation (fig. 1).

Consistent with our hypothesis, several papers have been published on the pro-apoptotic or anti-apoptotic role of *Chlamydia* spp. infections. In fact, we have to distinguish between intracellular changes, which lead to an anti-apoptotic effect [31], and immune system responses to *Chlamydia* spp. infection, which naturally lead to immune system-mediated cell death, during host inflammatory response [32, 33].

CT infection has an anti-apoptotic effect in vitro, *Chlamydia*-infected host cells are cross-resistant to apoptosis induced by a wide spectrum of pro-apoptotic stimuli, including the kinase inhibitor staurosporine. Moreover, during chlamydial infection, activation of caspase-3, poly (ADP-ribose) polymerase (PARP) hydrolysis and release of mitochondrial cytochrome c are inhibited [31]. In addition, there is a difference between active and persistent infections. Only during the latter, i.e. infection maintained up to 120 h, cells in culture resist pro-apoptotic stimuli and expression of CHSP60 increases [30].

Furthermore, *Chlamydia pneumoniae* infection protects host cells against cytochrome c release-induced apoptosis, during which it has been reported that the blockade of apoptosis lies upstream of caspase cascade activation, when both cytochrome c release and cytochrome c-dependent caspase activation are inhibited. It also seems that early bacterial protein synthesis is required for this protection [34].

Confirmation of our hypothesis that CHSP60, a bacterial anti-apoptotic protein synthesized during persistent infection may be one of the pathogenic mechanisms by which *Chlamydia* spp. can mediate the development of chronic diseases comes from the evidence that a heat-labile component is released during infection [35, 36].

All these considerations suggest that the heat-labile component released during CT infection, interacting with the caspase-dependent apoptotic machinery, may be CHSP60-1.

Concluding remarks

Complex organisms have evolved at least two cellular mechanisms to suppress the proliferation of cells at risk for oncogenic transformation: apoptosis and cellular senescence.

Cellular senescence is considered an important mechanism to irreversibly arrest the growth of cells at risk for tumorigenesis; mutations that disrupt the senescence response in human generally lead to increased cancer incidence [37]. Cellular senescence and apoptosis have a similar function: whereas apoptosis kills and eliminates potential neoplastic cells, cellular senescence irreversibly arrests their replication. Furthermore, many proteins involved in apoptotic pathways, such as p53 and p21, are common to the pathway of replicative senescence [37].

Our hypothesis is that *Chlamydia* spp. infection may disrupt both apoptotic and cellular senescence pathways, leading to cancer when cells are simultaneously infected by oncoviruses or are damaged by carcinogenic agents (fig. 2). To elucidate the functions of CHSP60-1 during

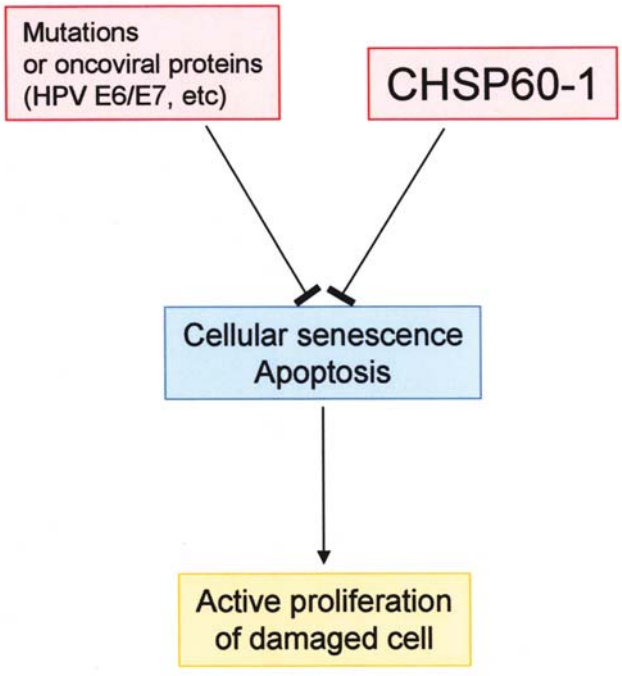

Figure 2. Hypothesis of neoplastic transformation during *Chlamydia* spp. infection. Mutations or oncoviral proteins and the production of CHSP60-1 may cooperate to inhibit cellular senescence or apoptosis; the inhibition of these two processes may induce active proliferation of damaged cells.

infections it may be useful to clone the CHSP-60-1 gene and transfect senescent human cells with a mammalian expression vector bearing the CHSP60-1 gene, either with or without the concomitant expression of the HPV E7 gene. It would be interesting to observe pathophysiological changes, replicative senescence regression and apoptosis inhibition before and after transfection with both oncogenic proteins.

Such an in vitro system could easily help to achieve a better understanding of the mechanisms responsible for the high probability of developing cervical or ovarian cancer in women who suffer CT infection.

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