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Heat shock proteins and drug resistance

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Summary

Heat shock proteins (hsp's) are induced in cells when exposed to different environmental stressful conditions. We have found that breast cancer cells sometimes express high levels of several hsp's, which may both augment the aggressiveness of these tumors and make them more resistant to treatment. We have shown that hsp70 is an ominous prognostic sign as detected by Western blot assays in node-negative breast tumors, and that hsp27 increases specific resistance to doxorubicin in breast cancer cell lines. These findings have direct clinical application, and suggest that modulating hsp expression may be a therapeutic target for reversal of hsp-associated detrimental cellular effects.

Introduction

Heat shock proteins (hsp's) are a family of proteins whose function is to protect cells from toxic external stimuli. Breast cancer cells express high levels of several hsp's that may serve to augment tumor aggressiveness. We have been exploring the relationship of hsp's with drug resistance and cell proliferation in breast cancer cells and tumors, so that clinical strategies to monitor and circumvent drug resistance can be devised to improve patient survival.

We have been studying one of the hsp's, hsp27, for several years. We originally described [1] and purified [2] an estrogen-induced protein which we called "24K" from MCF-7 human breast cancer cells. We later developed monoclonal antibodies to it and, using these reagents, cloned and sequenced its cDNA from a library prepared from estrogen-stimulated MCF-7 cells. This showed that "24K" was identical to hsp27 [3]. We then determined that hsp27 mRNA levels were controlled by both heat shock and estrogen in estrogen-responsive breast cancer cells [3,4]. It now appears that many investigators have been studying this small molecular weight hsp without knowing that it was in fact hsp27. It has independently been isolated as a "25 kDa protein" associated with actin polymerization [5], a "29 kDa protein" phosphorylated in thrombin-activated platelets [6], the "p29 ER-associated protein" [7], and the "25 kDa growth-related

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protein" in Ehrlich ascites tumors [8]. These multiple identifications of hsp27 from many different model systems are perhaps reflective of the many functions which hsp27 may be performing in cells.

Biological and prognostic implications of hsp expression in breast cancer

Hsp27 is very low in normal human breast tissue, and is variably expressed in benign breast lesions [9,10]. In contrast, hsp27 is found at high levels in human breast tumor cells; we [11] and others [12] have shown that elevated hsp27 expression correlates with the presence of estrogen and progesterone receptors, which are known predictors of disease-free survival and response to endocrine therapy in breast cancer patients. We hypothesized that since elevated hsp's in cancer cells might be an indicator of the biological stress experienced by tumors, hsp expression might predict a tumor's future behavior, i.e. the likelihood of early recurrence. In early preliminary studies, we did find that hsp27 overexpression in breast cancer, as measured by Western blot analysis, appeared to be associated with more aggressive tumors, i.e. with a shorter disease-free interval, in node-negative patients [13,14]. However, these results were in contrast to those reported by Thor et al. [15] who, though finding significant correlations between hsp27 expression and ER content, nodal metastasis, and lymphatic/vascular invasion, did not find that hsp27 was an independent predictor of patient outcome.

But much has been learned from past mistakes, including our own, in prognostic marker studies, and the late Dr. McGuire, who originally began these hsp27 studies and was a strong advocate of proper study design, published a rational approach to prognostic factor studies [16] championing the need for independent validation of apparently promising factors. We therefore embarked upon a larger study in node-negative breast cancer specimens from the San Antonio Breast Tumor Bank, simultaneously measuring hsp27, hsp70, and hsp90 by Western blot analysis. The basic design was to measure the hsp's in two independent, blinded studies. The first, smaller study was used to identify the level of hsp expression which optimally distinguished a significantly different outcome in the patients, and we then applied this cut-point to a larger validation group of patients to confirm that the hsp was indeed a marker of prognosis. Our major finding was that only high hsp70 expression remained an independent predictor of a shorter disease-free survival in node-negative breast cancer patients ([17] and Oesterreich, manuscript in preparation).

The association between hsp's and drug resistance in breast cancer

The selection of specific chemotherapeutic agents for the treatment of patients with breast cancer is unfortunately a process of elimination. Unlike the selection of patients for hormonal therapies, where steroid receptor status is a predictor of endocrine response, there are few tests available, other than in vitro chemosensitivity assays, which can predict the likelihood of response to a particular chemotherapeutic agent.

The most effective cytotoxic agents in breast cancer are doxorubicin (Adriamycin), taxol, cyclophosphamide, methotrexate, and 5-fluorouracil. Each of these agents if given alone can induce regression of metastatic breast cancer [18-22], and these drugs now constitute the core of most combination chemotherapy programs used in breast cancer. However, the effectiveness of all of these regimens is hindered both by intrinsic tumor drug resistance and by the development of drug-resistant tumor subpopulations. Determining the mechanisms involved in clinical drug resistance is critical to overcoming resistance and improving patient survival.

An emerging body of research suggests that the heat-shock/stress-response proteins may be involved in drug resistance. The synergistic

cytotoxic effects of hyperthermia when *combined* with a number of chemotherapeutic agents, such as doxorubicin, cyclophosphamide, and the nitrosoureas, has been appreciated for a long time [23,24]. However, it is now apparent that when hsp's are induced *prior* to drug treatment, by elevated temperatures or by exposure to agents such as arsenite, cadmium, or ethanol, then *resistance* to doxorubicin and actinomycin D may be conferred [24-28]. It is also recognized that either chronic anoxia or 2-deoxy glucose administration, conditions which induce another family of stress response proteins termed the glucose-regulated proteins (grp's), can also lead to doxorubicin resistance [29], again suggesting a relationship between stress proteins and drug resistance.

Direct evidence for specific hsp expression being involved in the drug-resistant phenotype comes from the elegant studies of Huot et al. [30], who transfected human hsp27 into Chinese hamster ovary cells and tested for sensitivity to a variety of cytotoxic agents. Cells overexpressing hsp27 were cross-resistant to doxorubicin, colchicine, and vincristine, but not 5-fluorouracil and the nitrosoureas. This strongly suggests that hsp27 might be involved in some forms of clinical resistance. Interestingly, this resistance appeared to result from mechanisms unrelated to cellular drug transport, unlike the well known P-glycoprotein-mediated multidrug resistance.

Our own data also suggest that hsp27, and possibly hsp70, are involved in resistance to a specific agent, doxorubicin, in breast cancer cells. Our first evidence that hsp's may be involved in clinical resistance came from observations that heat shock treatment of human breast cancer cell lines increased their resistance to doxorubicin killing [31]. However, in contrast to the results of Huot et al. in hamster cells [30], these human cells were not cross-resistant to other agents such as colchicines. We found as expected that both hsp27 and hsp70 were significantly induced in these experiments, implicating their expression in the development of heat-induced resistance to doxorubicin.

We next used transfection of hsp27 in both sense and antisense orientations to determine whether this gene is directly involved in doxorubicin resistance in human breast cancer cells. MDA-MB-231 cells, which normally produce only low levels of hsp27, were transfected with hsp27, resulting in an increase in resistance. Conversely, MCF-7/MG cells, which express high constitutive levels of hsp27, were transfected with antisense hsp27, and were thereby rendered more sensitive to doxorubicin [32]. We feel that this data directly implicates hsp27 expression in doxorubicin resistance.

Hsp function

Why should hsp's confer resistance to cytotoxic agents? Numerous studies have explained the importance of the hsp's for cell survival under stress conditions; they are somehow involved in "protecting" the cell from various stress-induced alterations. Thus it is not a great extension to propose that they might also enable the cell to recover from drug-induced damage. The exact functions of the hsp's are unknown, but an emerging picture is that they may play essential roles in many cellular activities. The best analyzed is their chaperonin function. For instance, the hsp70 family of proteins are thought to be important for the synthesis and translocation of proteins that localize to the endoplasmic reticulum and mitochondria, and hsp70 appears in complexes with SV40 large T antigen, the cellular oncogene c-myc, and mutated p53 suppressor proteins. Hsp70 and 90 may also be involved in modifying the activity of steroid hormone receptors by maintaining the receptors in an inactive state. Also, we found in our transfection experiments that hsp27 overexpression augmented the growth of the cells in both anchorage-independent and dependent assays [32]. Hsp's may be important in cell proliferation by interacting with proteins essential for the cell cycle, again suggesting how the hsp's could play a role in recovery from drug-induced disruption of cellular processes.

Therapeutic implications and future directions

We have recently embarked on a detailed study of the regulation of hsp genes, with the goal of ultimately manipulating their expression in patients. This work has taken two different directions. The first approach is directed at understanding the transcriptional factors involved in regulating hsp27 expression, and a second, pharmacological approach is aimed directly at inhibition of hsp expression. We would like to suggest that the use of inhibitors of transcriptional factors specific for hsp expression may be a new approach in drug resistance reversal strategies.

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