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Elevated levels of heat shock factor 1 indicate a poor prognosis in breast cancer

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Summary

Heat shock factor 1 (HSF1) is the transcriptional activator of heat shock protein (HSP) genes in both cell stress and cancer. The studies of Santagata et al clearly establish that HSF1 levels are increased in the nuclei of mammary cancer cells both at the *in situ* and invasive stages, and that these levels are closely correlated with increased mortality. HSF1 levels were elevated in ER-positive cells as well as HER2 expressing and triple negative breast cancer cells and higher levels of nuclear HSF1 were associated with a poor prognosis. These studies establish a clear role for HSF1 in human mammary carcinoma and suggest the potential for targeting HSF1 in breast cancer treatment.

Keywords

Heat shock factor 1; nuclear; human mammary carcinoma; patient survival; prognosis

Summary of methods and results

The authors aimed to evaluate the significance of intracellular HSF1 levels in the prognosis of human breast cancer patients. They developed a highly specific antibody cocktail for examining HSF1 levels by immunoblot analysis (IB) or immunohistochemistry (IHC). Using the antibody cocktail they were able to detect HSF1 by IHC and to demonstrate the potential significance of HSF1 as a characteristic of invasive carcinoma, showing elevated HSF1 levels by IB analysis of seven breast cancer samples compared to matched normal breast tissue controls. In addition IHC analysis of forty matched samples from breast epithelium or mammary tumors indicated that HSF1 was located in nuclei within the malignant tissues but was largely cytoplasmic in normal tissues. The differential intracellular partitioning may be significant, as HSF1 requires nuclear localization for transcriptional activity. The authors noted that HSF1 staining of nuclei in malignant cells was remarkably uniform throughout the fields, compared with many other prognostic markers evaluated in human breast cancer. The degree of staining remained uniform at the stromal interfaces or regions of necrosis, making unlikely a role for microenvironmental stress in HSF1 activation. Interestingly, HSF1 distribution to the nucleus was similar in *in situ* tumors (ductal carcinoma in situ- DCIS) and invasive carcinoma suggesting that the factor is elevated and transported to the nucleus in the earlier and later stages of tumorigenisis.

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These initial studies prompted a more in depth investigation and 1841 cases of invasive breast cancer from the nurse's health study (NHS) were evaluated by IHC for nuclear HSF1. This analysis indicated 21.9 % cases negative for nuclear HSF1, with 78.1 % positive. 47.9 % of these cases showed low-level staining, with 30.2% at high levels. In addition, levels of HSF1 expression differed by histological grade with only 14.4 % of low-grade carcinomas showing elevated nuclear HSF1 compared with 48.1 % in high-grade cancers. Differences were also observed between mammary tumors of different histological origin, expressing alternative molecular markers; the more aggressive HER2 positive and triple negative cancers were more likely to have elevated levels of nuclear HSF1 than estrogen receptor (ER)-positive tumors. These findings were of high statistical significance indicating great confidence in their validity.

When the results of nuclear HSF1 expression were compared with median survival assessed from Kaplan-Meier curves, it was shown that women with HSF1 positive tumors had significantly ($P<0.0001$) reduced survival rates compared with women whose tumors stained HSF1-negative and survival was correlated with the level of HSF1. They were also able to show a strong association between HSF1 positivity and reduced survival in patients with ER positive tumors. In this group, 74 % of patients had been treated with the ER antagonist tamoxifen and, according to a multivariate analysis, response to the drug was related to HSF1 status. Unfortunately samples sizes of HER2 positive and triple negative cancer did not permit accurate assessment of correlation between HSF1 levels in tumors and prognosis of breast cancer in patients. Interestingly however, Kaplan-Meier and multivariate analyses suggested an association between HSF1 status and survival in patients with HER2 positive tumors.

These effects were also manifest at the mRNA level. RNA expression profiling data, publicly available from the van de Vijver cohort was interrogated for HSF1 mRNA status [1]. Findings were largely consistent with the trends from the IHC studies. HSF1 mRNA was higher in ER negative, compared with ER positive tumors and elevated levels of this RNA species correlated with reduced survival.

Discussion

HSF1 was previously suspected to be involved in cancer as the incidence of a number of malignancies, induced by a wide spectrum of carcinogens is strongly decreased in HSF1 knockout mice [2, 3]. In addition, activation of cancer-related signaling in mammary cells by the oncogenic cytokine heregulin requires HSF1 and leads to HSF1 activation and increased survival [4]. The study under discussion here establishes HSF1 as a strong prognostic factor in breast tumorogenesis and as a potential target for novel therapies to treat breast cancer.

Activation of HSF1 in breast cancer appears to be regulated by different mechanisms compared with its induction during cell stress. Stress-induced HSF1 activation appears to be independent of the overall levels of the factor and instead involves protein-protein interactions as well as posttranslational modifications [5]. However HSF1 activation in breast cancer is associated with increases in its concentration [4, 6]. The analysis of Santagata et al also shows elevated levels of HSF1 mRNA in breast cancer samples that correlate with poor survival, suggesting that increased transcription of the *hsf1* gene or stabilization of its mRNA may play a role. Few studies have been carried out on the regulation of the HSF1 promoter, although it is known to contain Sp1 and CCAAT box motifs important in function [7]. In addition the *hsf1* promoter contains a large number of CpG dinucleotides that could be potentially methylated under resting conditions, leading perhaps to down regulation of expression [8]. As transcriptional silencing by CpG island

methylation is powerful mechanism of tumor suppression, reduced methylation of the HSF1 promoter could play a role in its upregulation in breast cancer [9]. HSF1 accumulation could also be related to reduced turnover at the protein level. HSF1 contains a consensus site for the E3 ubiquitin ligase Fbw7 known to regulate proteasomal degradation of other oncogenic proteins such as c-Myc, cyclin E and notch after their phosphorylation by Glycogen Synthase Kinase 3 (GSK3) [10]. We have shown that HSF1 levels are decreased in cells after overexpression of FBW-7 and increased by a dominant negative construct derived from FBW-7 (M.D. Khaleque and S. K. Calderwood, unpublished data). It is notable that HSF1 levels are increased in mammary cancer cells exposed to heregulin through a mechanism involving GSK3 inhibition [4].

The influence of elevated HSF1 expression in breast cancer carcinogenesis may involve induction of heat shock proteins (HSP) that could permit the emergence of tumors due to ability to inhibit apoptosis and senescence and to chaperone the abundant oncoproteins arising during tumorogenesis [11]. Indeed Hsp27 and Hsp70 were shown to be elevated in mammary cancer biopsy samples in a number of studies [12]. However, the correlation between breast cancer patient survival and HSF1 levels found in the studies of Santagata et al are considerably more impressive than have been found for the individual HSPs [6, 12]. This finding may reflect the position of HSF1 at the head of the HSP transcriptional cascade. However, HSF1 appears to play roles in tumorogenesis over and above those mediated by HSPs, and has been shown to influence cancer cell signaling, polyploidy, ER mediated transcription, and glucose metabolism apparently by mechanisms independent of HSPs [11].

Future Perspectives

These convincing findings of reduced survival in breast cancer patients with elevated nuclear HSF1 levels thus indicate further study of this factor as a marker for malignancy in breast tissues and mediator of mammary tumorogenesis. It will also be important to discover the molecular mechanisms behind HSF1 upregulation in breast carcinogenesis and the consequences for tumor progression of such an increase. These findings also support the future development of agents that could target HSF1 expression or activity in mammary cancer. A number of HSF1 inhibitors have been developed in recent years, although their effectiveness in treating breast cancer is as yet unknown.

Conclusions

These studies show conclusively that elevated expression and nuclear localization of HSF1 in tumor cells is closely correlated with a poor prognosis for women with breast cancer. HSF1 is therefore a valid marker for mammary carcinoma and a candidate for novel approaches to breast cancer treatment.

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Executive Summary

Study Design

- A study of HSF1 protein and mRNA expression in breast cancer biopsy samples from patients of known medical history.
- HSF1 protein levels were assessed by IHC using anti-HSF1 antibodies in samples from 1841 cases of invasive breast cancer from the NHS.
- In addition, HSF1 mRNA levels were determined by microarray-based gene expression profiling, using data from the van der Vijver cohort.
- 78.1 % of invasive breast cancers were positive for nuclear HSF1.
- HSF1 levels were significantly elevated (48.1%) in high-grade cancers compared to low-grade carcinomas (14.4 %).
- Women with higher levels of HSF1 had a significantly reduced survival rate compared with women whose tumors stained negative for HSF1 ($P<0.0001$).
- There was a strong association between HSF1 positivity and reduced survival in patients with ER positive tumors.
- The findings with RNA profiling were consistent with the IHC studies, indicating that high HSF1 mRNA levels correlate with reduced survival in breast cancer patients.