HET/SAF-B Overexpression Causes Growth Arrest and Multinularity and Is Associated with Aneuploidy in Human Breast Cancer

Steven M. Townson, Toby Sullivan, QingPing Zhang, Gary M. Clark, C. Kent Osborne, Adrian V. Lee, and Steffi Oesterreich

Breast Center, Departments of Medicine and Molecular and Cellular Biology, Baylor College of Medicine, Houston, Texas 77030 [S. M. T., Q. P., G. M. C., C. K. O., A. V. L., S. O.], and Texas Tech University Health Science Center, Lubbock, Texas 79430 [T. S.]

ABSTRACT

HET/SAF-B was originally cloned as a nuclear matrix protein that bound to matrix attachment regions and as a transcriptional repressor of the small heat shock protein hsp27. In addition, we have found recently that HET/SAF-B is also a corepressor of estrogen receptor activity. Estrogen receptor has a very well-described role in breast cancer, and aberrant expression of nuclear matrix and heat shock proteins has also been implicated in breast tumorigenesis. Therefore, we asked whether HET/SAF-B itself could be important in breast cancer. Toward this goal we examined its expression in breast cancer cell lines and asked whether HET/SAF-B can affect breast cancer cell proliferation. Finally, we studied HET/SAF-B expression in clinical breast cancer samples.

HET/SAF-B protein and mRNA were detected at varying levels in all of the eight breast cancer cell lines examined. Using a number of different approaches to modulate the level of HET/SAF-B protein in the cell, we found that HET/SAF-B levels are inversely correlated with cell proliferation. In addition, transfection of HET/SAF-B fused to the green fluorescent protein led to the formation of multinucleated cells not observed in cells transfected with green fluorescent protein alone, suggesting that this effect is a direct result of HET/SAF-B overexpression. Western blot analysis of HET/SAF-B in 61 human breast tumors revealed widely varying levels of HET/SAF-B expression, with some tumors (16%) lacking any detectable HET/SAF-B. Statistical analysis showed that high HET/SAF-B expression in these tumors was associated with low S-phase fraction and with aneuploidy, consistent with our results from transfection experiments in tissue culture cells. We conclude that HET/SAF-B plays an important role in breast cancer, and we discuss possible mechanisms of the involvement of HET/SAF-B in cell proliferation and division.

INTRODUCTION

HET/SAF-B was originally cloned as a protein binding to matrix/scaffold attachment regions (1) and as a NMP-binding to the hsp27 promoter in human breast cancer cells (2). Subsequently, it was shown to bind to the COOH-terminal domain of RNA polymerase II and to a subset of serine/arginine-rich RNA processing factors (SR proteins) and to function in mRNA splicing (3). This suggests that HET/SAF-B is involved in the formation of a “transcriptional” complex, bringing transcription and mRNA processing together. These macromolecular complexes have been shown previously to be associated with the nuclear matrix (4, 5).

The nuclear matrix consists of a protein-RNA network that is involved in structural organization of DNA within the nucleus, thereby controlling important regulatory processes such as transcription and DNA replication (reviewed in Ref. 6). Not surprisingly, many NMPs have been shown to be important in cell transformation. The NMP pattern of expression shows significant differences between normal and cancer tissue in bladder (7), colon (8), head and neck (9), prostate (10), and breast (11). Consistent with this, various NMPs were found to have potential as prognostic markers for cancer add (12, 13). Additionally, a role for the nuclear matrix in steroid hormone action was postulated many years ago (14–18) but only recently have specific NMPs been characterized that directly bind to hormone receptors and modulate their activity (19). For example, recently, the glucocorticoid receptor-interacting protein GRIP 120 has been identified as the NMP hnRNPU (20). We have shown recently that the NMP HET/SAF-B regulates the activity of the estrogen receptor (21).

HET/SAF-B binds to the ER and functions as an ER corepressor. In this way, HET/SAF-B is similar to several other
recently identified ER-interacting proteins, REA (repressor of estrogen receptor activity; Ref. 22), SMRT (the silencing mediator of retinoid and thyroid receptors; Refs. 23 and 24), and NcoR (nuclear receptor corepressor; Ref. 25), all of which also act as corepressors. Because estrogen is one of the most potent mitogens for breast cancer cells and is a known risk factor for breast cancer, a role of HET/SAF-B in estrogen action implies a role in ER-positive breast cancer cell growth control. Alternatively, it is also possible that HET/SAF-B can act as a transcriptional repressor independent of ER by interacting with other transcription factors. It has been shown that known steroid receptor-interacting proteins such as the coactivator SRC1 (27, 28), which was originally cloned as a steroid receptor cofactor, also mediates transactivation by other transcription factors including AP1 (28), serum response factor (29), nuclear factor-kB (NFKB) (29), cyclic AMP-responsive element binding protein, and signal transducers and activators of transcription (30). Moreover, SRC1 has also been found to bind to p53 and potentiate its transactivation, whereas two other ER coactivators, amplified in breast cancer (AIB1) and Xenopus steroid receptor coactivator-1 (xSRC-3), were found to repress p53-mediated transactivation (31). This suggests that these factors might have important and distinct roles in tumorigenesis independent of their function as a steroid hormone receptor regulator.

HET/SAF-B is involved in a number of cellular processes that are associated with tumorigenesis. These include its role in the repression of hsp27, which has been shown to positively regulate breast cancer cell proliferation (32), as well as its role as a NMP and as an ERE corepressor. Therefore, we have set out to analyze whether HET/SAF-B plays a role in breast cancer. Here we report that overexpression of HET/SAF-B causes growth inhibition and multinuclearity in cultured cells. Consistent with these findings from tissue culture, HET/SAF-B expression is associated with lower proliferation but also with aneuploidy in human breast tumor specimens. Thus, as predicted, HET/SAF-B plays a role in breast tumor behavior. Possible mechanism(s) will be discussed in more detail.

MATERIALS AND METHODS

Plasmid Constructs and Chemicals. The cloning of the HET/SAF-B expression construct in pcDNA1 has been described previously (2). To generate an antisense construct, the HET/SAF-B expression construct in pcDNA1 has been described in detail previously (34, 37). The tetracycline responsive element was cloned from pcDNA1 sense and antisense, respectively, along with 1 μg of pcDNA1 vector control or HET/SAF-B-pcDNA1 sense and antisense, respectively, along with 1 μg of pcDNA1 control or HET/SAF-B-pcDNA1 sense and antisense, respectively, along with 1 μg of pcDNA1 sense and antisense constructs. All transfections were performed using methods described previously (35).

Transfections, Cell Growth, and Cell Cycle Analysis. All transfections were performed using Lipofectamine (Life Technologies, Inc.) or Fugene (Roche Molecular Biochemicals, Indianapolis, IN). Transient transfections were analyzed 48 h after transfection. To establish stable cell lines, NIH3T3 cells were cotransfected with pcDNA1 only or HET/SAF-B-pcDNA1 (2) and pSVneo, and transfected clones were selected in 1000 μg/ml G418.

For growth analysis, cells were plated in quadruplicate at 2500 cells/well in a 96-well plate. The next day (day 0), cell number was assessed by MTT assay as described previously by us (36). Cells were then incubated in SFM or medium with 10% FBS, and cell number was determined at days 2, 4, and 6.

For colony formation assays, MCF-7/MG cells were transfected with 20 μg of pcDNA1 vector control or HET/SAF-B-pcDNA1 sense and antisense, respectively, along with 1 μg of pSVneo. After 3 weeks incubation in 400 μg/ml G418, colonies were stained with 1% crystal violet. 293 cells, which display very high transfection efficiency, were used for proliferation assays measuring [3H]thymidine incorporation into DNA. Cells (8 × 10^4) were plated in triplicate in six-well plates and transfected on day 2 with increasing amounts of pcDNA1 or HET/SAF-B-pcDNA1 antisense constructs. On day 4, the cells were incubated for 1 h with 1 μl of [3H]thymidine (Amersham; 1 mCi/ml). After washing in cold PBS and cold 5% trichloroacetic acid, the cells were kept on ice for 30 min in the presence of 5% trichloroacetic acid and finally lysed in 0.5 M NaOH.

For generation of inducible HET/SAF-B-expressing cells, we used the tetracycline inducible expression system, which has been described in detail previously (34, 37). The tetracycline inducible MDA-MB-453 cells were given to us by Dr. Douglas Yee (University of Minnesota). Briefly, cells were stably transfected with a plasmid (pUHD172–1-neo) expressing a protein termed rTA (VP16 linked to a tetracycline binding protein). Stable clones were selected in 1000 μg/ml G418, expanded, and then tested for expression of rTA by transient transfection with a reporter plasmid (pUHD16–3) consisting of
seven tetracycline operator sequences upstream of a luciferase gene. Treatment of cells with doxycycline (an analogue of tetracycline) at 1 μg/ml for 24 h indicated inducible luciferase expression (2–10-fold) in a number of clones. We used the clone with the highest inducibility (MDA-435rtTA1) for transfection with an expression plasmid containing HET/SAF-B under the control of a tetracycline-inducible CMV promoter (pUHHDHET). After transfection, these cells were selected in 600 μg/ml hygromycin and analyzed for inducible HET/SAF-B expression by Western blot analysis.

For the analysis of cell cycle distribution, cells were harvested, washed with PBS, fixed in 70% ethanol, and stored at −20°C. Immediately before analysis on a FACS STAR PLUS (Becton Dickinson, San Jose, CA), propidium iodide and RNase were added to the cell pellet to final concentrations of 0.1 and 0.5 mg/ml, respectively. Data were analyzed using CellQuest software.

The expression of HET/SAF-B throughout the cell cycle was investigated in T24 bladder carcinoma cells grown in IMEM containing 5% FBS by first growing the cells to confluence and then leaving them for 3 days to arrest in G0–G1 (37). The cells were then subcultured into 10-cm plates and plated at a density of 1 × 10^6 per well. Cells lysates were produced at different time points after subculture by first washing the cells in PBS and then lysing them in high salt buffer [50 mM Tris-HCl (pH 7.8), 0.2 mM EDTA, 0.4 M NaCl, 10% glycerol, and 1% NaPO_4] containing Protease Inhibitor Cocktail Tablets (Roche Molecular Biochemicals, Indianapolis, IN) used at the concentration suggested by the manufacturer. T24 cells were blocked in IMEM containing 5% FBS by first growing the cells to confluency and then leaving them for 3 days to arrest in G0–G1 (37).

RNA and Protein Analysis. The RPAs were performed as described previously (32) using a HET/SAF-B-specific probe as well as a probe for 36B4 as a loading control. For Western blot analysis, cell pellets were resuspended in 5% SDS or high salt buffer and sonicated, and 50 μg of total protein were analyzed by Western blotting using HET/SAF-B antibody as well as a polyclonal antibody against the p85 subunit of PI3K (Upstate Biotechnology, Lake Placid, NY) as a loading control. For the quantitative Western blot analysis of human tumors, each gel contained 50 μg of MCF-7 SDS extracts as an internal standard. The HET/SAF-B bands were quantitated by densitometric scanning using NIH Image 1.6 software, and the levels were calculated in arbitrary units by the ratio of the integrated densitometry signal in the tumor sample relative to the internal standard on each gel. For the detection of GFP-HET/SAF-B fusion protein, an anti-GFP antibody was used at a 1:1000 dilution (Clontech, Palo Alto, CA).

Statistical Analysis. All statistical analyses were performed using SAS (Version 6.11; SAS Institute, Cary, NC) running on a Sun Microsystems SparcServer 1000. Relationships between HET/SAF-B expression and S-phase fraction and between HET/SAF-B and ER expression were analyzed using Spearman’s rank correlation coefficients. The relationship between HET/SAF-B expression and ploidy was analyzed using a t test.

RESULTS

Expression of HET/SAF-B in Breast Cancer Cell Lines. Renz and Fackelmayer (1) have shown previously that HET/SAF-B is a ubiquitously expressed gene. Northern blot analysis using a variety of different human cancer cell lines and different tissues detected HET/SAF-B mRNA in all analyzed samples. To see how it is expressed in various breast cancer cell lines, we performed Western blot analysis using the HET/SAF-B antibody and a p85 antibody as a loading control (Fig. 1). HET/SAF-B protein levels varied between cell lines, with the highest expression in MDA-MB-231, ranging to almost undetectable levels in ZR-75 cells. The subsequent RPA (Fig. 1B) indicated that ZR-75 cells do express HET/SAF-B mRNA. Thus, although HET/SAF-B is a ubiquitously expressed gene, the levels in breast cancer cell lines vary.

Overexpression of HET/SAF-B in Tissue Culture Cell Lines and Effect on Cell Growth. To establish the relationship between HET/SAF-B expression and cell proliferation, we attempted to transfect HET/SAF-B into cell lines. A number of initial efforts to isolate stable breast cancer cell lines that constitutively overexpressed HET/SAF-B were unsuccessful; after transfection with HET/SAF-B-pcDNA1, some drug-resistant clones formed, but none survived further passaging in culture. In contrast, we were able to select several hundred control clones transfected with pcDNA1 alone. These results indicate that HET/SAF-B either inhibits proliferation or is toxic to the cells. To circumvent this problem, we used a tetracycline-inducible...
We transfected MDA-MB-435 cells using the GFP-HET/SAF-B construct and GFP only as a control. Of 20 fluorescent clones that were transfected with GFP alone, all 20 were still fluorescent after expanding them (2–3 weeks). In two of those clones, HET/SAF-B localized to the cytoplasm (data not shown), which has not been described before and which might represent an “escape mechanism” from the growth-inhibitory effects of HET/SAF-B overexpression. GFP-HET/SAF-B is functional, because it was able to corepress ER activity (data not shown), similar to our findings using the HET/SAF-B construct (21).

We transfected MDA-MB-435 cells using the GFP-HET/SAF-B construct and GFP only as a control. Of 20 fluorescent clones that were transfected with GFP alone, all 20 were still fluorescent after expanding them (2–3 weeks). In two of those clones, HET/SAF-B localized to the cytoplasm (data not shown), which has not been described before and which might represent an “escape mechanism” from the growth-inhibitory effects of HET/SAF-B overexpression. GFP-HET/SAF-B is functional, because it was able to corepress ER activity (data not shown), similar to our findings using the HET/SAF-B construct (21).

We transfected MDA-MB-435 cells using the GFP-HET/SAF-B construct and GFP only as a control. Of 20 fluorescent clones that were transfected with GFP alone, all 20 were still fluorescent after expanding them (2–3 weeks). In two of those clones, HET/SAF-B localized to the cytoplasm (data not shown), which has not been described before and which might represent an “escape mechanism” from the growth-inhibitory effects of HET/SAF-B overexpression. GFP-HET/SAF-B is functional, because it was able to corepress ER activity (data not shown), similar to our findings using the HET/SAF-B construct (21).
again we detected many polynucleated cells in the GFP-HET/SAF-B-overexpressing cells but not in the GFP cells. Finally, we repeated the transient transfection in 293 cells, which display very high transfection efficiency. As seen in MDA-MB-435 and CHO-K1 cells, we again observed many polynucleated cells among the HET/SAF-B-overexpressing cells. Depending on the cell line used, we detected multinucleated cells in 1–5% of the cells. We analyzed the cell cycle distribution of the transfected 293 cells by sorting the fluorescent cells and subjecting them to DNA histogram analysis (Fig. 4D). As shown previously (in the NIH3T3 transfection in Fig. 3), the number of cells in S-phase was decreased, from 34% in control cells to 12% in GFP-HET/SAF-B-overexpressing cells. We also observed a block in G2-M in the HET/SAF-B-overexpressing cells (23–44%). Thus, overexpression of GFP-HET/SAF-B was associated with multinuclearity and significant changes in cell cycle.

Because overexpression of HET/SAF-B seemed to block cells in G2-M, we asked whether HET/SAF-B protein levels vary through the cell cycle. To answer this question, we used T24 human bladder carcinoma cells that can be easily synchronized by contact inhibition as described previously (38). They reenter the cell cycle upon replating at a lower dilution. Breast cancer cells do not synchronize upon confluence but can be synchronized by withdrawal of serum. However, reentry into the

---

Fig. 3  Overexpression of HET/SAF-B and growth inhibition in NIH3T3 cells. A, for the Western blot, 50 μg of SDS-protein extracts were loaded onto 6% SDS-PAGE, transferred to nitrocellulose, and blotted with HET/SAF-B-specific antibodies. B, cells were plated in quadruplicate in 96-well plates in 10% serum or SFM, and MTT assays were performed on the next day (day 0) and on day 2, 4, and 6. Bars, SD.

Fig. 4  Generation of multinucleated cells after GFP-HET/SAF-B transfection. A, MDA-MB-435 cells were transfected with GFP only or with GFP-HET/SAF-B. Cells were observed using a FITC filter on an Olympus CK40 fluorescence microscope. Representative pictures of one control clone (GFP) and two GFP-HET/SAF-B clones are shown. B, 293 cells were transiently transfected with GFP or GFP-HET/SAF-B, and representative pictures were taken. C, transfected 293 cells were sorted on a FACS, and DNA histogram analysis was performed on fluorescent cells only.
cell cycle by serum stimulation may produce artifactual results because serum stimulation may affect HET/SAF-B levels or phosphorylation. Thus, we used synchronized T24 cells. In addition, we analyzed T24 cells that were blocked in G2-M by treatment with nocodazole. Cell cycle analysis was performed by FACS, and as expected, we were able to obtain cells synchronized in G0-G1, G1-S, and G2-M (Fig. 5A). Subsequent Western blot analysis demonstrated that HET/SAF-B was expressed throughout the cell cycle, but the levels of HET/SAF-B protein increased during S-phase and peaked in G2-M (Fig. 5B). The expression of HET/SAF-B in nocodazole-treated cells confirmed the increase of HET/SAF-B expression in G2-M. Thus, HET/SAF-B protein levels are at their highest in G2-M, which is the stage at which overexpressed HET/SAF-B blocks cells.

Transient Modulation of HET/SAF-B Levels in Tissue Culture Cell Lines. As shown in the above experiments, it was difficult to stably integrate HET/SAF-B into the genome of various cell lines. Thus, as a final approach to prove the effect of HET/SAF-B on proliferation, we used a transient tissue culture assay in breast cancer cells commonly used by other investigators (39) to demonstrate negative effects of genes on growth. In this assay, cells are transfected with the gene of interest and a selection marker and then grown in selection media until colonies can be stained and counted. We transfected MCF-7/MG cells with pcDNA1 plasmid and empty vector only (pcDNA1) or HET/SAF-B cDNA in sense or antisense orientation. Transfection of the antisense construct resulted in decreased HET/SAF-B expression at both RNA and protein levels (data not shown). The cells were selected for resistance to G418, and after 3 weeks, colonies were stained with crystal violet (Fig. 6A). The number of colonies was dramatically reduced after transfection of HET/SAF-B sense cDNA, whereas antisense transfection had no significant effect compared with pcDNA1 only. The inhibition of colony growth after HET/SAF-B overexpression confirms its growth inhibition. We were, however, surprised by the finding that antisense transfection did not increase colony formation in this experiment, and this could have several reasons. For instance, it is possible that we did not decrease the endogenous levels enough for generation of a phenotype in the transfected MCF-7 cells. Therefore, we repeated the transfection of the antisense DNA in 293 cells, which are known to display very high transfection efficiency. Determining [3H]thymidine incorporation into DNA as a direct measurement of cell proliferation, we were able to detect a dose-
dependent increase in the proliferative index after transfection of the antisense construct (data not shown), thus again supporting our other data describing HET/SAF-B as a negative growth regulator.

Expression of HET/SAF-B in Clinical Breast Cancer Specimens. Lastly, we asked whether in tumor samples we could detect an association of HET/SAF-B with proliferative markers in a way reflective of our findings from tissue culture experiments. Therefore, we analyzed HET/SAF-B expression in human breast cancer specimens and correlated this with proliferation rate as measured by S-phase fraction. We measured HET/SAF-B levels in 61 primary breast tumors by Western blot (a representative blot is shown in Fig. 7) and found that, as in the cell lines, HET/SAF-B protein content varied widely. Some tumors expressed high amounts (e.g., no. 5), some moderate (e.g., no. 6), and in 10 tumors (16%), no HET/SAF-B could be detected (e.g., no. 8), even after prolonged exposure of the film. The same extracts were analyzed for histone H3 levels, and statistical analysis revealed a trend toward a negative correlation with S-phase fraction, even after prolonged exposure of the film.

DISCUSSION

HET/SAF-B is a NMP that was cloned in our laboratory as a negative regulator of hsp27 expression (2) and in the laboratory of Renz and Fackelmayer (1) as a scaffold/matrix attachment site binding protein. The nuclear matrix was postulated many years ago to interact with nuclear hormone receptors (14–18), but only recently have specific NMPs been identified that bind directly to hormone receptors and modulate their activity (19). Because ER, like HET/SAF-B, is also involved in regulating hsp27 expression (40), we asked whether HET/SAF-B interacts with this receptor and modulates its activity and found that HET/SAF-B acts as an ER corepressor. Here we show more generally that HET/SAF-B is a growth inhibitor independent of its interaction with ER, blocking the cell in G2-M, and in some situations causing multinucularity.

We provide several lines of evidence that HET/SAF-B affects proliferation: (a) breast cancer cells with inducible HET/SAF-B expression grew significantly slower than their appropriate control clones; (b) NIH3T3 cells overexpressing HET/SAF-B also showed a significant decrease in growth; and (c) transient overexpression and underexpression of HET/SAF-B results in decreased colony formation and increased cell proliferation, respectively. Although interpretation of results from a single overexpressing NIH3T3 clone is limited because of the possible unpredictable effect of integration, the parallel evidence from HET/SAF-B-inducible MDA-MB-435rtTA cells and additional results from transient transfection assays in MCF-7 and 293 cells allow us to conclude that HET/SAF-B overexpression results in growth inhibition. In addition, in cultured cells as well as in clinical breast tumors, HET/SAF-B protein levels were inversely correlated with S-phase fraction, which is a direct measure of proliferation rate. In the present relatively small sample of breast tumors (n = 61), this correlation did not quite reach statistical significance (P = 0.08), and we are currently designing a larger study to address how well HET/SAF-B correlates with S-phase and other prognostic factors and whether HET/SAF-B levels could predict clinical outcomes of breast cancer patients. We have also shown that down-regulation of endogenous HET/SAF-B can lead to a higher growth rate, consistent with HET/SAF-B being a negative growth regulator, the absence of which may lead to excessive growth in tumors. Indeed, we have found that some breast tumors did not express HET/SAF-B protein at a detectable level.

The growth-inhibitory effects of overexpressed HET/SAF-B in ER positive cells can be readily explained by HET/SAF-B being an ER corepressor. It is feasible that HET/SAF-B suppresses estrogen-dependent transcriptional pathways related to breast epithelial cell proliferation, thus resulting in growth arrest. It is of interest to mention that the breast cancer susceptibility gene BRCA1 was shown recently to inhibit ER activity in transient transfection assays (41).

The exact mechanism of estrogen-induced proliferation is yet to be defined, but we think that HET/SAF-B could play a role. When cells are primed to respond to estrogen in G0-G1 and in G1-S-phase transition (42), HET/SAF-B levels are at their...
lowest. In contrast, when cells do not respond to estrogen (in M phase), HET/SAF-B levels are high. Overexpression of HET/SAF-B, for instance as a result of our transfection studies, results in high HET/SAF-B levels at all parts of the cell cycle, including the phases when cells are primed to respond to estrogen. This presumably leads to inappropriate ER corepression, thus resulting in growth arrest.

As described by us (21) and others (1–3), HET/SAF-B clearly has other functions, independent of its ER corepressor activity. These characteristics or other functions of HET/SAF-B yet to be defined might explain the growth-inhibitory effect of HET/SAF-B in ER-negative cells. For instance, hsp27 has clearly been shown to be associated with increased breast cancer cell growth, so that its down-regulation by HET/SAF-B could result in growth inhibition. It is also likely, just as discovered for other steroid receptor cofactors (42), that HET/SAF-B does not interact exclusively with ER. Indeed, our preliminary data indicate that HET/SAF-B can also inhibit the activity of other members of the steroid receptor as well as the retinoic acid/thyroid receptor families. Again, this regulation of other proteins besides the ER could explain the observed ER-independent effects.

HET/SAF-B has also been shown to bind to the COOH-terminal domain of RNA polymerase II and to a subset of serine/arginine-rich RNA processing factors (SR proteins; Refs. 1–3). This suggests that HET/SAF-B is involved in the formation of a transcriptosomal complex, bringing transcription and pre-mRNA processing together. The role of HET/SAF-B in this complex might be to prevent processing of mRNA transcripts, which would be consistent with the function of HET/SAF-B as a transcriptional repressor. Such an activity has been shown for CstF-50, which also binds to RNA polymerase II and is thought to prevent the processing of mRNA transcripts containing error (43). Overexpression of HET/SAF-B may disrupt the balance in the interaction with these splicing proteins, leading to a decrease in RNA processing and possibly resulting in growth arrest.

Finally, the attachment of HET/SAF-B to the nuclear matrix should be mentioned. The nuclear matrix organizes DNA into loop domains, the bases of which contain the S/MAR DNA sequences. It is the scaffold attachment factors such as HET/SAF-B that bind these sequences, thus connecting the chromatin to the NMP structures. The high levels of HET/SAF-B seen in G2-M may also reflect its role in the packaging of chromatin for mitosis. When cells are in G2-M, most transcription is repressed, and there is a dramatic increase in HET/SAF-B protein levels, which suggests that HET/SAF-B could be a major factor in the general repression of transcription at this phase of the cell cycle. The effect on cell proliferation when HET/SAF-B is overexpressed in both ER-positive and ER-negative cell lines may be the result of high intracellular HET/SAF-B levels throughout the cell cycle, leading to disruption of the organization of the transcriptionally active chromatin normally seen in G0-G1 and S-phase, thus producing transcriptionally inactive chromatin as seen in G2-M. This repression then leaves cells without the appropriate protein machinery to continue through the cell cycle and they become blocked.

In addition to the growth-inhibitory effects of overexpressed HET/SAF-B, our experiments with GFP-HET/SAF-B show that overexpression causes cells to be multinucleated. Furthermore, in breast tumors, HET/SAF-B was strongly associated with aneuploidy. Multinuclearity and aneuploidy are two of the most common features of tumor cells, but the exact molecular basis for these phenotypes is unknown (44, 45). Because HET/SAF-B overexpression results in a block in G2-M, one could imagine that overexpressed HET/SAF-B somehow disrupts mitosis, either directly by altering the condensation of chromatin or indirectly by repressing genes involved in spindle formation and cytokinesis.

In summary, we have provided evidence that the NMP HET/SAF-B plays several roles in human breast cancer. Our current studies are aimed at further identifying the mechanism(s) of HET/SAF-B-mediated growth inhibition and multinuclearity.

REFERENCES


HET/SAF-B Overexpression Causes Growth Arrest and Multinuclearity and Is Associated with Aneuploidy in Human Breast Cancer

Steven M. Townson, Toby Sullivan, QingPing Zhang, et al.


Updated version Access the most recent version of this article at: http://clincancerres.aacrjournals.org/content/6/9/3788

Cited articles This article cites 41 articles, 22 of which you can access for free at: http://clincancerres.aacrjournals.org/content/6/9/3788.full.html#ref-list-1

Citing articles This article has been cited by 11 HighWire-hosted articles. Access the articles at: /content/6/9/3788.full.html#related-urls

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.