Fatty Acid Synthase Expression Defines Distinct Molecular Signatures in Prostate Cancer

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Abstract

The androgen-regulated enzyme fatty acid synthase (FAS), required for de novo lipogenesis, is overexpressed in several cancers including prostate carcinoma and has been associated with aggressive disease. FAS expression was assessed in 81 prostate carcinomas, both by immunohistochemistry in tissue microarrays and by Affymetrix Hu95Av2 oligonucleotide arrays. Both FAS mRNA and protein were significantly overexpressed in prostate carcinomas compared with the corresponding normal tissue. FAS mRNA and protein expression increased substantially from normal to prostatic intraepithelial neoplasia, to low grade, to high grade, and to androgen-independent bone metastases. A significant correlation between FAS mRNA and protein expression was found in two thirds of the cases. In 17% of the cases, FAS protein levels were high despite low mRNA levels, and these tumors exhibited a distinct molecular signature when compared with tumors that did not express FAS protein. Whereas the latter group of tumors expressed some proapoptotic genes, tumors with high FAS levels overexpressed, among other genes, its transcriptional regulator, steroid regulator binding protein, and apolipoprotein E. These data demonstrate (1) the consistent overexpression of FAS in prostate carcinoma compared with the adjacent normal tissue, (2) a strong association between FAS and prostate tumor initiation and progression, (3) the highest FAS expression occurring in androgen-independent bone metastases, (4) the transcriptional and posttranscriptional regulation of FAS in the majority and in a subset of prostate cancers, respectively, and (5) most importantly, the identification by FAS expression of prostate tumors with unique molecular signatures and potentially diverse biologic behavior.

Introduction

Fatty acid synthase (FAS) is a 250–270-kd cytosolic multifunctional polypeptide chain that is involved in the metabolism of glucose to fatty acids (1). In particular, FAS is responsible for the last step of the endogenous fatty acid synthesis, generation of fatty acid palmitate from malonyl-CoA and acetyl-CoA. This step is dependent on NADPH produced during the glycolysis. Because fatty acids are mostly supplied by the dietary fat, their endogenous synthesis is minimal in an individual consuming a balanced diet.

Despite its apparently marginal physiologic role under normal conditions, FAS has been found to be overexpressed at both protein and mRNA levels in many tumors (1), including prostate carcinoma (2–8). In addition, androgen-dependent transcriptional FAS regulation has been previously established in prostate cancer cell lines (5, 9–11). Despite this fact, FAS is overexpressed in the androgen-independent (AI) setting as well (12, 13). However, whether FAS has a distinct role in prostate cancer progression toward androgen independence has not been established.

FAS inhibitors lead human cancer cells to apoptosis (1) and decrease the size of prostate xenographs (12). Thus, FAS overexpression is likely not only an epiphenomenon of the dysregulation of the lipogenic pathways in cancer cells but is also associated with tumor cell survival.

In order to address FAS regulation in human tumors, we analyzed FAS mRNA and protein expression by oligonucleotide arrays and tissue microarrays (TMA), respectively, in a large series of paired normal and tumor prostate tissue samples. We confirmed a transcriptional FAS regulation in most of the tumors, but we also identified a subset of carcinomas with nonconcordant FAS mRNA/protein levels. Importantly, these tumors displayed a unique gene expression pattern. In addition, by nearest neighbor analysis on the array data, the FAS transcriptional regulator steroid regulator binding protein (SREBP) was found in high FAS mRNA expressors. Finally, FAS protein and mRNA expression was highest in AI metastatic prostate tumors (to bone), suggesting a hormone-independent FAS regulation in advanced prostate cancer.

Results

FAS Messenger RNA Expression by Oligonucleotide Array

FAS mRNA showed a differential expression across the 52 tumor/normal samples (Fig. 1A). A 3-fold overexpression in prostate tumor samples (mean 158) in comparison with the corresponding normal tissue samples (mean 53) was detected.
(P < 2.9 × 10⁻⁷) (Fig. 1B). When the tumor samples were subdivided into three distinct groups by different total Gleason scores (TGS), we observed a general trend, although not statistically significant, for increasing expression levels of FAS mRNA as TGS increased from TGS 6, to TGS 7, and to TGS >7 tumors (Fig. 1C). Furthermore, 12- and 36-fold FAS mRNA expression levels were seen in metastatic AI tumors in comparison with nonmetastatic tumors (P < 0.008) and with the normal prostate (P < 4.6 × 10⁻¹⁰), respectively (Fig. 1C).

In order to identify FAS nearest neighbor genes, we calculated the Pearson correlation coefficient between FAS mRNA expression and the 7206 genes passing the variation filter. There were 87 features (i.e., genes or ESTs) with a Pearson correlation coefficient of greater than 0.80, the top 20 of which are presented in Fig. 2. Included in the top 10 genes with the greatest correlation with FAS was sterol regulatory element binding transcription factor 1 (SREBP-1), a known transcriptional regulator of FAS (5, 9).

**FAS Protein Expression by Immunohistochemistry (IHC) on TMAAs**

Among 64 normal prostate specimens, 44 (69%) did not react with the anti-FAS antibody and 20 (31%) showed positivity at least in one of the five arrayed cores. The mean of FAS expression was weak in 18 (28%) cases and moderate in 2 (3%) of these normal tissue samples.

FAS expression in tumor tissue, when present, was homogeneous in all cells. Among the 64 tumor samples, 58 (91%) expressed FAS (Fig. 2), although the mean of the immunostaining intensity scores was variable: strong (3+) in 5/64 (8%), moderate (2+) in 19/64 (30%), and weak (1+) in 34/64 (53%). Six (9%) tumor samples did not react with anti-FAS antibody. In 56 (97%) of the cases that expressed FAS in the tumor tissue, the FAS intensity score was higher in the tumor tissue than in the corresponding normal tissue. In one (2%) case, no difference between tumor and normal samples was detected, and in another case, the intensity score was higher in the normal tissue.

Among the 21 cases in which areas of high-grade prostatic intraepithelial neoplasia (HG-PIN) were present, 18 (86%) showed FAS protein expression in glands with HG-PIN (Fig. 2): weak reactivity was detected in 14 (67%) cases, moderate reactivity in 3 (14%), and strong reactivity in 1 (2%). When the mean of FAS intensity scores in the normal samples was compared with the mean expression in HG-PIN or cancer, a

![FIGURE 1](https://example.com/fig1.png)

**FIGURE 1.** FAS expression at the mRNA level in prostate carcinomas by oligomicroarray analysis. **A.** Differential expression level of FAS mRNA across the 52 tumor (T)/normal (N) samples. **B.** Mean values of FAS mRNA expression in normal and tumor samples. A 3-fold FAS mRNA overexpression (mean ± SEM 158.5 ± 17.1) was observed in tumor samples in comparison with the corresponding normal tissue (mean ± SEM 53.9 ± 7.2; P < 2.9 × 10⁻⁷). **C.** FAS mRNA expression and tumor progression, illustrated (logarithmic scale). Increasing expression levels of FAS gene from normal prostate (mean 53) to TGS 6 (mean ± SEM 146.5 ± 27.4), to TGS 7 (mean ± SEM 149.2 ± 21), to TGS >7 (mean ± SEM 240.8 ± 71.7), and to AI metastatic tumors (mean ± SEM 1960.8 ± 480) are shown.
A significant difference was found ($P < 2.54 \times 10^{-4}$ and $P < 8.57 \times 10^{-10}$, respectively) (Fig. 3, B and C). When areas of HG-PIN and areas of cancer with different Gleason scores were compared, the intensity of FAS immunoreactivity increased as tumor cells progressed from HG-PIN (mean $F_{SEM} 0.74$), to TGS 3 (mean $F_{SEM} 1.06$), to TGS 4/5 (mean $F_{SEM} 1.16$) (Fig. 3C). However, the differences were not statistically significant.

Six of the seven AI metastatic tumors, which had been analyzed by oligomicroarrays, were also stained for IHC. FAS was expressed in three of these cases, and the intensity score was strong in two cases and moderate in the other one. Two of the six cases did not express FAS protein, and in one case, no residual tumor was detected.

Comparison Between FAS Messenger RNA and Protein in Individual Cases

We compared FAS mRNA and protein levels in each of the 42 primary tumors studied by both oligomicroarrays and TMAs. Metastatic cases were excluded from this analysis. As expression cutoff, we used the median values for both mRNA and protein, calculated among the 52 and 64 samples, respectively. We found a statistically significant correlation between FAS mRNA and protein levels ($P < 0.01$), as 14 (33%) and 15 (35%) cases showed concomitant low and high levels, respectively, of both FAS mRNA and protein. Interestingly, in 13 (30%) cases, a correlation between mRNA and protein was not found (Table 1). To investigate whether the cases with discordant levels of FAS protein and mRNA were biologically different, the tumors were divided into four groups based on the high or low level of FAS mRNA and protein using the median values as cutoff. Between the two groups of tumors with high FAS mRNA expression and different intensity of FAS immunostaining, there were no significant differences in gene expression ($P$ values all $> 0.05$ by permutation testing) (Supplemental Tables 2, 3, and 4). However,

![Figure 2](http://mcr.aacrjournals.org)

**FIGURE 2.** Nearest neighbors of FAS by oligomicroarray analysis. The top 20 genes with the greatest correlation with FAS in 52 local prostatic tumors (Local), 12 metastatic prostate cancers (Met), and 3 normal bone marrow samples (BM) are shown. Rows, individual genes; columns, individual samples. The expression of each gene in each sample is represented by the number of SDs above (red) or below (blue) the mean for that gene across all the samples. Included in the top 10 genes is SREBP-1.

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[1] Supplemental data are available online at http://mcr.aacrjournals.org.
distinct molecular signatures were found in the two groups of tumors with low FAS mRNA levels and different FAS protein expression (Fig. 4). For these tumors with low mRNA levels of FAS, there were a large number of genes differentially expressed between the tumors with high IHC staining (>500 genes overexpressed at $P < 0.05$) and those with low IHC staining (315 genes overexpressed at $P < 0.05$; Supplemental Table 3). The tumors with high FAS protein expression potentially represent carcinomas in which posttranscriptional FAS stabilization occurs. All but one of these tumors overexpressed a variety of genes, such as mixed lineage leukemia (MLL1), previously implicated in tumor initiation or progression. Interestingly, apolipoprotein E was also up-regulated in the high FAS protein group. Among the genes up-regulated in the low FAS protein group, there were the proapoptotic tumor necrosis factor ligand (TRAIL) and the gene encoding for the ribosomal protein S5.

**Discussion**

Using oligonucleotide arrays and IHC on TMAs, we found that the enzyme FAS was overexpressed in the vast majority of prostate tumors compared with the adjacent normal tissue. In addition, FAS expression is highest in poorly differentiated and metastatic AI cancers, suggesting that FAS may be an important target in the more aggressive forms of the disease.

Early FAS expression in tumor progression, as demonstrated by overexpression in areas of HG-PIN, is not an exclusive feature of prostate but is also well documented in *in situ* lesions of the breast, colon, and oral cavity (1) and suggests the possibility to use FAS inhibitors in the prevention of cancer progression in these sites as well. Interestingly, the enzyme α-methyl-acyl CoA racemase is also overexpressed in the early phases of prostate cancer (14–16). This enzyme catalyzes the conversion of branched fatty acids from $R$ to $S$ configuration and allows them to undergo peroxisomal β-oxidation with generation of hydrogen peroxide and potential procarcinogenic oxidative damage (17, 18). Thus, the data in the literature, together with our results, suggest that FAS overexpression, and altered fatty acid metabolism in general, may play an important role in promoting prostate carcinogenesis.

Transcriptional FAS regulation has been previously established in prostate cancer cell lines. In LNCaP cells, androgens and growth factors have been demonstrated to up-regulate FAS through SREBPs, which stimulate FAS transcription (9, 19). As expected, we found a strong correlation between the expression of FAS and that of its transcriptional regulator SREBP-1 in both

**Table 1.** Correlation Between FAS Messenger RNA and Protein Expression in Primary Prostatic Tumors

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<th>FAS cDNA &lt; 138, $n = 21$</th>
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<td>FAS IHC &lt; 1, $n = 20$</td>
<td>33.3%, $n = 14$</td>
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<td>FAS IHC ≥ 1, $n = 22$</td>
<td>16.7%, $n = 7$</td>
<td>35.7%, $n = 15$</td>
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$P < 0.01$.  

**FIGURE 3.** FAS expression in prostate carcinomas by TMA analysis. **A.** Representative elements of TMAs stained with the anti-FAS antibody. FAS intensity score was moderate in TGS 3 tumor and in PIN (a). FAS-negative TGS 3 prostate cancer (b). FAS-positive TGS 3 carcinoma adjacent to negative normal prostatic glands (c). TGS 5 tumor with significant FAS overexpression (d). **B.** Mean FAS protein expression immunohistochemistry scores in 64 normal (N)/tumor (T) samples. A differential expression in tumor samples (mean ± SEM 1.2 ± 0.13) in comparison with adjacent normal prostate (mean ± SEM 0.13 ± 0.03) was seen ($P < 8.57 \times 10^{-15}$). **C.** Mean FAS protein expression and tumor progression. Increasing FAS immunoreactivity from normal areas (mean 0.13) to areas with HG-PIN (mean ± SEM 0.74 ± 0.13) to areas with TGS 3 low-grade (LG) tumor (mean ± SEM 1.06 ± 0.18) to areas with TGS 4/5 high-grade (HG) tumor (mean ± SEM 1.167 ± 0.1) to AI metastatic (AI MET) tumors (mean ± SEM 1.4 ± 0.5) was observed. The differential expression between PIN and normal prostate was statistically significant ($P < 2.54 \times 10^{-8}$). **D.** FAS intensity score was high in this bone metastasis of AI prostate carcinoma.
primary and metastatic tumors analyzed by oligonucleotide arrays. Activation of the phosphatidylinositol 3'-kinase (PI3K) pathway, often activated in the late phases of human prostate cancers (20), increases FAS transcription at least in part through the activation of SREBPs in prostate cancer cell lines (10). In addition, human mammary epithelial cell lines overexpressing HER-2-neu or transformed with the oncogene H-ras lead to PI3K pathway activation and FAS induction (21, 22). We had previously determined that the expression of Her-2-neu in prostate cancer increases with progression toward androgen independence (23), being highest, as is the case for FAS, in prostate cancer metastatic to bone. It is thus possible that the oncogenic effects of PI3K activation by a variety of oncogenes known to be up-regulated in prostate cancer, such as Her-2-neu, may be mediated, at least in part, via the induction of FAS expression.

Interestingly, inhibition of PI3K in LNCaP cells causes a more dramatic decrease in FAS protein than in FAS mRNA or than in FAS promoter activity. Thus, it has been proposed that regulation of FAS may occur at least in part through posttranscriptional mechanisms, which may involve downstream targets of the PI3K pathway (10). In fact, 30% of our cases showed no concordance between mRNA and protein levels of FAS, supporting the hypothesis of a posttranscriptional regulation of FAS. Therefore, we reasoned that this lack of correlation might be reflected by a biological difference between tumors. Because the detection of different gene expression patterns in tumors, which otherwise displayed similar phenotypic characteristics, would have supported the existence of biologically different subgroups, we sought to identify the transcriptional signatures associated with the different FAS expression patterns. The tumors were divided into four groups based on the median level of FAS mRNA and protein expression and separately analyzed for potential differences in gene expression profiles. No significant differences in gene expression were detected between the two groups of tumors with high FAS mRNA separated on the basis of low and high FAS protein levels, suggesting that high transcriptional activity is sufficient in tumor cells to supply adequate levels of FAS despite a putative posttranscriptional protein down-regulation. In contrast, there was a marked difference in the gene expression profile of tumors with high and low protein FAS levels but relatively low mRNA. Interestingly, no genes involved in the PI3K cascade were part of the transcriptional signature of either group. However, among the genes up-regulated in tumors with low FAS protein were two proapoptotic genes: tumor necrosis factor-related apoptosis-inducing ligand TRAIL and ribosomal protein S5 Rps5. TRAIL, the transcripts of which have been previously detected in human prostate (24, 25), is capable of inducing apoptosis through the caspase cascade in a variety of cancer cells in vitro, including prostate AI cancer cell lines (26) and prostate normal epithelial cells (27–29). Rps5 has been shown to be overexpressed in murine erythroleukemia cells undergoing apoptosis when compared with differentiating murine erythroleukemia cells (30). In contrast, no proapoptotic genes were found to be significantly expressed in the tumors with high FAS protein expression.

Among the genes up-regulated in the tumors expressing high levels of FAS protein was MLL, previously found to be mutated or translocated with consequent generation of oncogenic fusion proteins in acute leukemias of either lymphoid or myeloid lineage (31). These translocations/mutations result in a gain of MLL transcriptional effector function and are associated with an extremely poor prognosis in leukemia (32). However, no association between MLL overexpression and prostate cancer has been found to date. Based on our findings, further studies are needed to explore the biologic significance of MLL overexpression in prostate cancer. Down-regulation of E-cadherin has been previously correlated with increasing tumor grade (33) and with bone metastases (34) in prostate cancer. Consistent with the hypothesis that FAS-overexpressing tumors may behave more aggressively (reviewed in Ref. 1), E-cadherin expression was significantly lower in the tumors with high FAS expression.

Overall, the gene expression profiles suggest that prostate tumors overexpressing FAS protein display a molecular signature that suggests an association with aggressive biologic behavior as a result of the absence of a proapoptotic signature, via the overexpression of oncogenic proteins, or both.

The increase in FAS expression in AI prostate cancers metastatic to bone, taken together with concomitant SREBP overexpression, suggests an AI transcriptional mechanism for the induction of FAS overexpression in this setting. Previous studies in animal models also underscore the relevance of FAS overexpression in AI tumors. In the CWR22 model of prostatic adenocarcinoma, FAS protein expression, tumor size, and proliferation rate were decreased after castration and returned to normal with androgen replenishment (13). However, tumors in castrated mice, which relapsed after a long latency, showed high levels of FAS protein. In addition, in nonrelapsed CWR22 tumors dormant in castrated mice, FAS, while mostly suppressed, was overexpressed in selected foci characterized by high Ki-67 and low p27 expression. One may speculate that these represent areas capable of full-fledged development of AI adenocarcinoma in an androgen-depleted environment (13). Finally, growth suppression by FAS inhibitors has been achieved in some AI prostatic cancer xenografts (12). Our results, taken together with the previous studies, point to FAS as a potential target in AI cancer and as in the prevention of cancer progression to androgen independence.

While the relationship between FAS and apoptosis appears to be well established, (21, 35–37), FAS may also play a role in tumor cell proliferation. FAS inhibition has been shown to decrease DNA synthesis and not to allow cells to progress into S phase (37, 38). It has been proposed that the link between FAS and DNA replication may be a result of the need for de novo synthesis of membrane phospholipids necessary for DNA replication. In fact, among the genes up-regulated in the group of tumors with high FAS protein was apolipoprotein E, which is a major regulator of phospholipid metabolism (39, 40). It is interesting to note that estrogens have been shown to down-regulate serum levels of apolipoprotein E in prostate cancer patients (41). In addition, the increase in the level of the enzymes responsible for fatty acid synthesis (FAS and acetyl-CoA carboxylase-α) is not associated with an increase in the level of the enzymes involved in cholesterol synthesis in prostate cancer tissue (5). The resulting altered cholesterol/phospholipid ratio may affect the fluidity of plasma membranes with possible...
consequences on the organization of membrane-bound proteins involved in the regulation of cell proliferation (42). Furthermore, membranous fatty acids have an important function as second messengers in pathways such as protein kinase C, mitogen-activated protein kinase, and PI3K pathways, all of which are involved in cell proliferation.

In summary, it is clear that FAS plays an important role in prostate carcinogenesis and tumor progression. Our data provide evidence of a different molecular signature of prostate cancers with or without FAS overexpression and pave the way for the investigation on the mechanism of action of FAS in prostate tumor cell survival, proliferation, and response to apoptosis-inducing chemotherapy agents.

Materials and Methods

Patients Population

Specimens from 1995 to 1997 were obtained from radical prostatectomy performed at Brigham and Women’s Hospital. Bone marrow biopsies from seven patients with metastatic AI prostatic tumors were obtained from the Beth Israel Deaconess Medical Center. Institutional Review Board approval was obtained for all cases.

The expression of FAS was assessed in a series of 81 patients including 74 primaries and 7 patients with AI bone metastases. Each of the primary samples was reviewed to determine the TGS that represents the sum of two dominant Gleason scores in the same tumor. Among the 81 patients, 59 (52 primaries and 7 metastases), in which a frozen tissue block had cancer present on opposing sides, were selected to assess mRNA FAS expression by Affymetrix Hu95Av2 oligonucleotide arrays (Affimetrix, Santa Clara, CA; 43). In 70 patients (64 primaries and 6 metastasis), in which paraffin-embedded tissue was available for the construction of TMAS, FAS protein was determined by IHC. FAS protein expression was also assessed in HG-PIN, which was present in 33% of cases.

Direct comparison between FAS mRNA and protein levels was determined in 48 patients (42 primary tumors with corresponding normal tissue and 6 metastases), in which a frozen tissue block with cancer on opposing sides and corresponding paraffin-embedded tissue were available.

FAS Messenger RNA Expression Measurements

Total RNA was extracted from OCT-embedded specimens after tissue homogenization using Trizol reagent. Biotin-labeled cRNA was synthesized from 10 to 20 µg of total RNA from each of the 52 tumor/normal sample pairs and from the 7 metastatic tumor samples and hybridized to Hu95Av2 microarrays containing 12,600 features (genes, ESTs, and controls) and using standard protocols as previously described (43). The complete gene array analysis of the primary tumors has been previously published by our group (43). The expression of FAS (accession number 38429_at) was determined for each sample after the samples were scaled together to minimize technical variability. To determine if significant gene expression differences exist between two classes of tumor samples (i.e., tumors with low or high expression of FAS protein in the setting of low RNA expression), we performed class distinction using a signal-to-noise (S2N) metric (44). In order to determine if the number of genes meeting the class distinction of interest was greater than that expected by chance alone, permutation testing was performed by randomizing class distinctions 1000 times and repeating the S2N measures. The observed S2N metric for each gene with the class distinction was compared with that from the permuted data at \( P = 0.05 \), and those genes with S2N metrics exceeding that from permuted cases were considered significant (43, 45).

FAS Nearest Neighbor Analysis

To determine which genes had expression most strongly correlated with FAS, the expression files for 52 local tumors, 7 metastatic prostate cancers, and 3 normal bone marrow samples were scaled together (based on the mean expression of all genes on each array) and analyzed. As a preliminary step in the analysis, gene expression, as measured by the microarrays, was set at upper and lower thresholds (16,000 and 10, respectively), and genes, which did not have at least a 5-fold difference in expression levels between any two samples, were excluded. For the 7206 genes that passed this filter, the correlation between each gene’s expression and that of FAS was determined using Pearson’s correlation coefficient. Genes were subsequently ranked according to the degree of correlation.

TMA Construction and IHC

TMAs were prepared from 42 patients, the tissue of which had been analyzed by oligonucleotide microarrays, and from 22 additional patients. Tissue samples were fixed in 10% buffered formalin, embedded in paraffin, and used to construct TMA. H&E-stained sections were made from each selected primary tumor block (donor blocks) to define representative tumor regions. With the use of the tissue arrays instrument (Beecher Instruments, Silver Spring, MD), five tissue cylinders (0.6 mm in diameter) were punched from two regions of the donor block representative of the two predominant Gleason-grade patterns, reflecting the overall Gleason score in the final pathology report of each patient. Five normal areas, five HG-PIN, when present,
and five tumor areas (745 cores) were arrayed for each patient. Tissue cylinders were placed on five 25 × 35-mm paraffin blocks to produce the TMA blocks used for IHC. Immunostaining was performed in all tissue samples using the primary monoclonal antibody against FAS (Transduction Laboratories, Lexington, KY) at 1:2000 dilution. Five-micrometer sections were cut from the TMA blocks and from six blocks of bone metastasis of AI prostatic tumors, which had been also analyzed by oligonucleotide arrays. The sections were deparaffinized, rehydrated, and subjected to microwaving in 10-mM citrate buffer (pH 6.0) in a 750-W oven for 15 min. The primary antibody was applied at room temperature in an automated stainer (Optimax Plus 2.0, Biogenex, San Ramon, CA). Standardized 3,3-diaminobenzidine development times allowed accurate comparison of all samples. Substitution of the primary antibody with PBS served as negative control. Staining for FAS was either uniformly positive or negative.

Scoring of IHC Status

Each tissue cylinder was evaluated for degree of cytoplasmic staining intensity (0 negative; 1 weak positive; 2 moderate positive; 3 strong positive), and the average of the five scores (one per TMA cylinder) was used as a single value for each specimen. If HG-PIN was present, this was also noted and scored for degree of immunohistochemical staining.

Statistical Analysis

A two-tailed t test was used to compare the average expression of FAS between tumor and normal samples and between tumors with different TGS at both mRNA and protein levels. The same test was also used to compare these values with the average FAS expression in areas with HG-PIN assessed by TMAs. FAS mRNA and protein levels were compared for each tumor sample using z² test for qualitative data, with the median values of mRNA and protein FAS, calculated among the 52 and 64, respectively, as cutoff. In all statistical tests, a P value of 0.05 was considered statistically significant.

References


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