IGFBP-3 Is a Metastasis Suppression Gene in Prostate Cancer

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Abstract

The insulin-like growth factor binding protein IGFBP-3 is a proapoptotic and antiangiogenic protein in prostate cancer (CaP). Epidemiologic studies suggest that low IGFBP-3 is associated with greater risk of aggressive, metastatic prostate cancers, but in vivo functional data are lacking. Here we show that mice that are genetically deficient in IGFBP-3 exhibit weaker growth of primary prostate tumors but higher incidence of metastatic disease. Prostates in IGFBP-3 knockout mice (IGFBP-3KO mice) failed to undergo apoptosis after castration. Spontaneous prostate tumors did not develop in IGFBP-3KO mice, but splenic lymphomas occurred in 23% of female IGFBP-3KO mice by 80 weeks of age. To assess the effects of IGFBP-3 deficiency on prostate cancer development, we crossed IGFBP-3KO mice with a c-Myc–driven model of CaP that develops slow-growing, nonmetastatic tumors. By 24 weeks of age, well-differentiated prostate cancers were observed in all mice regardless of IGFBP-3 status. However, by 80 weeks of age IGFBP-3KO mice tended to exhibit larger prostate tumors than control mice. More strikingly, lung metastases were observed at this time in 55% of the IGFBP-3KO mice but none in the control animals. Cell lines established from IGFBP-3KO:MyC tumors displayed more aggressive phenotypes in proliferation, invasion, and colony formation assays, relative to control Myc tumor cell lines. In addition, Myc:IGFBP-3KO cells exhibited evidence of epithelial–mesenchymal transition. Our findings established a function for IGFBP-3 in suppressing metastasis in prostate cancer, and they also offered the first reported transgenic model of spontaneous metastatic prostate cancer for studies of this advanced stage of disease. Cancer Res; 71(15); 5154–63. ©2011 AACR.

Introduction

The acquired capability of tumor cells for tissue invasion and metastasis has been defined as a ‘hallmark of cancer’ (1) and currently no curative therapies exist for prostate cancer (CaP) that has metastasized (2). A chemotherapeutic agent, docetaxel, is the only approved therapy that has been shown to prolong survival among men with this condition (3); although a recent study reports improved survival among men with this condition (3); although it was identified by microarray analysis as the most reduced transcript in CaP cells obtained through laser microdissection relative to neighboring normal prostatic cells (9). We published evidence that showed the antiangiogenic and proapoptotic effects of IGFBP-3 on CaP in vivo (10, 11).

Several clinical studies have shown that serum IGF-I is elevated and IGFBP-3 levels reduced in patients before the diagnosis of CaP and that increased serum levels of IGFBP-3 are associated with a decreased risk and better prognosis of CaP (12–14). Importantly, an IGFBP-3 promoter polymorphism has been associated with increasing risk for CaP metastasis and for having tumors with a biologically more aggressive phenotype (15).

We studied the effect of crossing mice with a genetic deletion of IGFBP-3 with mice expressing human Myc in the prostate (16). These Myc mice reliably develop murine prostatic intraepithelial neoplasia, then locally invasive adenocarcinomas. In addition, these tumors share molecular features with human prostate cancer. We describe an important requirement for IGFBP-3 in prostate apoptosis induced...
by androgen deprivation and identify a role for IGFBP-3 as a metastasis suppression gene.

Materials and Methods

Mouse colony, castration, tissue collection, preparation, and necropsy

Animal experiments were approved by the Animal Research Committee (ARC) of the University of California, Los Angeles and according to NIH guidelines. IGFBP-3-KO (knockout) and WT (wild-type) mice were bred and homozygous littersmates were used in the study. Male and female pups were weaned, weighed, and genotyped at 3 weeks of age and maintained on normal chow.

Ten-week-old adult male mice were castrated or sham operated in survival surgeries and the ventral and dorsolateral prostate was removed after 2 or 3 days. Briefly, animals were anesthetized with methoxyflurane (generic name), a lateral incision was made into the scrotum, and the testes ligated and removed. The incision was closed with surgical silk.

Prostates and testes were fixed and stained with hematoxylin and eosin (H&E) for morphologic analysis and quantification of apoptosis by using a terminal deoxynucleotidyltransferase dUTP nick-end labeling (TUNEL) strategy (see below). TUNEL staining was done according to established procedures (10). Apoptosis was quantitated by scoring 500 epithelial cells per field in 5 random fields per tissue section.

Tissues collected at necropsy were routinely fixed in 10% (v/v) phosphate-buffered formalin for 6 hours and then transferred to 70% ethanol. Sections were cut from paraffin-embedded tissues and mounted on slides. Routine sections were stained with H&E.

Generation of IGFBP-3-KO:Myc and WT:Myc mice

The PB-Hi-Myc (Myc) mouse was derived at UCLA and was a kind gift of Dr. Charles Sawyers (16). These were bred with IGFBP-3KO mice, originally developed in collaboration with Lexicon Pharmaceuticals, Inc. (17). The Myc model was generated by utilizing the probasin promoter (ARR2PB) to transfer the c-myc gene to the mouse prostate. To avoid potential genetic variations, only F3 generation of male offspring was used in this study.

Genotyping

Female Myc mice were crossed with male IGFBP-3-KO mice to produce the F1 generation. F1 mice, heterozygous for myc transgene and igfbp-3 gene (IGFBP-3+/+ Myc+/−) were mated with heterozygous IGFBP-3 (BP-3+/− Myc−/−) mice to generate F2 mice. The expected F2 genotypes were IGFBP-3−/− Myc−/−, IGFBP-3−/− Myc+/−, IGFBP-3−/− Myc−/−, IGFBP-3−/− Myc+/−, and IGFBP-3−/− Myc−/−. IGFBP-3−/− Myc−/− was characterized in this study.

Genotypes of all offspring were analyzed by PCR with genomic DNA isolated from ear and tail clippings by using the following primer pairs: For Myc-5′-ATGATAGCATC-TTGTTCTTATGCCTTCTTTTATTAAG-3′ and 5′-GGTATC-TGGACCTCAGTA CAAGGTCGAGAG-3′ (PCR product will consist of 2 bands 400 bp and 200 bp). For IGFBP-3-KO, 5′-TAAGGTCTCAGCATCAGAG-3′ and 5′-CCCTAG-GAATGCTGTCACAAGA-3′ (PCR product = 288 bp). For IGFBP-3-WT, 5′-TGCGAGCGCATACATCCT-3′ and 5′-CCCCAGGCTATTTCACCCAATT-3′ (PCR product = 164 bp). Only the male IGFBP-3-KO:Myc and IGFBP-3-WT:Myc of F4 or later generations were used for experiments.

Biochemical analysis

Mouse circulating IGF-1 and IGFBP-3 were measured by using an in-house generated ELISA as previously described (18). Antibody and standard for IGF-1 and IGFBP-3 were obtained from R&D Systems.

Tissue RNA isolation and RT-PCR

Total RNA was isolated from 80-week-old male WT:Myc prostate and lung tissue and from IGFBP-3-KO:Myc prostate and lung metastasis by using Trizol reagent following the manufacturer's protocol (Invitrogen) followed by purification by using the Qiagen RNeasy kit (Qiagen). One microgram of total RNA was treated with DNase I and reverse transcribed by using Superscript III and oligo dT following the manufacturer recommendations (Invitrogen). Human c-myc 420-bp fragment and mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH) 600-bp fragment were amplified by standard PCR by using 5′ HotMasterMix (primers) and specific primers. Primers for human c-myc were 5′-CTCCTTGCCAAAAGGTCAGAG-3′ (forward) and 5′-ACGTTTTGCTCTTCGTGCTTG-3′ (reverse). Primers for mouse GAPDH were 5′-TTCCACCACATTGGAAGGCG-3′ (forward) and 5′-GGGAAATGAGCTGGTCAA-3′ (reverse). The PCR conditions were as follows: 94°C for 3 minutes; 94°C for 1 minute, 50°C for 1 minute, 72°C for 1 minute for 35 cycles followed by 10 minutes at 72°C for final elongation by using a Peltier Thermal Cycler PTC 200 (MJ Research). PCR products were run on a 1.5% agarose/ethidium bromide gel.

Initiation and isolation of WT:Myc and IGFBP-3-KO:Myc mouse primary prostate cancer cell lines

WT:Myc and IGFBP-3-KO:Myc mouse primary prostate cancer cell lines were derived from 2 each of 37-week-old WT:Myc and IGFBP-3-KO:Myc mice. The different lobes of prostate were dissected under the dissecting microscope and then transferred to Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS plus 100 μg/mL penicillin and 100 μg/mL streptomycin. After washing with PBS 3 times, the tumor tissues were cut into 1 mm3 pieces and incubated with collagenase for 1 hour to eliminate fibroblast contamination in the 37°C incubator containing 5% CO2. The cells were resuspended in DMEM containing 10% FBS and 0.1 nmol/L R1881 after centrifugation at 800 × g for 5 minutes and grown as monolayers in flasks at 37°C with 90% humidity and 5% CO2. The culture medium was changed twice weekly until a cell line was obtained.

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confluent monolayer was established. The cells were passaged 4 to 10 times until use.

**Immunofluorescence confocal microscopy**

Thirty thousand cells per chamber were plated. Cells were fixed for 10 minutes in 4% paraformaldehyde in PBS and permeabilized in 0.2% Tween 20. Incubations with primary antibody and secondary reagent were done for 1 hour at room temperature. Endogenous IGFBP-3 was detected with anti-human IGFBP-3 goat polyclonal antibody (R&D Systems), followed by Alexa Fluor 488 (Molecular Probes). Counterstaining of nuclei was done with 4′,6-diamidino-2-phenylindole and cells were mounted in ProLong Gold antifade reagent (Molecular Probes). Acquisitions were done by using a Leica DM IRE2 Microscope and Leica Confocal Software.

**Western immunoblots**

Protein extracts were prepared by homogenizing the cells in NP-40 lysis buffer supplemented with Complete Protease (Roche) and phosphatase inhibitors. Soluble proteins were boiled in 2× SDS sample buffer, resolved on a 4% to 20% Tris-HCl gel (Biorad), and transferred to polyvinylidene difluoride membranes. Membranes were incubated with antibodies against Myc, E-cadherin, and vimentin (BD Biosciences); N-cadherin, fibronectin, and SM22 (Abcam); Smad4 (Cell Signal- ing); androgen receptor (AR; Invitrogen); and β-actin (Sigma). Membranes were incubated with horseradish peroxidase–linked secondary antibodies, washed, and antigen–antibody complexes were visualized by using enhanced chemiluminescence detection (Millipore).

**In vitro invasion assay**

The ability of prostate cancer cells to invade was examined in 24-well cell culture chamber inserts (BD Matrigel Invasion Chamber, 8.0 Micron; BD Biosciences). WT:Myc and IGFBP-3KO:Myc mouse primary tumor cells were harvested by trypsinization, washed, and resuspended in phenol red–free medium containing 0.1% (w/v) bovine serum albumin. The number of living cells was counted by using 0.4% trypan blue (Sigma) exclusion. Only cells with viability 95% or more were used for further experiments. About 7 × 10^4 tumor cells in 200 μL of medium were added to the upper chamber with or without recombinant human IGFBP-3 (kind gift of Insmed Incorporated) in the concentrations indicated. The lower compartments were filled with 700 μL of conditioned medium, used as a chemoattractant, collected from 3 × 10^6 NIH/3T3 fibroblasts incubated overnight in growth medium containing 10% fetal calf serum. After 24 hours of incubation at 37°C under 5% CO2, the unattached cells in the upper chamber were swabbed and the invasive cells in the lower chamber were stained with 0.01% crystal violet and then random fields were photographed under a microscope. After elution with 0.5 mL of 0.01% crystal violet solution for 10 minutes and counted by using a dissecting microscope (50× magnification) for each triplicate sample. The average values are presented as mean ± SD.

**In vitro cell growth kinetics**

To determine cell growth kinetics, cells were grown in DMEM medium containing 10% FBS and 0.1 mmol/L R1881 (PerkinElmer Life Sciences) in 96-well dishes at an initial density of 2,000 cells per well. Media was changed every 3 days. On days 1 through 5, cells were incubated with 10 μL of Cell Counting Kit-8 (CCK-8; Dojindo) for 3 hours, then absorbance at 450 nm was measured by using an ELISA plate reader (Spectra MAX 190; Molecular Devices).

**Gene expression microarray analysis**

Whole-genome expression analysis was done with the Affymetrix Mouse Gene Array ST 1.0 (Affymetrix) at the UCLA DNA Microarray Core. RNA from WT:Myc and IGFBP-3KO: Myc primary prostate tumor cell lines was prepared by using manufacturer recommended protocols and RNeasy (Qiagen) columns. Each sample was labeled by using standard protocols and reagents from Affymetrix. Probes were fragmented and hybridized to the Affymetrix Human Gene 1.0 ST Array. Standard wash, stain, and scanning protocols were used.

**Data analysis**

GeneSpring GX 10.0.2 software (Agilent Technologies) was used to analyze the raw Excel files. The Exxon RMA16 probe summarization algorithm, with a transcript level of core was used. The lower 20th percentile of the data were filtered out, leaving 23254/28815 transcripts. We compared the experimental group to the control group with a fold change of 3, to obtain lists of genes with differential expression. The genes were clustered with the following parameters: Clustering Algorithm: Hierarchical; Similarity Measure: Euclidean; Linkage Rule: Centroid. We used the DAVID program (19) to identify functional groups represented in these lists. Gene lists were tested for significant pathways by using the MetaCore pathway web application by GeneGO, Inc.

**Statistics**

All in vitro experiments were repeated at least 3 times. Means ± SD are shown. Statistical analyses were done by using t test, ANOVA, or Fisher’s exact tests by using Instat as indicated (GraphPad). Differences were considered statistically significant when *, P < 0.05 and when **, P < 0.005.

**Results**

**IGFBP-3 is required for androgen deprivation–induced apoptosis**

Multiple genes, including IGFBP-3 (20, 21) are upregulated in the prostate in response to androgen deprivation; however, many of them [including Clusterin (22) and Bax (23)] have been shown not to be essential to prostatic apoptosis utilizing
KO mouse models. To assess the functional contribution of IGFBP-3 to prostate apoptosis in response to androgen deprivation, we utilized the IGFBP-3KO mouse (17). Ten-week-old male WT mice and IGFBP-3KO mice were surgically castrated and prostates were harvested after 48 and 72 hours. The histologic appearance of both testes and prostates from IGFBP-3KO mice precastration did not differ from their control littermates at baseline as determined by H&E staining (data not shown). As expected, WT mouse prostates showed a dramatic 6-fold increase in the number of TUNEL-positive nuclei at 48 hours postcastration (Fig. 1A). However, IGFBP-3KO mice prostates failed to show any significant increase in TUNEL staining at 48 hours. By 72 hours, TUNEL staining returned to near baseline levels in WT mice and remained near baseline levels in IGFBP-3KO mice. Serum IGFBP-3 levels were undetectable in the KO mouse and remained unchanged in WT mice postcastration (Fig. 1B). Whereas data are conflicting on whether apoptosis secondary to androgen ablation is modified in p53 deficient mice (24, 25), we have now shown that IGFBP-3, which is activated downstream of p53 (26), is required for this process.

We did not observe spontaneous prostate tumor development with aging in IGFBP-3KO mice. However, the incidence of splenic lymphomas in female IGFBP-3KO mice was 23%; no splenic lymphomas were observed in WT mice (data not shown).

**Paradoxical progression of CaP in IGFBP-3KO:Myc mice**

To investigate the significance of endogenous IGFBP-3 in the development of prostate cancer in vivo, we crossed IGFBP-3KO mice with the Myc model of prostate cancer (expressing human c-Myc driven by a prostate-specific promoter; ref. 16). These Myc mice reliably develop murine prostatic intraepithelial neoplasia (PIN), then adenocarcinomas. Myc mice develop relatively slow-growing, nonmetastatic tumors. Surprisingly, at 17 weeks of age, the majority of prostates of IGFBP-3KO:Myc mice did not develop cancer and only displayed PIN, whereas control Myc mice had well-differentiated prostate cancer (Fig. 2A). We measured serum IGF-1 levels in these mice and found that it was decreased by more than 40% in the IGFBP-3KO:Myc mice when compared with controls (Fig. 2B), thus providing a possible mechanism for the decreased cancer observed in the KO animals. This finding correlates well with clinical studies showing serum IGF-1 levels to be an important determinant of early CaP (12, 27) in humans and mice (28, 29). By twenty-four weeks of age, well-differentiated cancer was observed in all mice, irrespective of IGFBP-3 status (Supplementary Fig. S1A). By eighty weeks of age, prostate carcinoma was observed in all mice; however, tumors tended to be bigger in IGFBP-3KO:Myc mice (Supplementary Fig. S1B) with some very large tumors in IGFBP-3KO:Myc mice, 20% of which weighed more than 4 g (Supplementary Fig. S1C).
Increased metastasis in IGFBP-3KO:Myc mice

When IGFBP-3KO:Myc mice were necropsied at eighty weeks of age, prostate tumors tended to be larger in the IGFBP-3KO:Myc mice when compared with WT (Supplementary Fig. S1B). In addition, we identified lung metastases in 55% of IGFBP-3KO:Myc mice, although none of the WT:Myc mice exhibited any metastases (Fig. 3A and B). This is a remarkable finding, as metastases in the Myc model of prostate cancer have not been described to date, and suggests a major antitumorigenic role for endogenous IGFBP-3 as a metastasis suppressor.

We characterized the presumptive lung metastases by immunohistochemical staining for Clara Cell 10 (CC10) protein, a marker of bronchiolar epithelial cells (30). CC10 is expressed in the lung in nonciliated airway epithelial cells and is not expressed in prostate cells. Most primary lung adenomas and adenocarcinomas are of Clara cell origin.

As shown in Figure 4A, normal lung parenchyma stains positive for CC10 and the lung nodule does not, showing that the observed lung nodules are not of lung origin. Occasionally, some mice developed metastatic nodules in visceral organs, including liver and intestine (Fig. 3C and D). Reverse transcriptase PCR (RT-PCR) analysis for the human Myc gene verified expression in the lung nodules, thereby confirming their metastatic nature (Fig. 4B).

Establishment and characterization of prostatic epithelial cell lines from the WT:Myc and IGFBP-3KO:Myc mouse models

To further our investigation of the molecular mechanisms underlying IGFBP-3-controlled prostate tumorigenesis and metastasis, we isolated primary prostatic cells from WT:Myc and IGFBP-3KO:Myc tumors. After serial passages (see Materials and Methods for details), several clonally derived cell lines were established and from which 2 lines each were characterized and studied herein. A representative cell line of each is presented. Confocal immunohistochemistry for IGFBP-3 confirmed absence of IGFBP-3 in the IGFBP-3KO:Myc line (Supplementary Fig. S2).

To determine the properties of these established cell lines, we carried out Western blot analysis by using antibodies corresponding to phenotypic markers in the prostate epithelium. As shown in Figure 5A, both WT:Myc and IGFBP-3KO:Myc cell lines maintained AR expression. Protein expression of E-cadherin (31) did not change and IGFBP-3KO:Myc phenotype was associated with a loss of fibronectin expression. Expression of N-cadherin (32) increased with decrease in expression of Smad4 (33). This change in markers is consistent with the more aggressive phenotype of the IGFBP-3KO:Myc cells. In addition, vimentin, a well-known marker of epithelial-mesenchymal transition (34), was significantly induced in the IGFBP-3KO:Myc cell line. Whereas many of the observed epithelial-to-mesenchymal transition (EMT) markers are consistent with the more invasive phenotype of the IGFBP-3KO:Myc cells (vimentin, N-cadherin, Smad4), others are not consistent (E-cadherin, fibronectin); implying a complex contribution of IGFBP-3 loss to EMT that may be complicated in part by low serum IGF-1 levels (35).

To measure the biological effects of igfbp3 gene deletion in Myc CaP cells, we first compared cell growth properties of the
WT:Myc and IGFBP-3KO:Myc cell lines. As shown in Figure 5B, cell proliferation rates were significantly increased to over 3-fold at 120 hours in the IGFBP-3KO:Myc cell line as compared with WT:Myc cells, suggesting that IGFBP-3 plays a role in the cell-cycle progression of Myc prostatic epithelial cells.

**IGFBP-3 loss leads to increased colony formation and invasiveness of CaP cells in vitro**

To determine whether genetic deletion of IGFBP-3 leads to enhanced transformation potential, we compared the colony forming efficiency of WT:Myc and IGFBP-3KO:Myc cell lines. After 14 days in culture, colonies were photographed and counted. IGFBP-3KO:Myc cells formed over 4-fold more colonies when compared with WT:Myc cells (Fig. 6A).

In addition, to evaluate the relation between cell migratory ability and the expression of IGFBP-3, we conducted a migration assay by using a modified Boyden chamber method. The invasiveness of IGFBP-3KO:Myc cells when compared with WT:Myc cells was increased over 3-fold (Fig. 6B). In a separate experiment, invasiveness was increased even more significantly in IGFBP-3KO:Myc cells when compared with WT:Myc cells. Addition of recombinant IGFBP-3 to IGFBP-3KO:Myc cells culture reversed this phenotype in a dose-dependent manner (100 and 1,000 ng/mL; Fig. 6C). Thus taken together, these data support a role for IGFBP-3 in metastasis suppression in prostate cancer.

**Differential gene expression in WT:Myc and IGFBP-3KO:Myc primary prostate tumor cell lines**

Differential gene expression was examined by using microarray analysis. Microarray analysis (28,815 genes) revealed that 462 genes were upregulated and 760 genes were downregulated (fold-change >3.0; \( P < 0.05 \); see Supplementary Table S1 for complete list; Fig. 7A). Gene lists were tested for significant pathways by using the MetaCore pathway web application by GeneGO, Inc. The most significant pathway maps were ranked by the negative log of the calculated hypergeometric \( P \) value. Genes differentially expressed fell into many different pathways, but the 2 most significant pathways include cell adhesion and extracellular matrix remodeling, and development/regulation of EMT, important pathways in the development of cancer metastasis (Fig. 7B). SM22 (also known as Transgelin) was downregulated 60-fold in IGFBP-3KO:Myc cells compared

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**Figure 4.** A, CC10 immunohistochemical staining of a representative lung nodule and adjacent normal lung tissue in a IGFBP-3KO:Myc mouse. B, RT-PCR of human Myc of primary tissue RNA from mouse necropsy confirming metastatic nature of lung nodules.

**Figure 5.** A, expression of characteristic proteins in primary WT:Myc and IGFBP-3KO:Myc cell lines by Western immunoblot. B, cell proliferation curves of WT:Myc and IGFBP-3KO:Myc cell lines by MTT assay. *, \( P < 0.05 \) for entire curves by ANOVA.
with WT:Myc cells in the array. A significant decrease in SM22 expression was confirmed by immunoblot (Fig. 7C). SM22 is an actin cross-linking protein and functions as a tumor suppressor. Its expression in prostate cancer is decreased (36).

**Discussion**

The tumor-suppressive properties of IGFBP-3 have been intensely studied in our laboratory and by others in vitro and include IGF sequestration (6), mediation of antigrowth signals...
(37, 38), induction of cancer cell apoptosis (39), senescence association (7), and its effects as an antiangiogenic agent (11). Molecular mechanisms underlying these effects in CaP cells include rapid cellular internalization via Caveolin-1 binding and nuclear localization (40, 41) as well as activation of the intrinsic apoptosis pathway via association with the nuclear receptors RXRα and Nur77 and nuclear export with subsequent mitochondrial translocation (39, 42, 43). Importantly, we have established an important physiologic role for IGFBP-3 in apoptosis induced by androgen deprivation.

Recent studies have hinted at a role for IGFBP-3 as a metastasis suppression gene. In a study of ovarian epithelial cancer cell with invasion-related sublines, microarray analysis revealed IGFBP-3 to be one of the most suppressed genes in the most invasive subline P4. Re-expression of IGFBP-3 in P4 effectively inhibited cell migration, invasion, and metastasis but did not affect cell proliferation. In patients with EC tumors, low IGFBP-3 expression correlated clinically with higher tumor grade, advanced stage and poor survival (44). Moreover, in non–small cell lung cancer (NSCLC) cells, non-cytotoxic doses of adenoviral or recombinant IGFBP-3 significantly decreased the migration, invasion, and metastatic potential of H1299 and A549 NSCLC cells in vitro and in vivo (45). Furthermore, in patients with previously untreated metastatic colorectal cancer, higher baseline circulating levels of IGFBP-3 were associated with a significantly greater response rate to chemotherapy and a longer time to tumor progression and overall survival, even after adjusting for other potential predictors of patient outcome (46).

In men with prostate cancer, plasma IGFBP-3 levels were lowest in patients with bone metastases with lower preoperative IGFBP-3 levels and biopsy Gleason score being independent predictors of biochemical progression and failure related to cancer aggressiveness (14). Importantly, an A/C polymorphism at position –202 in the promoter region of IGFBP-3 was correlated to increased risk of metastatic disease in a Japanese study, with those men with CC and AC genotype having a significantly increased risk of metastatic disease (stage D) than those with AA genotype. Therefore, the authors concluded that the presence of the C allele may cumulatively increase the risk for tumor metastasis and for having tumors with a biologically more aggressive phenotype (15).

In genetic mouse models of prostate cancer, many are aggressive and metastasize at an early age. These include the following: prostate PTEN deletion (12–29 weeks; ref. 47), TRAMP (12 weeks; ref. 48), and prostatic p53 and Rb inactivation (28 weeks; ref. 49). However, of the published genetic studies utilizing the Myc mouse, none have reported metastasis (16, 50, 51), making our observation of metastasis in IGFBP-3KO:Myc mice remarkable. Interestingly, a list of 18 prostate cancer "signature" genes delineated in metastasis-prone prostatic p53 and Rb inactivated mice (49) includes the IGFBP-3 binding partner Caveolin-1 (40), the polycomb protein EZH2 that is known to repress IGFBP-3 expression (52, 53), the proto-oncogene and IGFBP-3 regulator Myb (54), and the homeobox tumor suppressor NKX3.1 that activates IGFBP-3 expression and whose actions are partially mediated by IGFBP-3 (55).

These important findings expand and confirm a novel role for IGFBP-3 (previously considered to function as a tumor suppressor), as an antimetastasis gene, further supporting the targeting of IGFBP-3 as therapy in prostate cancer patients. Clearly, IGFBP-3 expression could represent a common signaling nexus in the CaP tumor suppression and further work into the molecular mechanisms that underlie its tumor-suppressive effects may reveal targets for therapy in men with prostate cancer.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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