Global \textit{Igfbp1} deletion does not affect prostate cancer development in a c-Myc transgenic mouse model

Ashley Gray\textsuperscript{1}, William J Aronson\textsuperscript{2}, R James Barnard\textsuperscript{1}, Hemal Mehta\textsuperscript{3}, Junxiang Wan\textsuperscript{3}, Jonathan Said\textsuperscript{4}, Pinchas Cohen\textsuperscript{3} and Colette Galet\textsuperscript{2}

\textsuperscript{1}Department of Integrative Biology and Physiology, University of California at Los Angeles, Los Angeles, California, USA
\textsuperscript{2}Department of Urology, \textsuperscript{3}Division of Pediatric Endocrinology and \textsuperscript{4}Department of Pathology, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, California, USA

(Correspondence should be addressed to W J Aronson; Email: waronson@ucla.edu)

Abstract

Circulating insulin-like growth factor binding protein 1 (IGFBP1) levels vary in response to nutritional status, and pre-clinical studies suggest that elevated IGFBP1 may be protective against the development and progression of prostate cancer. We hypothesized that global deletion of \textit{Igfbp1} would accelerate the development of prostate cancer in a c-Myc transgenic mouse model. To test our hypothesis, c-Myc transgenic mice (Myc/BP-1 wild-type (WT)) were crossed and interbred with the \textit{Igfbp1} knockout mice (Myc/BP-1 KO). The animals were placed on a high-protein diet at weaning, weighed every 2 weeks, and euthanized at 16 weeks of age. Prostate histopathology was assessed and proliferation status was determined by Ki-67 and proliferating cell nuclear antigen analyses. IGF-related serum biomarkers and body composition were measured. No significant difference in the incidence of prostate cancer was observed between the Myc/BP-1 KO and the Myc/BP-1 WT mice (65 and 80\% respectively, \(P=0.48\)). Proliferation was significantly decreased by 71\% in prostate tissue of Myc/BP-1 KO mice compared with Myc/BP-1 WT mice. Myc/BP-1 KO mice exhibited a significant 6-7\% increase in body weight relative to the Myc/BP-1 WT mice that was attributed to an increase in fat mass. Fasting insulin levels were higher in the Myc/BP-1 KO mice without any difference between the groups in fasting glucose concentrations. Thus, contrary to our hypothesis, global deletion of \textit{Igfbp1} in a c-Myc transgenic mouse model did not accelerate the development of prostate cancer. Global \textit{Igfbp1} deletion did result in a significant increase in body weight and body fat mass. Further studies are required to understand the underlying mechanisms for these metabolic effects.


Introduction

Prostate cancer is one of the leading causes of cancer death among men in the United States (Kohler \textit{et al.} 2011). Insulin-like growth factor 1 (IGF1) has been implicated as a possible factor contributing to prostate cancer risk. The crucial role played by IGF1 in regulating cell proliferation, differentiation, and apoptosis has been shown to contribute to the growth and progression of prostate cancer and normal cells \textit{in vitro} (Cohen \textit{et al.} 1991, 1994, Yu & Rohan 2000). In epidemiologic studies, elevated IGF1 levels have been reported to be associated with increased prostate cancer risk in several prospective, case–control, and meta-analysis studies (Mantzoros \textit{et al.} 1997, Chan \textit{et al.} 1998, Wolk \textit{et al.} 1998, Harman \textit{et al.} 2000, Statin \textit{et al.} 2000, Chokkalingam \textit{et al.} 2001, Rowlands \textit{et al.} 2009). However, other investigators have found serum IGF1 levels to have no prognostic value for prostate cancer (Shariat \textit{et al.} 2000, Yu \textit{et al.} 2001).

The majority of IGF1 is bound to one of six soluble, high-affinity IGF-binding proteins (IGFBPs 1–6) that antagonize IGF1 activity by sequestering it away from the IGF1 receptor, thereby inhibiting its actions (Ferry \textit{et al.} 1999, Firth 2002). IGFBP1 binds IGF1 with an affinity higher than that of the IGF1 receptor allowing for a reduction of free IGF1 levels and inhibition of IGF1 receptor signaling (Wheatcroft & Kearney 2009). In addition, IGFBP1, which is primarily expressed in the liver and to a lesser degree in the kidney (Meinbach & Lokeshwar 2006), is acutely regulated in response to diet (Katz \textit{et al.} 1998, Meinbach & Lokeshwar 2006). During hyperglycemic states, IGFBP1 levels are downregulated by elevated insulin levels (Yki-Jarvinen \textit{et al.} 1995, Katz \textit{et al.} 1998).

Fasting sera measurements from men (age 60±3 years) that participated in an 11-day low-fat diet and exercise program were found to have a 20\% reduction in IGF1 levels, 53\% increase in IGFBP1 levels, and 30\% reduction in serum-stimulated LNCaP cell growth. These results remained similar in men that strictly adhered to this lifestyle intervention for over 14 years (Ngo \textit{et al.} 2002). Utilizing the same cohort of men, the addition of IGFBP1 to...
pre-intervention serum induced a significant reduction in cell growth and increased apoptosis of LNCaP cell cultures in an IGF1−dependent manner (Ngo et al. 2003b). Similarly, in a pre-clinical study, SCID mice consuming an isocaloric low-fat diet had reduced LAPC-4 prostate cancer xenograft growth along with 55% increased serum IGFBP1 levels when compared with the high-fat diet group. Serum IGF1 was also positively correlated with serum-stimulated LAPC-4 growth in vivo (P<0.05; Ngo et al. 2003a). These results were reproduced in a transgenic mouse model of prostate cancer in which 7-month c-Myc transgenic mice fed an isocaloric low-fat diet displayed elevated IGFBP1 levels compared with c-Myc mice fed a high-fat diet. Using an ex vivo assay, LNCaP and Myc-CaP cells incubated in media containing serum from the low-fat diet decreased growth compared with cells incubated in serum from mice fed a high-fat diet (Kobayashi et al. 2008). The ability of IGFBP1 to regulate IGF1 activity in response to nutritional status along with epidemiological and experimental evidence of the role of the IGF1 axis in prostate cancer leads us to consider IGFBP1 as a potential target for decreasing prostate cancer risk.

To study whether global deletion of Igfbp1 accelerates the development of prostate cancer, we used the c-Myc transgenic mouse model that overexpresses the human c-MYC oncogene in a prostate-specific manner (Ellwood-Yen et al. 2003). These mice were crossed and interbred with Igfbp1 knockout (KO) mice (C57BL/6; Leu et al. 2003). c-Myc mice develop murine prostatic intraepithelial neoplasia (mPIN) as early as 2–4 weeks of age and the transition to invasive adenocarcinoma occurs between 3 and 6 months. The goal of this study was to evaluate the role of IGFBP1 in prostate cancer development.

Materials and Methods

Animal husbandry and feeding protocol

The experimental protocol was approved by the University of California at Los Angeles Chancellor’s Animal Research Committee, and the animals were cared for in accordance with institutional guidelines. Mice carrying the probasin c-myc transgene (balbc/FVB; Kobayashi et al. 2008) were crossed and interbred with Igfbp1 KO mice (C57BL/6; Leu et al. 2003). c-Myc mice develop murine prostatic intraepithelial neoplasia (mPIN) as early as 2–4 weeks of age and the transition to invasive adenocarcinoma occurs between 3 and 6 months. The goal of this study was to evaluate the role of IGFBP1 in prostate cancer development.

DNA isolation and genotyping

At weaning, ear or tail clips were collected for genotyping purposes. DirectPCR Lysis Reagent (Viagen Biotech, Los Angeles, CA, USA) was used for DNA extraction according to the manufacturer’s instructions. Primers AGR2PB (5’-ATGATAGCATCTTGTCTTAGTCTTATTGGAAGGGG-3’) and probasin (5’-GGAAGAGAAGTTGCCGCAC-3’) were used in PCR analyses to identify the human c-MYC gene. Primers (5’-GAAACAATGGCATTGTCACGG-3’) and (5’-TGCAATCAATACCTGTGGCGCGAC-3’) were used in PCR analyses to identify the Igfbp1 WT gene. Primers (5’-GAAACAATGTGGCATTGTCACGG-3’) and (5’-TTCCATTGTGTCAGCTGCGAC-3’) were used in PCR analyses to identify the Igfbp1 gene deletion (Leu et al. 2003).

Blood and prostate tissue collection

Mice were anesthetized at 16 weeks of age. Blood was collected via cardiac puncture after a 14 h fast. Sera were placed in Capiject Termo (t-mg) tubes (#3T-MG, Terumo Medical Corporation, Elkton, MD, USA) for 15 min at 4°C to allow for complete coagulation of blood specimens. Sera were spun at 3500 g for 15 min to separate blood components. Sera were stored in aliquots at −80°C. Genitourinary organs were harvested all together, immediately rinsed, and placed in ice-cold PBS as described previously (Kobayashi et al. 2008). Using a dissecting microscope, the ventral, dorsal, lateral, and anterior prostate lobes were dissected and were either flash frozen or fixed in 10% buffered formalin for 12 h, washed in running water, and transferred to 70% ethanol before embedding in paraffin blocks.

Pathology

Sections (4 μm) were obtained from paraffin-embedded blocks and stained with hematoxylin and eosin. Histopathologic analysis to determine the presence of mPIN and invasive adenocarcinoma was performed in a blinded fashion by a single pathologist (J S), as described previously (Kobayashi et al. 2008).

Immunohistochemistry

Ki-67 immunostaining was performed as described previously (Kobayashi et al. 2006). The area of adenocarcinoma and the PIN was circled by a single pathologist. A total of five 20X fields were counted for each mouse prostate, and the number of Ki-67-positive cells was scored by a single pathologist. Adenocarcinoma and PIN areas were measured using the ImageScope software (Aperio Technologies Inc., Vista, CA, USA).
**Measurement of mouse serum IGF1, IGFBPs, and insulin levels**

The levels of murine IGF1, IGFBP1, IGFBP2, and IGFBP3 were measured using in-house mouse-specific ELISAs as described previously (Watson et al. 2006, Yakar et al. 2006) using mouse-specific antibodies and recombinant mouse IGF and IGFBP standards. The mouse IGF1 assay has a sensitivity of 0.1 ng/ml and no cross-reactivity with mouse IGF2 or human IGF1. The intra-assay and inter-assay coefficients of variation (CV) were <10% in the range from 1 to 10 ng/ml. The mouse IGFBP1, IGFBP2, and IGFBP3 assays have sensitivities of 0.2 ng/ml and no cross-reactivity with other IGFBPs or the human homologues. The intra-assay and inter-assay CV were <6% and <8%, respectively, in the range 1 to 6 ng/ml. All measurements were taken from the same mice to reduce comparison variation.

Serum insulin levels were measured using an insulin (mouse) ultrasensitive ELISA (Alpco, Salem, NH, USA). Twenty-five µl of sera were run in duplicate. The sensitivity of the assay is 0.019 ng/ml. The intra-assay CV were <7% in the range 0.21 to 0.54 ng/ml. The inter-assay CV were <6% in the range 0.214 to 0.558 ng/ml. The range of recovery is 79–106% with an average of 96%. There is no cross-reactivity with mouse c-peptide 1 and 2, IGF1, IGF2, or the human and rat homologues.

**Glucose measurements**

Fasting glucose concentrations were measured in duplicates using the Freestyle Freedom Lite Blood Glucose Monitoring System (Abbott Laboratories). Glucometer measurement range was 20–500 mg/dl. Blood was obtained from tails prior to anesthesia. If duplicate concentrations varied broadly, a third measurement was obtained and averaged.

**Quantitative magnetic resonance measurements**

Minispec (Bruker Optics, Woodlands, TX, USA) analyzer and software from Echo Medical Systems (Houston, TX, USA) were utilized to precisely determine the fat mass, lean body mass, and free fluid in conscious live mice using quantitative magnetic resonance (QMR; Taicher et al. 2003). This method provides noninvasive and rapid measurements without anesthetics. Mice were placed in a clear cylinder tube with a stopper placed inside the tube to restrict the movement of the mouse during analysis. The procedure lasted ≤2 min/mouse. No observable changes in mouse behavior were observed after measurement was made. All measurements were taken on the day prior to euthanasia in a non-fasted state. This technique has a CV of 0.9–1.6% for body fat and 2.2–3.7% for lean body mass along with a strong correlation of 0.81 between QMR and dual-emission X-ray absorptiometry (DEXA) measurements in C57BL/6 mice (Taicher et al. 2003).

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**Western blot analysis**

Mouse prostate tissue lysates were prepared in 100 µl radioimmunoprecipitation assay buffer supplemented with an EDTA-free protease inhibitor cocktail and Phos-STOP phosphatase inhibitor tablets (Roche Applied Bioscience). The lysates were clarified by centrifugation and equal amounts of protein (12.5 µg) from each lysate were resolved on SDS polyacrylamide gels and transferred electrophoretically to polyvinylidene fluoride (PVDF) membranes. Proliferation was assessed by detection of proliferating cell nuclear antigen (PCNA) in mouse prostate tissue lysates. Briefly, the PVDF membranes were incubated overnight at 4°C with a 1:5000 dilution of a PCNA antibody (#2586, Cell Signaling, Danver, MA, USA) in 5% milk/Tris-buffered saline (TBS) blocking solution followed by 1 h incubation at room temperature with a 1:3000 dilution of a goat anti-mouse antibody HRP conjugated (Bio-Rad Laboratories Inc., #170-6516). All immune complexes in the western blots were visualized using enhanced chemiluminescence detection kit (Fisher Scientific, Pittsburg, PA, USA) and exposed to film (Kodak Bio Light Max). The quantitative analysis presented was obtained using Image J (http://rsbweb.nih.gov/ij/index.html). The membranes were then stripped using a mild stripping solutions (15 g/l glycine, 1 g/l SDS, and 1% Tween 20 at pH 2.2). Briefly, the membranes were incubated for 10 min at room temperature in mild stripping solution, washed for 10 min in TBS, and washed for an additional 10 min in NTBS. The membranes were then blocked in 5% milk/TBS solution for 2 h and incubated overnight at 4°C with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibody at a 1:5000 dilution (# 2118, Cell Signaling) followed by 1 h incubation with a goat anti-rabbit IgG antibody HRP conjugated as described above (Bio-Rad Laboratories, Inc., # 170-6515). GAPDH was used as a protein loading control. Western blots were run twice.

**Statistical analysis**

Quantitative measures were compared between the groups (Myc/BP-1 WT vs Myc/BP-1 KO) using two-tailed Student’s t-test. The proportions of mice with prostate cancer vs mice with mPIN were compared between the two groups using the Fisher’s exact test. To determine the effect body weight had over time between the Myc/BP-1 WT and Myc/BP-1 KO groups, a two-way repeated measures ANOVA was performed followed by Bonferroni post-hoc test. Correlation between serum insulin concentration and fat mass along with serum insulin concentration and pre-fasting weight were analyzed using the Pearson correlation coefficient. All statistical analysis was performed using GraphPad Prism 5.0 Software (San Diego, CA, USA). In all cases, statistical significance was considered when P<0.05. Data are presented as mean ± S.E.M.

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Results

Igfbp1 deletion does not accelerate the development of prostate cancer

To evaluate whether global deletion of Igfbp1 accelerates the development of prostate cancer in the c-Myc transgenic mice, prostate histopathology was evaluated at 16 weeks of age. Contrary to our initial hypothesis, no significant change in prostate cancer incidence was observed in the Myc/BP-1 KO mice (65%) compared with the Myc/BP-1 WT mice (80%; P = 0.48, Fig. 1A). Histological appearances of mPIN and invasive prostate cancer at 16 weeks of age are shown in Fig. 1B and C.

Reduction in prostate tissue proliferation in Myc/BP-1 KO mice

To further investigate the effect of global deletion of Igfbp1 on prostate cancer development, proliferation in prostate tissue from Myc/BP-1 KO and Myc/BP-1 WT mice was determined by western blot using PCNA as a proliferation marker. Myc/BP-1 KO mouse prostate tissue displayed a significant reduction of 71% in proliferation compared with Myc/BP-1 WT mouse prostate tissue (P < 0.05, Fig. 2A and B). These data were further confirmed by Ki-67 immunostaining of mouse prostate sections. As shown in Fig. 2C and D, epithelial cells in adenocarcinoma lesions in the Myc/BP-1 KO group had a significantly lower proliferative index (6.3 ± 0.6% Ki-67) than that of the Myc/BP-1 WT group (14.4 ± 1.1% Ki-67). No significant differences were observed in the proliferation index of PIN lesions (Myc/BP-1 WT: 4.2 ± 1.2% vs Myc/BP-1 KO: 3.2 ± 0.4%; P = 0.3) or benign tissue (Myc/BP-1 WT: 1.4 ± 0.5% vs Myc/BP-1 KO: 1.6 ± 0.6%; P = 0.6). Areas of adenocarcinoma tended to be smaller in Myc/BP-1 KO (4.78 ± 1.36 vs 7.67 ± 1.82 mm²) but the difference was not statistically significant (P = 0.13). PIN lesions areas were similar in both groups. Finally, Myc/BP-1 KO mice showed a decrease in prostate weight (P = 0.05) with no difference in genitourinary weight between the groups (Table 1). Taken together, Myc/BP-1 KO mice displayed no significant change in prostate cancer incidence but a significant decrease in prostate tissue weight and proliferation index was seen.

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Figure 1 Effect of Igfbp1 deletion on the transition from mPIN to cancer in Myc/BP-1 WT and Myc/BP-1 KO mice prostates. (A) Qualitative statistical analyses were performed comparing the presence or absence of prostate cancer phenotype in the Myc/BP-1 WT and Myc/BP-1 KO groups. Fisher's exact test (two-tailed) yielded a P value of 0.48 (n = 20 for each group). Dissection of prostates was performed at 4 months. (B) mPIN involving the lateral lobe of the prostate that was characterized by tufted proliferation of epithelial cells with enlarged nuclei and prominent nucleoli. A peripheral basal layer of flattened epithelial cells is evident. The amorphous particulate and eosinophilic secretions are characteristic of the basal layer of flattened epithelial cells is evident. The amorphous cells with enlarged nuclei and prominent nucleoli. A peripheral prostate that was characterized by tufted proliferation of epithelial performed at 4 months. (B) mPIN involving the lateral lobe of the prostate. (C) Well-differentiated carcinoma consisting of increased numbers of small neoplastic glands with back-to-back configuration. Neoplastic differentiated carcinoma consisting of increased numbers of small neoplastic glands with back-to-back configuration. Neoplastic glands infiltrate outside of the prostate lobe and into adjacent fibrous tissue (magnification, 20×). Full colour version of this figure available via http://dx.doi.org/10.1530/JOE-11-0240.

Figure 2 Effect of Igfbp1 deletion on prostate tissue proliferation in Myc/BP-1 WT and Myc/BP-1 KO mice prostates. (A) PCNA expression was assessed by western blot analysis on prostate tissue lysates as described in the Materials and Methods section. GAPDH was used as a loading control. (B) Western blot analysis of the Myc/BP-1 KO prostate tissue lysates displayed a decrease in proliferation index of PIN lesions (Myc/BP-1 WT: 4.2 ± 1.2% vs Myc/BP-1 KO: 3.2 ± 0.4%; P = 0.3) or benign tissue (Myc/BP-1 WT: 1.4 ± 0.5% vs Myc/BP-1 KO: 1.6 ± 0.6%; P = 0.6). Areas of adenocarcinoma tended to be smaller in Myc/BP-1 KO (4.78 ± 1.36 vs 7.67 ± 1.82 mm²) but the difference was not statistically significant (P = 0.13). PIN lesions areas were similar in both groups. Finally, Myc/BP-1 KO mice showed a decrease in prostate weight (P = 0.05) with no difference in genitourinary weight between the groups (Table 1). Taken together, Myc/BP-1 KO mice displayed no significant change in prostate cancer incidence but a significant decrease in prostate tissue weight and proliferation index was seen.
Table 1 Fasting serum biomarkers, prostate, and genitourinary tract weights

<table>
<thead>
<tr>
<th></th>
<th>Myc</th>
<th>Myc/BP-1 KO</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGFBP1 (ng/ml)</td>
<td>14.7±4.1 (n=11)</td>
<td>ND (n=9)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>IGFBP2 (ng/ml)</td>
<td>341±24 (n=9)</td>
<td>400±54 (n=8)</td>
<td>NS</td>
</tr>
<tr>
<td>IGFBP3 (ng/ml)</td>
<td>1137±73 (n=9)</td>
<td>1275±99 (n=8)</td>
<td>NS</td>
</tr>
<tr>
<td>IGFl (ng/ml)</td>
<td>258±21 (n=9)</td>
<td>304±24 (n=9)</td>
<td>NS</td>
</tr>
<tr>
<td>Prostate weight (g)</td>
<td>0.177±0.01 (n=17)</td>
<td>0.144±0.01 (n=15)</td>
<td>0.05</td>
</tr>
<tr>
<td>Genitourinary weight (g)</td>
<td>0.35±0.01 (n=52)</td>
<td>0.37±0.01 (n=41)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are presented as mean±s.e.m. ND, non-detectable; NS, not significant.

IGF axis is unaffected by Igfbp1 deletion

To determine whether global deletion of Igfbp1 affected other IGF-related proteins, fasting serum IGFBPs and IGF1 levels were measured. As presented in Table 1, no changes were observed in IGFl, IGFBP2, and IGFBP3 serum levels. Validation of genotyping and breeding techniques were confirmed by observing undetectable serum protein levels of IGFBP1 in Myc/BP-1 KO mice (Table 1).

Increased body weight and fat mass in Myc/BP-1 KO mice

Biweekly body weight measurements illustrated a significant difference between Myc/BP-1 KO and Myc/BP-1 WT mice. Myc/BP-1 KO mice displayed a significant increase in body weight from weeks 14 through 16 (P=0.04, Fig. 3A). To assure that the increase in body weight observed in the Myc/BP-1 KO mice relative to the Myc/BP-1 WT mice was not due to the crossing of the BP-1 KO mice with the Myc/BP-1 KO mice, we compared the body weight of BP-1 KO mice with the Myc/BP-1 KO mice. No difference was observed between WT/BP-1 KO mice and Myc/BP-1 KO (31±1.2 vs 30.7±0.7 g, P=0.83). At time of euthanasia, Myc/BP-1 KO mice showed a significant increase of 6.7% in body weight when compared with the Myc/BP-1 WT mice (P<0.05, Fig. 3A). To define whether increase in body weight was attributed to fat mass or lean mass, QMR analysis was performed. Myc/BP-1 KO mice displayed a significant increase in fat mass compared with Myc/BP-1 WT mice (P<0.01, Fig. 3B). No difference in lean mass between the two groups was observed (Fig. 3C).

Myc/BP-1 KO mice display increased fasting serum insulin levels with no difference in fasting glucose

Given that increased body fat has been associated with insulin resistance, fasting serum insulin levels and fasting glucose levels were measured (Reaven 1998). Myc/BP-1 KO mice showed increased fasting serum insulin levels after a 14 h fast (P<0.05, Fig. 4A). However, there was no significant difference in fasting glucose levels between the groups after a 14 h fast (P=0.7, Fig. 4B). There was a significant positive correlation between serum insulin levels and body weight (r=0.56, P<0.01) and fat mass (r=0.53, P<0.01; Fig. 5A and B).

Discussion

Whereas pre-clinical and short-term clinical studies support a possible link between IGFBP1 levels and development of prostate cancer, epidemiologic studies have presented conflicting results. In nested case–control studies, no association was found between serum IGFBP1 and prostate cancer risk (Johansson et al. 2009, Gill et al. 2010). In contrast, Signorello et al. reported an increase in serum IGFBP1 levels in prostate cancer patients. A fivefold increase in prostate cancer risk was observed for patients with circulating IGFBP1 levels above 17 ng/ml after controlling for age, body mass index (BMI), and height (Signorello et al. 1999). Our study was designed to further elucidate the possible role of IGFBP1 in the development of prostate cancer utilizing a global Igfbp1 KO model and the c-Myc transgenic mouse model of prostate cancer.

Contrary to our hypothesis, the global deletion of Igfbp1 in c-Myc transgenic mice did not affect prostate cancer incidence. However, a decrease in prostate weight was observed in the Myc/BP-1 KO mice compared with the Myc/BP-1 WT mice. This decrease was associated with a reduction of 71% in prostate tissue proliferation as measured by PCNA western blot quantification. Further analysis allowed us to define that this reduction in proliferation index was due to a lower proliferation index of epithelial cells within adenocarcinoma lesions. Interestingly, the deletion of Igfbp1 led to significant effects on body composition and metabolic markers. There was a significant increase of 6-7% in body weight in the Myc/BP-1 KO mice relative to the Myc/BP-1 WT mice due to
than IGFBP1 (IC50 are 0.05, 0.25, and 5.0 for IGFBP1, 2002). Although IGFBP2 and IGFBP3 have a lower affinity for
Tripathi et al 2002). Even though we did not see any evidence of hyperglycemia and increased body weight. Increased insulin levels have been associated with increased prostate cancer risk (Rodriguez et al. 2001, 2007, Lehner et al. 2002, Hammarsten et al. 2010) while other studies found no association with prostate cancer (Stattin et al. 2000, 2001, Hubbard et al. 2004). Insulin is thought to be the primary regulator of IGFBP1 expression (Yki-Jarvinen et al. 1995); however, in the current study, the deletion of Igfbp1 leads to a positive correlation between serum insulin levels and adiposity (Fig. 5A and B). This association may suggest that insulin resistance is induced by a distinct mechanism. Further studies are needed to investigate potential mechanisms.

Literature on the association between obesity, high BMI, and prostate cancer risk has also been inconsistent. An association between obesity and increased risk of prostate cancer has been proposed (Rodriguez et al. 2001, 2007) while others have shown no association or an association with lower risk of prostate cancer (Giovannucci et al. 2003, Davies et al. 2009, Wallstrom et al. 2009). Similar to our results, one study found that BMI and insulin levels correlated inversely with IGFBP1 levels independently of insulin resistance in borderline hypertensive subjects (Lemne & Brismar 1998). This inverse correlation remained significant after adjustment for fasting insulin levels in healthy subjects in another study (Janssen et al. 1998). These results mirror those found in this study. One could argue that increase in body weight may have played a protective role against the development of prostate
cancer.

Figure 4 Fasting insulin and glucose concentrations. (A) Myc/BP-1 KO mice showed increased fasting serum insulin compared with Myc/BP-1 WT mice. Serum was obtained after a 14 h fast. Serum insulin concentrations were quantified by ELISA (n=28 for each group). Student’s t-test (two-tailed, unpaired) yielded a P=0.01. Data are presented as mean ± S.E.M. (B) No significant difference was observed in fasting glucose between the groups after a 14 h fast. Glucose concentrations were measured in duplicates. Data are presented as mean ± S.E.M. (Myc/BP-1 WT, n=12; Myc/BP-1 KO, n=11).

We initially postulated that the deletion of Igfbp1 in the c-Myc transgenic mice would lead to an increase in ‘free’ IGF1, thereby leading to an increase in prostate cancer incidence. However, prostate cancer incidence was similar and no difference in total IGF1 levels was observed between the groups (Table 1). The half-life of IGF1 in the ‘free’ form is 10 min, but when bound in ternary complexes such as IGF1/IGFBP3/acid-labile subunit (ALS), the half-life is more than 12 h (Zapf et al. 1995). One plausible explanation for similar total IGF1 levels between the groups is that Myc/BP-1 KO mice continued to produce normal levels of IGF1, but without IGFBPs to increase the half-life of IGF1 in the circulation, unbound ‘free’ IGF1 is rapidly degraded. Total IGF1 measurements included mostly bound IGF1 as only 1% of IGF1 is ‘free’ in plasma (Baxter 1994, Jones & Clemmons 1995). This suggests that Myc/BP-1 KO mice may be able to maintain comparable levels of total IGF1 compared to Myc/BP-1 WT mice possibly by compensation mechanisms through binding to IGFBP2, IGFBP3, and/or IGFBP5 (Tripathi et al. 2009). Even though we did not see any significant change in IGFBP2 and -3 levels, IGFBP5 may have been upregulated to compensate for the lack of IGFBP1. Approximately 80 to 85% of circulating IGF1 is bound in a ternary complex with IGFBP3 and ALS (Baxter 1994, Firth 2002). Although IGFBP2 and IGFBP3 have a lower affinity for IGF1 than IGFBP1 (IC50 are 0·05, 0·25, and 5·0 for IGFBP1, 2, and 3 respectively), IGFBP1 does not sequester the majority of IGF1 in circulation under normal conditions (Oh et al. 1993). Thus, the deletion of Igfbp1 in our intervention played no role in regulating circulating IGF1. Finally, Myc/BP-1 KO mice had smaller prostates than Myc/BP-1 WT mice accompanied with a decrease in adenocarcinoma epithelial cells proliferation index. A trend toward smaller areas of adenocarcinoma was also observed in Myc/BP-1 KO mice, which may explain the significant decrease in prostate weight. Interestingly, lean body mass was unaffected, suggesting that there was no reduced growth of skeletal muscle. Taken together, these data may suggest that the deletion of Igfbp1 had a prostate-specific effect on lessening the c-Myc-driven malignant growth. In summary, global KO of Igfbp1 leads to a decrease in prostate weight and adenocarcinoma proliferation index without affecting cancer incidence. Our results indicate that intracellular IGFBP-1 may play a pro-survival role as previously suggested (Leu & George 2007). Further studies will be required to elucidate the potential mechanism.

Global deletion of IGFBP1 leads to elevated insulin levels without evidence of hyperglycemia and increased body weight. Increased insulin levels have been associated with increased prostate cancer risk (Rodriguez et al. 2001, 2007, Lehner et al. 2002, Hammarsten et al. 2010) while other studies found no association with prostate cancer (Stattin et al. 2000, 2001, Hubbard et al. 2004). Insulin is thought to be the primary regulator of IGFBP1 expression (Yki-Jarvinen et al. 1995); however, in the current study, the deletion of Igfbp1 leads to a positive correlation between serum insulin levels and adiposity (Fig. 5A and B). This association may suggest that insulin resistance is induced by a distinct mechanism. Further studies are needed to investigate potential mechanisms.

Figure 5 Correlation analysis of serum insulin concentrations to pre-fasting body weight and fat mass. (A) Serum insulin correlates with pre-fasting weight as determined by the Pearson correlation coefficient, r=0·56 (P<0·0001). Pre-fasting weight was taken immediately before beginning the 14 h fast. (B) Correlation between serum insulin and fat mass was determined by the Pearson correlation coefficient (two-tailed), r=0·53 (P=0·0009).
enlargement in Myc/BP-1 KO mice. For example, protective adipokines from the increased fat mass, such as adiponectin, may have protected the Myc/BP-1 KO mice from exhibiting an increase in prostate cancer incidence. Adiponectin has been shown to be an 'anticancer' adipokine in prostate cancer as well as in other cancers (Kadowaki & Yamauchi 2005).

In summary, our study found that the global KO of Igfbp1 in c-Myc transgenic mice resulted in the following: a) no change in prostate cancer incidence, b) decreased prostate tissue proliferation and prostate weight, c) increased body weight and fat mass, and d) hyperinsulinemia. These studies suggest that IGFBP1 may play a role in prostate tissue proliferation while also affecting other metabolic parameters in vivo. Further studies are necessary to elucidate the underlying mechanisms for the metabolic effects seen in this model.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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